JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Alkali and Enzymatic Pretreatments of *Eucommia ulmoides* Leaves and Barks on the Extraction of Gutta Percha

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This paper exploited a novel method of single solvent recycling plus enzymatic pretreatment. Petroleum ether (60−90 °C) was an ideal solvent to extract *Eucommia* gum at high temperature (~ 80 °C) and to precipitate the gum (flocculent) as the temperature of the solution fell to below 40 °C. The gutta percha was almost completely precipitated from solution as the temperature cooled down to below 0 °C. After filtration and recovery of the precipitated gum, the filtrate, petroleum ether, was applied to an activated carbon column to remove dissolved impurities and reused for next gutta percha extraction. Because impurities were kept in solution, the precipitated gutta percha was highly pure. In an experiment of enzyme hydrolyzing the plant cell wall, cuticle layers on the surfaces of leaves prevented cellulase from approaching to and hydrolyzing the cellulose of the cell wall. NaOH (1%) at 70 °C efficiently degraded the cuticle layer, which greatly improved the enzymatic hydrolysis of cellulose within eucommia leaves. Gutta percha that was extracted from the alkali- and cellulase-treated leaves had a degree of polymerization as high as in the leaves, and the yield increased from 2.5% of milled leaves up to over 3.2%. The tensile strength of obtained gutta percha increased from 0.02 MPa of milled *Eucommia* leaves to 60.5 MPa, the breakage extensibility ranged from about 0 to 24%, and the tearing strength ranged from 0.5 to 36 kN/m.

KEYWORDS: ***E. ulmoides*** **cuticle layer; cellulase; petroleum ether; gutta percha**

INTRODUCTION

Within the leaves, bark, and seeds of *Eucommia ulmoides* (*E. ulmoides*) Oliv., a large xylophyta mainly grown in China, is a special biomaterial, *Eucommia* (*E. ulmoides*) gum. The gum, also called gutta percha or balata, is a rubber with a molecular structure of *trans*-polyisoprene, an isomer of hevea rubber, *cis*-polyisoprene (*1*) (**Figure 1**). Gutta percha has a distinct nature; that is, because of the transconfiguration, it can crystallize at room temperature, but hevea rubber [or natural rubber (NR)] can not. NR has high flexibility, while gutta percha, as a material possessing the duality of rubber and plastic, has both flexibility and plasticity.

With these properties, gutta percha can mix with NR and plastics, and the mixture will have some special functions (*2, 3*), which do not present in individual rubbers and plastic. With hardness at room temperature, shaping at ordinary temperature, a high Mooney's viscosity, highly anti-impact strength, and hot melt binding properties, gutta percha is a promising material and has potential applications in many fields.

To date, however, the application of gutta percha is limited, much less than that of hevea rubber. Besides a low harvest of gutta percha from individual trees, the main cause is that no cost-effective and high-efficient method has been developed for the extraction of gutta percha from *Eucommia* bark and leaves. Current extraction methods include two classes (*3*−*12*): dry process and wet process (or solvent extraction). The dry process refers to the process that obtains gutta percha under mechanical force through the breaking of plant structures and cells containing gutta percha. Z. B. Chen (*4*) reported in 1987 that *Eucommia* bark or leaves that were subjected to fermentation were boiled in 2% NaOH solution in an autoclave at 80−120 °C for 120−135 min, and then, the treated bark or leaves were shattered with high pressure water and dried. The gutta percha was obtained by screening out plant residuals. The wet method means that organic solvents are applied to extract gutta percha from *Eucommia* bark and leaves, such as Yan's patent (*6*). He developed a method to expose gutta percha; *Eucommia* bark or leaves were ground and screened for the removal of nongum fragments, and then, toluene, benzene, or dichloroethane was used as an extraction solvent, ethanol was used as a precipitant, and acetone was used as a deimpurity agent to obtain gutta percha. Comparatively, the wet method is superior to the dry method in extraction yield. Gutta percha is a high molecular polymer, in which a great deal of double bonds exist that hold certain polarity, so it is insoluble in both strongly and weakly

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10.1021/jf800642y CCC: $40.75
Published on Web 08/30/2008

polar solvents but soluble in moderately polar solvents, such as chloroform, diethyl ether, and benzene, as well as hot petroleum ether. Chloroform and benzene are the most often-used solvents. However, when such small molecule solvents serve as solvents, they will disperse between gutta percha molecules, hampering the crystallization and precipitation of gutta percha from solvent and forming gel at low temperature. To recover the gutta percha from those moderately polar solvents, it is necessary, as current methods have described (*3, 6, 7, 13*), to add a small amount of strongly polar solvent, such as ethanol or acetone, to induce precipitation. However, after the gutta percha precipitate is separated by filtration, the filtrate, mixed solution, can not be reused in the next extraction of gutta percha since the solution can never dissolve gutta percha again, which greatly increases the extraction cost and may cause environmental impacts. On the other hand, if the separation of dissolved gutta percha is done by evaporation of the solvent, impurities within obtained gutta percha will be very hard to remove.

Furthermore, gutta percha, as a polymer, shows many unique properties and functions because of its high degree of polymerization and long molecular chain. However, current extraction methods require *Eucommia* bark and leaves to be crushed up before the extraction to improve the permeability of raw materials and the dissolution of gutta percha in solvents (*3−6, 9, 10, 12−14*). However, such crushing badly damages the long chain and the degree of polymerization of gutta percha, impairing its nature and functions, such as elasticity, toughness, and tensile strength.

In the work reported here, we developed a simplified method "recycling solvent for dissolving gutta percha from *Eucommia* leaves and separating it at different temperature" (*15*). The favorable aspects of the method include lower cost, less solvent usage, less pollution impact, and purer gutta percha. After gutta percha is collected by filtration, the solvent is treated through an activated carbon column, compounds that dissolved in solvent are removed by adsorption, and thus, the treated solvent can be reused in the later extraction of gutta percha from other leaves. Because in the whole gutta percha extraction process only a single solvent is applied for extraction and precipitation, no other solvents are added, no solvent is discarded in the process, and so no environmental impact is caused by the solvent. Significantly, the application of enzymes instead of mechanical crush in this method for the pretreatment of *Eucommia* bark and leaves basically avoids cutting of gutta percha polymer and maintains its original degree of polymerization. The gutta percha obtained through the alkali and enzymic pretreatments holds the original nature of itself.

MATERIAL AND METHODS

Sample and Solvent. *Eucommia* leaves and bark were harvested from large trees 20 years old in Zunyi district, Guizhou province, China, where *E. ulmoides* Oliv. abundantly grows. The fresh bark and leaves were dried in air followed by a cabinet drier at ∼50 °C overnight. Some of those dried materials were milled, and some were cut into big pieces, ∼2 cm in diameter, with scissors or a big puncher. Methanol, ethanol, acetic ether, nitrobenzene, chloroform, acetonitrile, acetic acid, acetone, isopropanol, phenol, butanol, 1,2-dichloroethane, carbon tetrachloride, and hexane of analytical grade were purchased from Tianjin Kermel Chemical Reagents Development Centre (Tianjin, China). Petroleum ether (60−90 °C) for the extraction of gutta percha was purchased from Kelong Chemical Reagent Co. (Chengdu, China). All solvents were used as received. Sodium hydroxide and sulfuric acid of analytical grade were obtained from Shanghai Chemicals Co., Chinese Medicine Group. Water was supplied by a Milli-Q water purifier system (Millipore). Two commercial enzyme preparations, cellulase and pectinase, were obtained from Jinyuan Biological Co., Zhangjia Gan, China.

Figure 1. Molecular structures of *Eucommia* gum and hevea rubber.

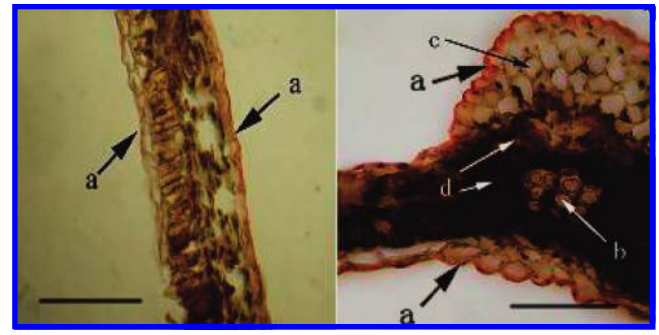

Figure 2. Sections of *Eucommia* leaves, after they were macerated in acetonitrile for 5 days, are stained by Sudan III. Points: a, stained cuticle layer; b, vasculature; c, thin wall cell; and d, gutta percha. Pictures were taken under a light microscope (ECL IPSE-E200, Nikon, Japan). The bar is 120 μm.

Degradation of Cuticle on Leaf. On the surface of the leaf, there is a cuticle layer that protects the leaf and hampers the accessibility of enzymes to the cellulose cell wall. To check the cuticle layer on the leaves, the dried leaves were cut into big pieces and macerated in acetonitrile for 5 days, and then, the macerated pieces were stained by Sudan III, sections of which were pictured under light microscope (ECL IPSE-E200, Nikon, Japan) and are shown in **Figure 2**. Cuticle removal studies were carried out by dispersing leaf pieces in different solvents to dissolve the cuticle or in alkali solution and acid solution according to its degradation as it reacts with chemicals. In the degradation, *Eucommia* leaf pieces (100 g) were macerated and degraded in 350 mL of aqueous solution containing an increasing amount of NaOH (1−5%) in a 500 mL conical flask at 30−70 °C on a laboratory shaker (120 r/min) for 6 h. Control groups were macerated the same way in 350 mL of Milli-Q water without any chemicals. Subsequently, the mixture was filtered, and the filter residue was washed with deionized water to neutrality and dried in an oven at 50 °C.

Enzymatic Hydrolysis of Cellulose−Wall of Plant Cell. Five grams of enzyme preparation powder was weighed in 500 mL of acetate buffer solution (pH 4), and the mixture was stirred constantly for 45 min. Then, the extract was separated from insoluble fractions by filtration through absorbent cotton. The enzyme solution was kept at 4 °C in a sealed glass container until further use. Sodium carboxymethyl cellulose (S-CMC), purchased from Damao Chemicals Co. of Tianjin, China, was used as a standard substrate for the investigations of working conditions of enzyme. Two grams of sodium carboxymethyl cellulose was weighed and dissolved in 200 mL of buffer solution (40 g of $NaAc \cdot 3H_2O$, 268 mL of 6 mol/L HAc, and water made up 1 L solution, pH 4.0) using a team bath to heat it. The solution was transferred to a glass container after it was filtered through #1 filter paper (Whatman) to study the working conditions of enzymatic hydrolysis later.

Alkali-pretreated *Eucommia* leaf pieces were then hydrolyzed in 100 mL of cellulase solution and 200 mL of acetate buffer (pH 4). The hydrolysis was conducted on a shaker (120 r/min) at 50 °C for 2−4 h. The pretreated leaves were hydrolyzed in the same way a second time using fresh cellulase solution if necessary. Certain leaves were also hydrolyzed using pectinase. After the solution was filtered, the residue was dried in a hood for the gutta percha extraction. Sampling of the hydrolysis solution was carried out at every half-hour from the start of hydrolysis to determine glucose conversions.

Some *Eucommia* bark pieces were hydrolyzed in 4% NaOH solution at 30−40 °C for 2 days, and then, the treated pieces were hydrolyzed with cellulase at 50 °C and pH 4.0 for 6 h. The enzymolysis was repeated four times. It was very important to keep the experimental temperature at less than 55 °C, which is the melting point of gutta percha crystals. The obtained gutta percha crystals were white. Their images, without extraction by petroleum ether under a light microscope (ECL IPSE-E200, Nikon, Japan), are shown in **Figure 9A**. **Figure 9B** shows the images of silk spun from the crystals.

Analysis of Reducing Sugar. To determine glucose conversions in this study is to determine the produced reducing sugar. The analysis was conducted according to the colorimetric method of 3,5-dinitrosalicylic acid oxidizing glucose as described in ref *16*. An amino compound was given while such reducing sugars as glucose and cellobiose were oxidized by 3,5-dinitrosalicylic acid in an alkaline condition. The product was measured at 530 nm using a Shimadzu UV-2550 spectrophotometer (Shimadzu, Japan). A calibration curve was established by operations as follows. Ninety-one grams of potassium sodium tartrate was dissolved in 500 mL of water. To this were added 3.15 g of 3,5-dinitrosalicylic acid and 10.4 g of sodium hydroxide. After they dissolved completely, 2.5 g of redistilled phenol and 2.5 g of anhydrous sodium sulfide were further added to make a color-developing reagent. The reagent was then kept in a brown bottle for 1 week before use. A glucose standard solution (500 μg/mL) was prepared by dissolving 0.500 g of dry glucose of analytic grade (that had been dried at 105 °C for 2 h before use) in Milli-Q water. Prior to photometric analysis, the glucose standard solution was diluted 1:5−1:25 v/v in Milli-Q water, and 2.5 mL from each was taken to mix with 2.5 mL of color-developing reagent. The mixtures were heated in a boiling water bath for 5 min and measured at 530 nm for a calibration curve.

Extraction of Gutta Percha. The extraction of gutta percha from leaf pieces and bark pieces treated by alkali and enzyme was performed in a round-bottom flask using a single solvent, petroleum ether (60−90 °C), at material ratio of petroleum ether to treated leaves in volume (mL)/weight (g) of 10:1 to 15:1. The resulting mixture was heated at a temperature of 80−85 °C under reflux for 2 h and stirred constantly. While the extract was still hot, it was separated from insoluble fractions through a stainless steel screen (screen mesh 100). The extract (filtrate) was cooled to room temperature and then frozen in a refrigerator (MDF-436, SANYO Electric Biomedical Co. Ltd., Japan) at below 0 °C for 0.5−1.0 h to induce and speed up the precipitation of gutta percha. The precipitate that was obtained by filtration through 100 mesh stainless steel screen was a pure gutta percha, and the filtrate was petroleum ether that contained little gutta percha; after it was adsorbed by activated carbon to remove liposoluble substances, it was reused in the next extraction of gutta percha from other leaves or bark.

To check the novel method presented by us, *E. ulmoides* leaves pretreated in different ways were extracted under reflux at 80 °C in petroleum ether to obtain gums. The pretreatment ways of leaves involved just a milling treatment, a hydrolization in alkali solution without milling, a milling and enzymolysis by cellulase, and a hydrolization in alkali solution and enzymolysis by cellulase without milling. The gums obtained were captured with a digital camera (see **Figure 10**).

Determination of Physical Properties of Obtained Gutta Percha. The extracted gutta perchas from milled leaves and from the alkali- and cellulase-treated leaves were shaped into a thick sheet (5 × 15 cm^2 and 0.5 cm in thickness) on a polytetrafluoroethylene board. After they dried up in the air, the boards were placed in an oven at 80 °C for 1 h to allow the gutta percha to melt and stick together. The tensile strength, breakage extensibility, and tearing strength were measured on an Electronically Omnipotent Tester, WDW-10C, made by Hualong Test Instrument Co. Ltd., Shanghai, China.

RESULTS AND DISCUSSION

Gutta percha comprises as much as 3−5% by weight of the dried leaf material and 6−12% by weight of dried bark material in *Eucommia* plants (*17*). Although seed shell and root bark contain more gutta percha, *Eucommia* leaves and bark are the major resources because of their higher total yields, especially the yield of leaves, which are produced a great amount every year by *E. ulmoides* Oliv., a deciduous tree. Leaves are the renewable and nonexhausting resource for gutta percha. So, *Eucommia* leaves are our main research subject.

However, enzyme hydrolysis experiments showed cellulase and/or pectinase could not directly degrade cellulose, or the cell wall, within leaves even in the appropriate enzymatic working conditions. Experiments demonstrated that even little substances had been hydrolyzed from leaves by cellulase or pectinase, as compared to the control without enzyme loading. Losses of weight, or the amounts hydrolyzed by cellulase and pectinase, were 1.59 and 1.72 g or 31 and 33%, respectively, while the loss of weight of leaves in control, without enzymes, was 1.47 g or 29.5%. The residual weights of leaves were 3.52 g for pectinase, 3.48 g for cellulase, and 3.53 g for control, which suggested that the addition of enzymes did not evidently increase the loss of weight, or hydrolyzed amount, of leaves in water. Those imply that some kind of material may be present and protect cellulose from being degraded by enzymes, whereas the difference between enzymes and control, we supposed, is due to the dissolution and degradation of oligosaccharide and pectin within leaves.

A further study showed that the material that hampered the hydrolysis of cellulose is a compound that grows on the surface of leaves, known as a cuticle, or called a cuticular membrane, a protective material layer to protect the leaves from being destroyed by microorganisms in their living surroundings. The compound is a network recalcitrant macromolecular polymer and is mainly composed of hydroxyl fatty acid with 16 or 18 C. **Figure 2** confirms the existence of the cuticle that appears as a red color after being stained by Sudan III (symbolized as a in **Figure 2**).

Degradation of Cuticle. The recalcitrant structure of the cuticle and its close association with the leaf matrix make it highly resistant to the enzymic hydrolysis of cellulose. Therefore, the cuticle must be physically or chemically removed to improve the accessibility of the enzyme to cellulose and the cell wall. Various removal methods including organic solvents, dilute acid, and dilute alkali are assessed for their ability to improve enzymic hydrolysis. A number of solvents have been attempted to dissolve the cuticle on the surface of leaves. However, a long-term (more than 5 days) soak revealed that, of 14 organic solvents (see the list in the section of Sample and Solvent), no one could satisfactorily dissolve the cuticle from leaves at room temperature, although those polar solvents (methanol, ethanol, etc.) turned out to be green and phenol brown. It suggests that those often-used organic solvents are powerless in removing the cuticle of *Eucommia* leaves at ordinary temperature. However, leaves soaked in 30% H_2SO_4 solution turned its color into light dark, and the cuticle layer was able to be peeled off, while the leaves treated by 5% NaOH solution became soft and were soaked perfectly, and the cuticle dropped from the leaves and decomposed into tiny pieces. These indicated that alkali was clearly beneficial in removal of the cuticle layer, which is shown in **Figure 3**.

In addition to the level of alkali, during the hydrolysis of cuticle, temperature is another important parameter. As **Figure 4** indicated, the higher temperature is clearly beneficial in increasing the glucose conversion at all alkali levels (see the curves in **Figures 3** and **4**). However, the weight loss of leaves during enzymic hydrolysis, see **B** in **Figure 4**, suggests that the maximum glucose conversion is achieved at 70 °C, while the increase in glucose conversion levels off at temperatures

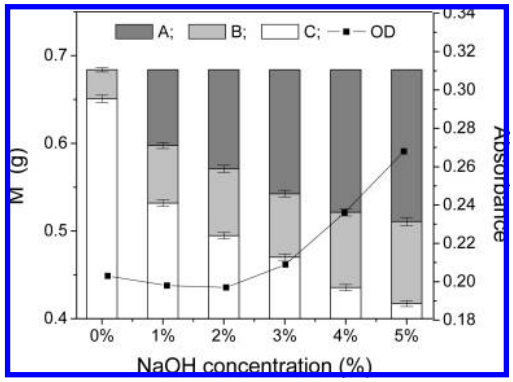

Figure 3. Effects of solutions with different alkali levels on the hydrolysis of *Eucommia* leaves at 40 °C for 3 h. (**A**) Loss of weight in alkali solution, (**B**) loss of weight in enzymic hydrolysis, and (**C**) residual weight of leaves. OD, the curve, is the absorbance at 530 nm of glucose in solution as the cellulase hydrolysis is in process (the concentration of cellulose is 0.0333 g/mL or 991 IU/mL).

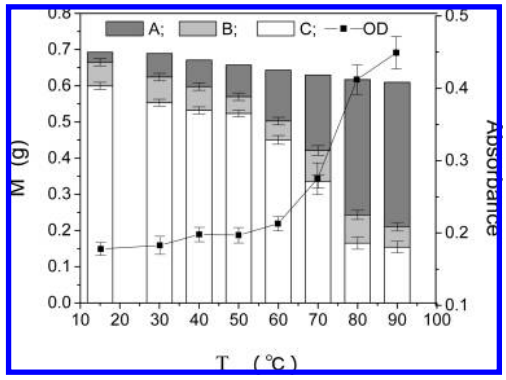

Figure 4. Effects of 1% NaOH on the hydrolysis of *Eucommia* leaves at different temperatures. The residence time is 3 h. (**A**) Loss of weight in basic solution, (**B**) loss of weight in enzymic hydrolysis, and (**C**) residual weight of leaves. OD, the curve, is the absorbance at 530 nm of glucose in solution as the cellulase hydrolysis is in process (the concentration of cellulose is 0.0333 g/mL or 991 IU/mL).

above 80 °C. It is shown from **Figures 3** and **4** that the pretreatment at higher temperature and higher level of NaOH increased the weight loss of leaves. However, a higher NaOH concentration impaired gutta percha quality, leading to a low elasticity, low yield, and greatly degraded natural compounds contained in leaves, some of which, such as chlorogenic acid, are important natural medicine. So, 1% NaOH was selected, and higher temperature (70 °C) was set to balance the efficiency and velocity of hydrolysis.

As the residence time extended, the mass loss of *Eucommia* leaves soaked in 1% NaOH become less and less. In the first 2 h, the hydrolysis of cuticle caused the weight of leaves to decrease sharply; during the period of 2−5 h, leaves were macerated and loosened, and substances within leaves diffused into solution, resulting in mass loss rapidly in 5 h of residence time and slowly after 5 h (see **Figure 5**). Thus, 5 h of residence time of *Eucommia* leaves being treated in 1% NaOH at 70 °C not only degrades the surface cuticle completely but also is beneficial in loosening the cell structure and in improving the enzymic hydrolysis as well, because alkali treatment also degrades some amounts of lignin and hemicellulose within leaves and bark. Although the amounts of lignin and hemicelluloses hydrolyzed in 1% NaOH at 70 °C are limited, their degradation increases the permeability of plant tissue for petroleum ether.

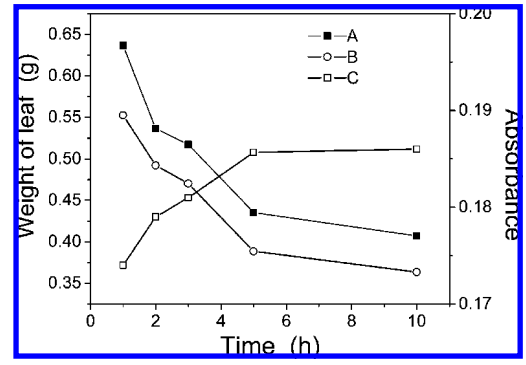

Figure 5. Effects of 1% NaOH on the weights of leaves in different residence times at 70 °C. (**A**) Loss of weight in basic solution, (**B**) loss of weight in enzymic hydrolysis, and (**C**) absorbance (at 530 nm) of glucose in a solution of cellulase hydrolysis (the concentration of cellulase is 0.0166 g/mL or 494 U/mL).

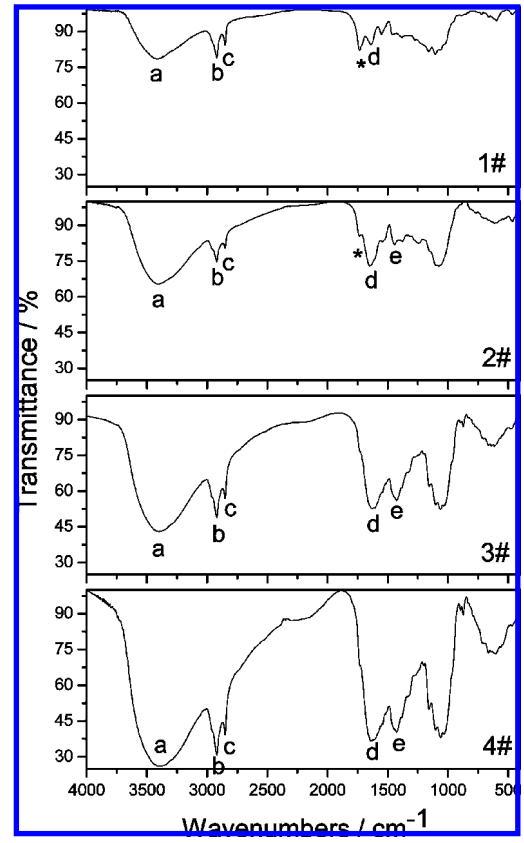

Figure 6. Infrared spectra: 1#, the cuticle layer peeled from the leaves in sulfuric acid solution; 2#, *Eucommia* leaves without alkali and cellulase treatment; 3#, *Eucommia* leaves treated by basic solution and cellulase solution; and 4#, *Eucommia* leaves treated only by basic solution. *Characteristic absorption of cuticle layer. The IR analysis was performed on a Nigaoli 5700 STIR spectrometer (MA).

Figure 6 shows infrared spectra of *Eucommia* leaves treated in different ways. The first part (1#) in **Figure 6** is an IR spectrum of cuticle peeled from leaves that was soaked in sulfuric acid for 2 days, which shows an obviously characteristic absorption peak of cuticle at 1730 cm^{-1}, sign * in the figure, which, we thought could be a reference to check if the alkali solution degraded the cuticle on the leaves. The characteristic absorption peak can also be seen in the spectra of leaves that were not pretreated (2#). However, the IR spectra of leaves hydrolyzed by alkali and cellulase or just by alkali, 3# and 4# in **Figure 6**, are indicative of the absence of cuticle. The

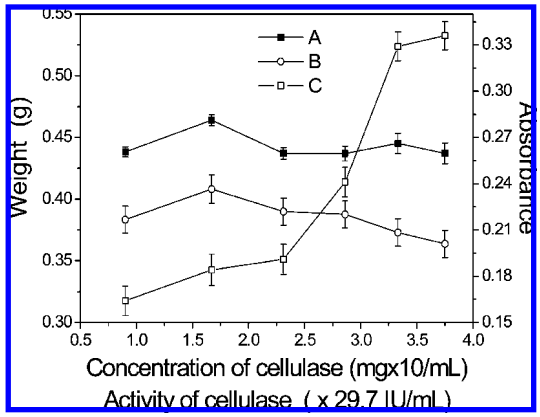

Figure 7. Effects of cellulase loading in solution on the hydrolysis at 50 °C and pH 4.0 for 3 h. (**A**) Loss of weight in base solution, (**B**) loss of weight in cellulase solution, and (**C**) absorbance (at 530 nm) of reducing glucose in a solution containing cellulase.

comparisons of IR spectra suggest that alkali pretreatment can degrade and remove cuticle layers efficiently from *Eucommia* leaves. These results indicated that 1% of alkali solution can evidently degrade the cuticle on the surface of *Eucommia* leaves at 70 °C.

Hydrolysis of Cellulose within Leaves. Activities and biologic functions of enzyme highly depend on its working circumstance: temperature, pH, and enzyme loading, the three vital factors. Cellulase was first used to hydrolyze carboxymethyl cellulose (CMC) to get the knowledge of enzyme working conditions, and then, on the basis of the conditions, the hydrolysis of *Eucommia* leaves by cellulase was investigated.

Temperature experiments showed that the optimum temperature was 50 °C, at which the remaining weight of *Eucommia* leaves is the minimum and the level of produced glucose is the highest (if interested, see Figure S-1 in the Supporting Information), implying the maximum degradation of cell wall. The temperature is identical with the optimum one of cellulase hydrolyzing CMC.

As the pH shifted to 4, the degradation of cellulose within leaves went up to the highest. The cellulase built of amino acids is amphiprotic substance. Its spatial conformation and combination state with substrate may change with the varied pH; hence, the enzymatic activity for hydrolyzing fiber modifies correspondingly. It is shown that the optimal pH value is 4.0, and at other desired conditions, the glucose conversion reaches its maximum (see Figure S-2 of the Supporting Information).

With the increase of cellulase loading, the level of reducing glucose produced in enzymatic hydrolysis increases. Actually, enzyme performance was much slower, and loading of cellulase needed more in the enzymatic hydrolysis of pretreated leaves as compared to that in the hydrolysis of CMC. As a loading of enzyme increases to 0.025 g/mL, the yield of glucose increased evidently and nearly reached its equilibrium after the concentration of cellulase went up to 0.033 g/mL as the absorbance curve of **Figure 7C** shows.

For the optimization of the temperature, enzyme loading, and pH, as shown previously, the residence time was kept fixed at 4 h. However, a shorter residence time might be enough to reach the same conversions considering the leaves had been macerated in alkali solution and the structure of cellulose might fall apart. In the initial residence time of 45 min, the produced reducing glucose increased significantly and glucose conversion reached the maximum. Beyond 1 h, little or no improvement was observed. Cellulase is an enzyme that its activity will be

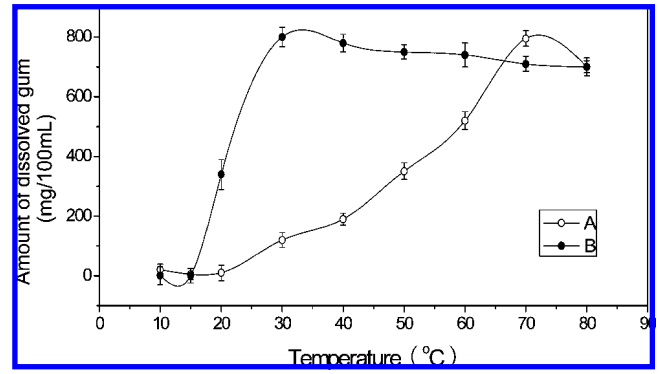

Figure 8. Amount of gum that was extracted from alkali- and enzymic-pretreated leaves dissolved in petroleum ether at different temperatures. Key: A, curve of temperature rising to dissolve gutta percha; and B, curve of temperature falling down to precipitate gutta percha.

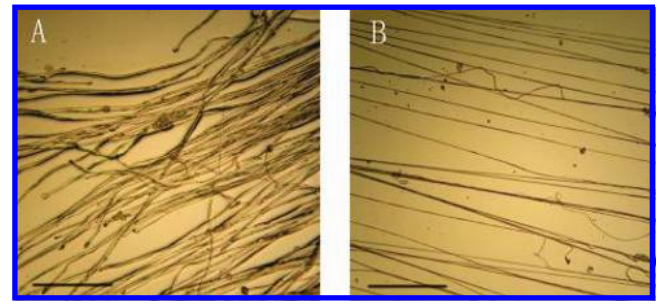

Figure 9. Images of (**A**) *E. ulmoides* gum crystals that grow in the cell and (**B**) silks that are spun from the crystal. Pictures were taken under a light microscope (ECL IPSE-E200, Nikon, Japan). The bar is 100 μm.

inhibited by hydrolysis product as the level of product goes up. Behaviors of the residence time affecting the enzymatic hydrolysis are shown in Figure S-3 of the Supporting Information. So, in the actual, the enzymatic hydrolysis might need to be repeated in fresh hydrolysis solution. The structure of cellulose within leaves is tighter than purchased CMC, so its residence time of enzymatic hydrolysis is longer than that of CMC.

Extraction of Gutta Percha. Gutta percha exiting in *E. ulmoides* Oliv. plants as crystals includes two kinds of crystal forms (*18*): an α-crystal with a melting point at 65 °C and a β-crystal with a melting point at 55 °C (*19*). To improve the dissolution of gutta percha in organic solvent, the crystal grating has to be broken first. Heating can provide energy (crystal grating energy) to break both gutta percha crystals, α and β, provided that the temperature rises up to over 65 °C. Chloroform is the best solvent for dissolving gutta percha because its molecule is small and easy to penetrate into and destroy the crystal grating of gutta percha. The same is true for diethyl ether. However, both of them have a lower boiling point (61.15 and 34.6 °C, respectively) than the melting point of gutta percha. Although bearing double bone and the boiling point is 80.10 °C, higher than 65 °C, benzene has a higher toxicity to operators. Petroleum ether consists of a series of solvents with different molecular weights, classified into four groups according to their boiling spreads. Of them, the petroleum ether with boiling spread at 60−90 °C possesses a suited boiling temperature range and smaller molecule as compared to the other three groups of petroleum ethers, which help to improve the resolution of gutta percha into the solvent. Although it dissolves gutta percha very slowly at ordinary temperature, petroleum ether (60−90 °C) will dissolve the gum quickly and greatly when the reflux temperature is fixed over 65 °C since melted gutta percha is facility to diffuse into the ether.

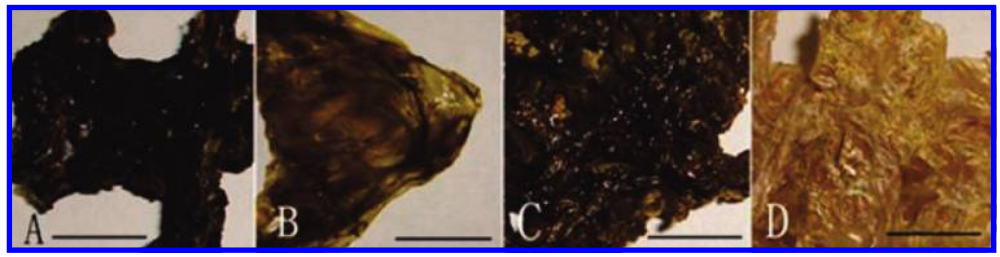

Figure 10. After they were pretreated in different ways, *E. ulmoides* leaves were extracted under reflux in petroleum ether to obtain gum. (**A**) Leaves were milled and extracted in petroleum ether; (**B**) leaves were treated by alkali solution and extracted; (**C**) leaves were milled and treated by cellulase; and (**D**) leaves were treated by alkali solution and then cellulase solution and extracted in petroleum ether. Pictures were taken with a digital camera. The bar is 1.5 cm.

Table 1. Effects of the Ratio of Leaves to Petroleum Ether on the Extraction of the Gum

ratio of leaves to petroleum ether (g/mL)	60/400	60/480	60/600	60/800	60/1000
recovery of gum (%) alkali and enzymic pretreatment	2.831	3.012	3.206	3.210	3.213
recovery of gum (%) (*14*) milled leaves	2.10	2.40	2.508	2.495	2.522

Table 2. Some Physical Properties of Gums Obtained from Milled *E. ulmoides* Leaves and from Alkali- and Enzymic-Pretreated *E. ulmoides* Leaves

	tensile strength (MPa)	breakage extensibility (%)	tearing trength (kN/m)
gum from milled leaves	0.02	~0	0.50
gum from alkali- and enzymic-pretreated leaves	60.5	24	36.0

Figure 8 indicates that the levels of gutta percha dissolving in petroleum ether increase as the reflux temperature rises. A higher temperature speeds up the diffusion of gutta percha from leaves to solution. However, at 80 °C, the content of gutta percha in solvent did not increase as compared to that at 70 °C, which demonstrated that the petroleum ether (60−90 °C) had approached to the saturation in dissolving gutta percha at such conditions. We set the reflux temperature at 80−85 °C in the actual extraction of gutta percha.

In the research on the extraction of gutta percha, the precipitation of gutta percha from extraction solution is as significant as the dissolution of gutta percha from leaves or bark to extraction solvent. Petroleum ether (60−90 °C) dissolves gutta percha a little at room temperature, suggesting that the ether has a different saturation capacity for gutta percha at a different temperature. Furthermore, experiments indicated that different pretreatment of leaves and bark has a different precipitation temperature curve: Gutta percha extracted from milled leaves or bark was precipitated from a solution at 40 °C, while the gum from alkali- and enzyme-treated leaves or bark began to precipitate from solution until the temperature fell to 30 °C (see **Figure 8**). The temperature at which gutta percha begins to precipitate is considered a critical temperature of precipitation. The lower temperature is clearly beneficial in increasing the amount of precipitated gutta percha. Therefore, a detailed study of the effects of different temperature on the precipitation amount was undertaken.

For the milled leaves and bark, the precipitation of gutta percha from extraction solution reached the maximum at 20 °C below zero. The flocculent gutta percha was very easily separated with solution through filter screen and the filtered solution contained only trace gutta percha and so could be reused for the next extraction of gutta percha from leaves and bark. Experiments showed the amount of gutta percha deposited from extraction solution increased sharply as the temperature was close to −20 °C (see Figure S-4 of the Supporting Information). Further temperature experiments indicated that if the temperature was lowered to −40 °C, the gum extracted from milled leaves precipitated as disperse fine grains that were very hard to filter through screen or filter paper. Detection of gutta percha remaining (or dissolved) in solution demonstrated that a filtered solution contained almost the same trace gutta percha as the filtered solution at −20 °C.

For the alkali- and enzymic-pretreated leaves and bark, behaviors of gutta percha in precipitation are clearly different from that from the milled leaves and bark. Experimental results imply that the gutta percha obtained has a much higher polymerization degree and much greater molecular weight. The gutta percha dissolved in extraction solution is more likely to form a supersaturated solution and its critical precipitation temperature is 30 °C, 10 °C lower than the gum from milled leaves and bark. Almost all gutta percha, as **Figure 8** shows, precipitated from extract at 15−10 °C. A further experiment demonstrated that the gutta percha (in dissolved form) remained in solution that approximated zero when the temperature was cooled down to 0 °C for 0.5−1 h. These results also imply that the gutta percha extracted from alkali- and enzymic-pretreated leaves and bark is different from the gutta percha from milled leaves and bark in their molecular structure and weight.

We detected that 100 mL of hot (80 °C) petroleum ether could dissolve about 0.8 g of pure gutta percha. However, in actual extraction, the content of gutta percha in extract is much lower than the level, which, we believed, was due to the dissolved impurity compounds. The level of gutta percha extracted from milled leaves was 0.318 g in 100 mL of hot petroleum ether at its maximum, while the level from alkali- and enzymic-pretreated leaves is much higher than that from milled leaves, reaching up to 0.425 g. We suppose that alkali and cellulase pretreatments have removed a great deal of impurity compounds from leaves by dissolving them in alkali and enzymatic solution. So, when such pretreated leaves were refluxed with petroleum ether, only a small amount of impurity compounds was dissolved in the ether, which was beneficial in increasing the amount of gutta percha dissolved in petroleum ether, and meanwhile, enzymatic pretreatment increased the permeability of plant cell, promoting solvent and gutta percha diffused to and from cells containing gutta percha. **Table 1** shows that the recoveries of both gutta percha extracted from alkali- and enzymic-pretreated leaves and from milled leaves reach the optimum at material ratio of the weight of leaves to the volume of petroleum ether, 60:600 or 1:10.

Figure 9 shows images of gutta percha from *Eucommia* bark. The bark was cut into big piece (1 cm × 3 cm) and soaked in 4% NaOH solution at 30 °C for 5 days during which the alkali

solution was changed three times to depolymerize lignin effectively prior to enzymatic treatment. After enzymatic treatment, gutta percha was directly recovered by filtering the enzymatic solution through a filter screen without solvent extraction. Those experiments were undertaken at a temperature lower than 50 °C (the temperature of cellulase working), so the gutta percha obtained kept its original appearance as they were in bark (**Figure 9A**). The silks appearing in **Figure 9B** are long silks that were spun from the crystalline gutta percha shown in **Figure 9A** under outside force.

The gutta percha grown in bark has a higher degree of polymerization and greater molecular weight than those grown in leaves because bark is perennial while leaf is renascent every year, because measurements showed that the breakage extensibility of the gutta percha from bark is about 31%, while the gutta percha from leaves is 24%, which, we believe, highly depends on the degree of polymerization of gutta percha from leaves and bark. Therefore, extraction of gutta percha from bark is simpler than the extraction from leaves.

To check the method presented in this paper, different methods were taken to compare the quality and efficiency of gutta percha extracted by them. Pictures of gutta percha were taken and are shown in **Figure 10**. **Figure 10B**,**D** are the gums extracted from the leaves without milling. The gums have much higher tensile strengths than the gums from milled leaves, as **Figure 10A**,**C** shows. The determination of physical properties of gums indicated that the tensile strength, breakage extensibility, and tearing strength of the gutta percha obtained from alkali- and enzymic-pretreated leaves had increased greatly against the gutta percha extracted from milled *E. ulmoides* leaves. These results shown in **Table 2** suggest that the gutta percha from milled leaves has basically lost the original properties of the polymer. On the other hand, the recoveries were different depending on the used methods. The recovery of gutta percha shown in **Figure 10C** is the highest, reaching up to 3.58% (W/W) of dry leaves; that shown in **Figure 10D** is 3.21%, being extracted using the new method, that is the leaves were pretreated by alkali and cellulase, while that shown in **Figure 10A** is 2.50%, extracted from milled leaves, and the one shown in **Figure 10B** is only 1.02% because the leaves were just treated by alkali without being milled.

We have mentioned in previous paragraphs that besides gutta percha, some other compounds within leaves were also dissolved into petroleum ether, which led to a substantial decrease in the dissolved capacity of gutta percha in petroleum ether and hence in the efficiency of gutta percha extraction. Activated carbon was used to remove these impurity compounds. The recovered petroleum ether was applied to an activated carbon column, and impurities contained in the ether were adsorbed on activated carbon. **Figure 11** gives the absorbed spectra of recovered petroleum ether before and after being treated by activated carbon. The use of activated carbon not only enhanced the extraction capacity for gutta percha in solvent recycling but also recovered impurities—fat-soluble nature compounds—that was able to be eluted from activated carbon by benzene. Some of these nature compounds are potentially medicines. **Figure 11** also shows that the used petroleum ether, after treatment by activated carbon, contains some impurity (see **Figure 11**, curve b) as compared to pure petroleum ether (see **Figure 11**, curve c). The residual impurity, however, will slowly accumulate as recycling times increase but can be removed by distilling the petroleum ether after recycling 5−7 times.

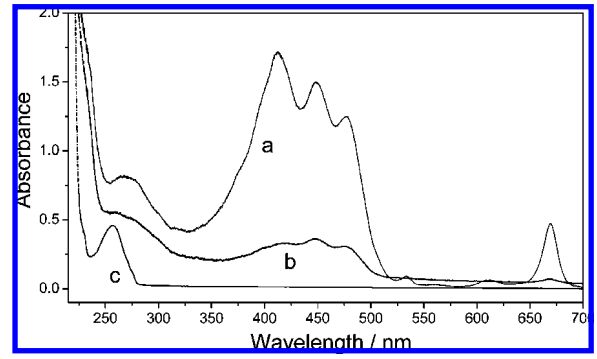

Figure 11. UV−vis absorbed spectra of petroleum ether solution before, curve a, and after, curve b, treated by activated carbon. Curve c is the spectra of pure petroleum ether.

Petroleum ether (60−90 °C) is not one of the best solvents for dissolving gutta percha at ordinary temperatures; however, such a characteristic of petroleum ether makes it become an ideally single solvent for recycling extraction of gutta percha through changing temperature only. The recycling single solvent for extraction gutta percha could be used for large-scale production based on its low costs and little environmental impact since no organic solvent is discarded. Treatment of activated carbon enhances the solvency of recycling petroleum ether and recovers lipid soluble substances, from which some potential medicines might be found. However, the time of the whole process from pretreatment of leaves to final gutta percha is prolonged due to the long period of cellulose hydrolysis by enzyme.

Supporting Information Available: Figures of the effect of temperature on the hydrolysis of *Eucommia* leaves in cellulase solution, effect of pH on the hydrolysis of cellulose, relationship between the residence time and enzymolysis, and influence of cooling temperature on the deposition amount of gum extracted from mille leaves. This material is available free of charge via the Internet at http://pubs.acs.org.

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Received for review March 2, 2008. Revised manuscript received July 30, 2008. Accepted July 31, 2008. Financial support from the Doctor Foundation Program, Guizhou University, China, and from the Social Key Project [No. (2000)1163] of Guizhou Province, China, is gratefully acknowledged.

JF800642Y