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INFLUENCE OF BIOPRETREATMENT ON THE CHARACTER OF CORN STOVER LIGNIN AS SHOWN BY THERMOGRAVIMETRIC AND CHEMICAL STRUCTURAL ANALYSES

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The effect of corn stover lignin structure alteration caused by white-rot fungi pretreatment on the pyrolysis kinetics was studied by FTIR and TG/DTA. Results showed that biopretreatment had a remarkable effect on lignin pyrolysis. Biopretreatment can decrease the activation energy and increase the pre-exponential factor in the initial stage of pyrolysis, which makes it possible to start the lignin pyrolysis at a relatively gentle condition and improve the availability of biomass pyrolysis as a renewable energy. Analysis by FTIR showed that white-rot fungi destroyed the aromatic skeletal carbons, which are the main ether and carbon linkages of lignin, converting lignin into compounds having relatively simple structures. The relationship between the pyrolysis characteristics and the structure alteration pretreated by white rot fungi showed that the deconstruction and depolymerization of recalcitrant linkages of lignin could accelerate the reaction and lignin pyrolysis with lower energy consumption.

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INTRODUCTION

Because of the accelerated rate of growth of energy consumption in Asia, and particularly in China, the incentives for developing renewable energy sources are of growing importance (Mohan et al. 2006). With the environmental benefits, biofuels (Searchinger et al. 2008) are produced by converting various biomass resources to provide renewable energy (Kim et al. 2007). Pyrolysis is a possible thermochemical conversion route, converting biomass to a huge number of chemical compounds that can be used as conventional fuels (Meier and Faix 1999).

However, little research work has been carried out on green pretreatment of biomass prior to pyrolysis. Recent studies have primarily focused on physical (steam explosion and hot compressed water treatment), and chemical (acid and alkali) (Hassan, Steele and Ingram 2009; Dobele et al. 2003) pretreatments. Both of physical and chemical processes require high temperature and operating pressure, and the usage of acid and alkali can cause serious environmental consequences. White rot fungi (Blanchette 1984), which have been considered to be the most efficient microorganisms during plant lignin degradation occurring in nature (Hatakka 1994), are playing a more and more important role in the biomass pretreatment for pyrolysis.

Lignin (Guo et al. 2001), which is a heterogeneous polymer in woody and vascular tissues, is considered to be the most complex cell wall component, and it is resistant to being decomposed and pyrolyzed, compared with cellulose and hemicellulose. Due to the complex structure of lignin and the difficulties in lignin isolation (Jiang and Argyropoulos 1999; Jung and Himmelsbach 1989), published research about the pyrolysis characteristics of pretreated lignin is scarce. After it is pretreated with white rot fungi (Kirk and Chang 1975), lignin can be significantly deconstructed, making the structure of biomass less compact and more easily pyrolyzed.

The purpose of the present work was to explore the thermogravimetric characterization of lignin pretreated by the white-rot fungus *Irpex lacteus* CD2, determining its influence on the pyrolysis of biomass. The structure alterations of lignin have also been studied to investigate the relationship between biopretreatment and thermogravimetric characterization of lignin pyrolysis. For studying how biopretreatment influences lignin pyrolysis, the changes were analyzed by TG/DTA and FTIR.

EXPERIMENTAL

Fungal Strain and Cultivation on Corn Stover

White-rot fungus *Irpex lacteus* CD2 was obtained from the Shennongjia Scenic Area in Hubei of China, and subsequently isolated by the authors in Key Laboratory of Molecular Biophysics, Ministry of Education in China, Huazhong University of Science & Technology. The organisms were maintained on potato extract agar slant cultures at 5°C. Two discs cut from actively growing cultures on potato extract agar plates were used to inoculate 100 ml of 20 % potato extract and 2 % glucose medium (pH 5.5) in 250 ml round flask incubated at $25\,^{o}$C for 3-5 days on a reciprocal shaker. The contents of the flask were gently homogenized, and 10 ml was used to inoculate the second generation grown culture for 3 days in the same condition. 10 g (dry mass) of chopped corn stover (40-50 mm long) collected from Henan in China was taken in a 500 ml Erlenmeyer flask, moistened with 25 ml distilled water, autoclaved (150 kPa, 1 h), inoculated with 10 ml of secondary generation homogenized seed culture, and incubated at 28°C. After 15 and 30 days, the biopretreated corn stover was dried to remove water in vacuum at $60\,^{o}$C.

Lignin Isolation from Biopretreated Corn Stover

An enzymatic and acidolysis method was used for isolating lignin from biopre-treated and unpretreated corn stover (Guerra et al. 2006; Wu and Argyropoulos 2003). EMALs were isolated from samples (10 g) of vibratory- or rotary ball-milled corn stover according to the procedure described by Wu and Argyropoulos. The ground corn stover meal was treated with cellulase and hemicellulase in a previously optimized ratio of 40 FPU/g. The enzymatic hydrolyses were carried out at $40\,^{o}$C for 48 h using 50 mM citrate buffer (pH 4.5) at 5% consistency in an orbital water bath shaker. The insoluble material that remained after the enzymatic hydrolysis was collected by centrifugation (2000g), washed twice with acidified deionized water (pH 2.0), and freeze-dried. The crude lignin obtained was further submitted to a mild acid hydrolysis using an azeotrope (bp, $86\,^{o}$C) of aqueous dioxane (dioxane/water 85:15, v/v, containing 0.01mol L^{-1} HCl) under an argon

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atmosphere. The resulting suspension was centrifuged (2000g), and the supernatant was carefully withdrawn, neutralized with sodium bicarbonate, and finally added dropwise to 1 L of acidified deionized water (pH 2.0). The precipitated lignin was allowed to equilibrate with the aqueous phase overnight, and it was then recovered by centrifugation, washed twice with deionized water, and freeze-dried.

Fourier Transform Infrared spectroscopy (FTIR) analysis

FTIR spectra were recorded with a NEXUS 670 spectrometer (Thermo Nicolet Corporation, Madison, WI). KBr pellets for FTIR spectroscopy were prepared using a Perkin Elmer pellet die (2 mg of lignin sample in 40 mg of KBr) (Pandey and Pitman, 2003). Peak height and area was measured using OMNIC software version 1.2a (Nicolet Instrument Corporation, USA).

Thermogravimetry-Differential Thermal Analysis (TG/DTA)

TG/DTA experiments were performed by using a sensitive thermobalance (PerkinElmer, Diamond) by Analytical and Testing Center, Huazhong University of Science and Technology. Temperature, furnace, and weight were calibrated according to the manufacturer's recommendations. Temperature calibration was performed by measuring the Curie points of indium, tin, and gold. Prior to thermogravimetric experiments, samples were ground to chips small enough to pass through a 0.15-0.2 mm screen. Initial sample masses of 5mg were placed in the pan of the TGA microbalance, which was enough to fill the pan because of the low density of the ground samples. Air was used as carrier gas. Experiments were carried out on a thermobalance at a linear heating rate of $10\,^{\circ}\mathrm{C}\ \mathrm{min}^{-1}$, with the temperature range from $25\,^{\circ}\mathrm{C}$ to $1000\,^{\circ}\mathrm{C}$, at a steady air flow of $100\ \mathrm{ml}\ \mathrm{min}^{-1}$.

RESULTS AND DISCUSSION

Structure Analysis of Lignin

Figure 1 shows several major absorption bands in FTIR representing the differences between corn stover lignin pretreated by *I. lacteus* CD2 for 15 and 30 days versus the control sample.

Results were analyzed according to Pandey and Pitman (2003), Collier et al. (1992), Faix (1986), and Pandey (1999). Strongly increasing intensities at the 1688-1708 cm^{-1} band were recorded in pretreated lignin, which indicated the existence of unconjugated carbonyl groups. The peak at 1657-1658 cm^{-1} band assigned to C=O in conjugated carbonyl groups was observed in biopretreated lignin. By degrading with white rot fungi, the aromatic skeletal vibration at 1509-1513 cm^{-1} decreased, and the intensity of aliphatic C-H stretching vibration increased. In sum, the results showed that during lignin biodegradation, white-rot fungi can generate a great amount of conjugated and unconjugated C=O, which may be significant to lignin biodegradation as an important oxidation procedure. In addition, decreasing intensity in aromatic skeletal vibrations and increasing intensity of C-H vibration in aliphatic carbon suggested that *I. lacteus* CD2 was able to destroy the aromatic skeletal carbon of lignin.
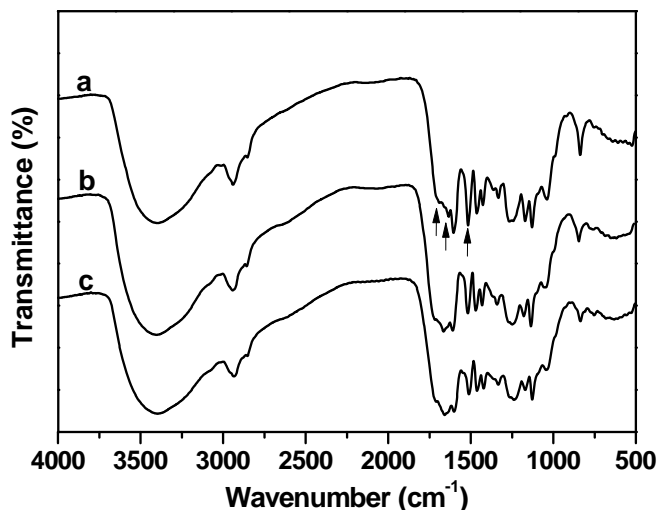
Fig. 1. FTIR spectra of biopretreated lignin (a = original corn stover lignin, b = corn stover lignin biopretreated by white rot fungi for 15 days, c = corn stover lignin biopretreated by white rot fungi for 30 days)

Thermal Decomposition Studies

There were significant differences in the TG-DTG-DTA curves between the biopretreated and the original lignin. As it shows in Fig. 2, at the heating rate of 10 $^{\circ}$C min^{-1}, the exothermic peak of biopretreated lignin increased compared with control sample, which indicated that biopretreatment with white rot fungi can increase the heat released in the pyrolysis reaction. And as the pretreated time was extended, more heat was released. TG curves (Fig. 3) of both biopretreated lignin and control sample showed three pyrolysis stages (Stage 1 a moisture weight loss from about 50 $^{\circ}$C to 130 $^{\circ}$C, Stage 2 active pyrolysis occurred around 190 $^{\circ}$C to 450 $^{\circ}$C, Stage 3 passive pyrolysis from about 500 $^{\circ}$C to the end).

Moreover, the peak shapes of the DTG curves (Fig. 4) were different for biopretreated lignin in comparison to unpretreated lignin. Two peaks occurred in the thermal decomposition of biopretreated lignin during pyrolysis within the temperature range from 200 $^{\circ}$C to 400 $^{\circ}$C, which was different from unpretreated lignin with only one peak. Also, the thermal degradation rates of biopretreated lignin were higher than original lignin from 280 $^{\circ}$C to 480 $^{\circ}$C, and the longer the lignin was biopretreated, the faster the thermal decomposition reaction was completed. Moreover, the maximum degradation rates (($-dX/dt)_{max}$) of biopretreated lignin were much higher than for unpretreated lignin. Biopretreated lignin could reach the maximum degradation rate at a relatively lower temperature compared with the original lignin. On the other hand, there were two ($-dX/dt)_{max}$ for biopretreated lignin (80.1ug min^{-1} and 89.5 ug min^{-1} at 281.6 $^{\circ}$C and 343.7 $^{\circ}$C for biopretreated lignin for 15 days; 95.9 ug min^{-1} and 103.4 ug min^{-1} at 283.7 $^{\circ}$C and 348.9 $^{\circ}$C for biopretreated lignin for 30 days) which was higher than that of original lignin (74.1 ug min^{-1} at 283.8 $^{\circ}$C) at a lower temperature.
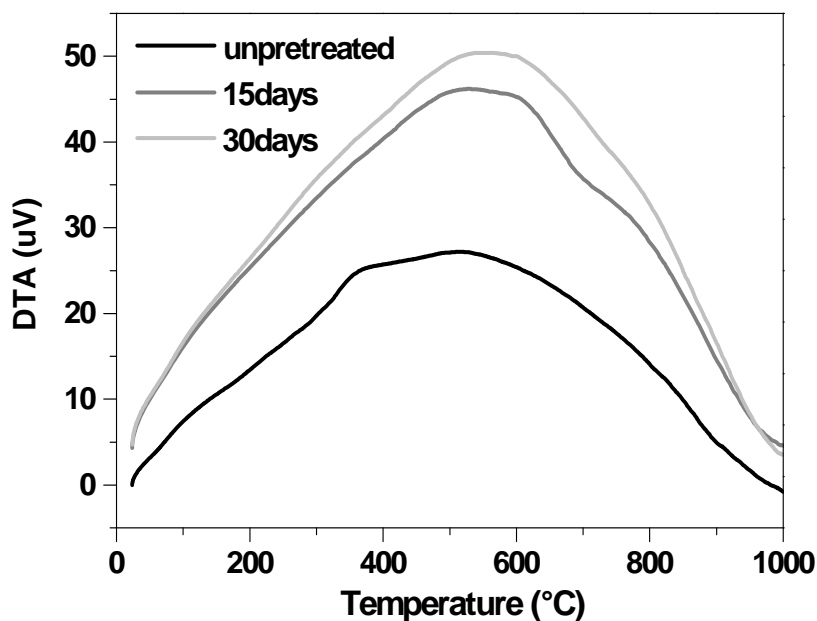
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Fig. 2. DTA curves of biopretreated lignin (unpretreated = original corn stover lignin, 15days = corn stover lignin biopretreated by white rot fungi for 15 days, 30days = corn stover lignin biopretreated by white rot fungi for 30 days)

Fig. 3. TG curves of biopretreated lignin (unpretreated = original corn stover lignin, 15days = corn stover lignin biopretreated by white rot fungi for 15 days, 30days = corn stover lignin biopretreated by white rot fungi for 30 days)
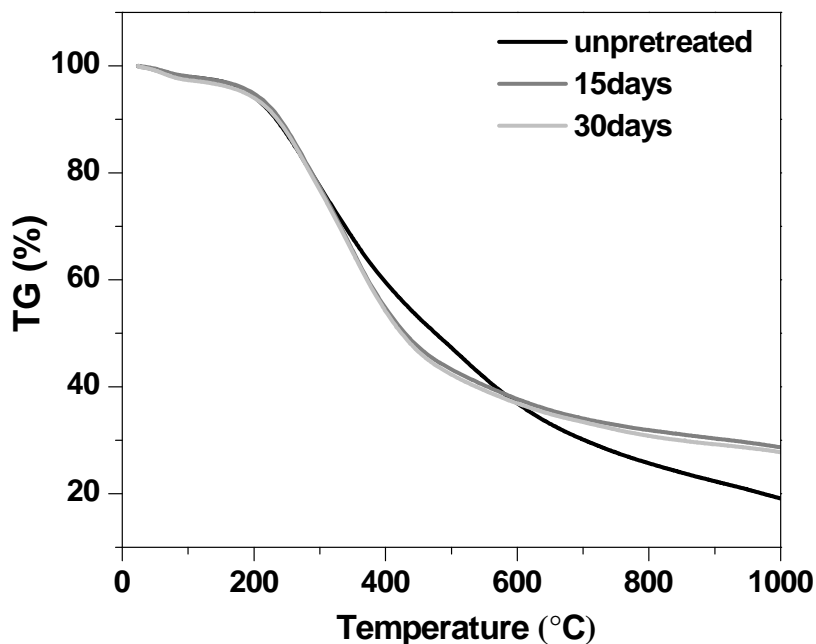
Fig. 4. DTG curves of biopretreated lignin (unpretreated = original corn stover lignin, 15days = corn stover lignin biopretreated by white rot fungi for 15 days, 30days = corn stover lignin biopretreated by white rot fungi for 30 days)
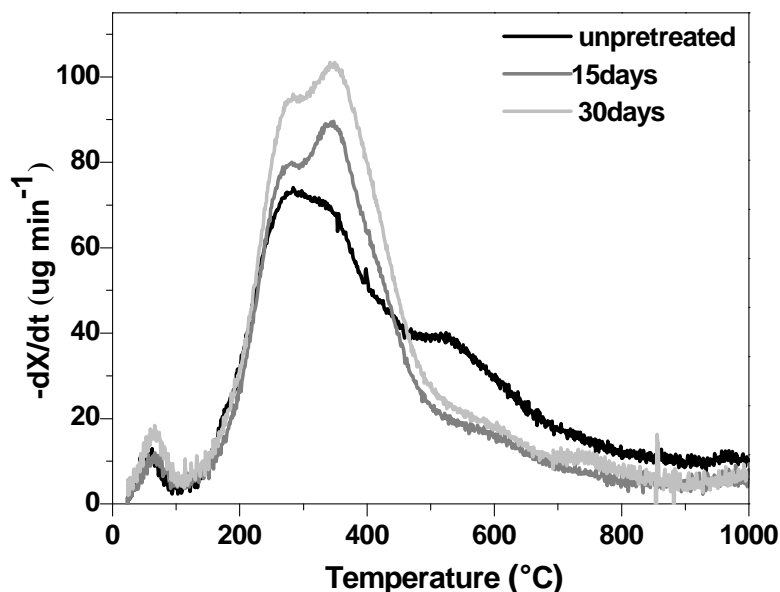
Kinetics Analysis of Biopretreated Lignin Pyrolysis

As was shown above, a simple one-stage model was not suitable for explaining the behavior of biopretreated and original lignin pyrolysis. Therefore, a model consisting of two independent simultaneous reactions was used to describe the pyrolysis behavior of lignin. In the formulation of the kinetic model, it is additionally assumed that the two reactions are of first order. Mathematical analysis was performed by Coats and Redfern's integral method (1964), to determine the kinetic parameters by using the data from TG and DTG curves. The method has been successfully used for studies on the kinetics of decomposition of solid substances (Girgis and Petro 1988; Khadikar 1987; Ninan 1989; Órfão and Martins 2002). Kinetic equations of a common type can be written as follows,

$$\frac{d\alpha}{dt} = k(T)f(a) \tag{1}$$

where $k(T)$ is the temperature dependent rate constant, T the absolute temperature, t the time, and α the degree of transformation. And α can be calculated from the corresponding TG curve by the formula,

$$\alpha = \frac{w_0 - w}{w_0 - w_f} \tag{2}$$

where w is the mass fraction present at any time, w_0 is the initial mass fraction, and w_f is the mass fraction at time infinity.

According to the Arrhenius equation,

$$k = A \exp\left(-\frac{E}{RT}\right) \tag{3}$$

where E is the activation energy, R is the gas constant, k is the rate coefficient, and A is the pre-exponential or frequency factor. Under a constant heating rate:

$$\frac{\mathrm{d}T}{\mathrm{d}t} = \beta = \text{constant} \tag{4}$$

Since the variation degree of conversion is a function of temperature, and the temperature depends on the time of heating, the reaction rate could be written as follows:

$$\frac{\mathrm{d}\alpha}{\mathrm{d}t} = \frac{\mathrm{d}\alpha}{\mathrm{d}T}\frac{\mathrm{d}T}{\mathrm{d}t} = \beta\frac{\mathrm{d}\alpha}{\mathrm{d}T} \tag{5}$$

After some transformations and a combination of Eqs. (1), (2), (3), (4) and (5):

$$\int_0^\alpha \frac{\mathrm{d}\alpha}{f(\alpha)} = \frac{A}{\beta}\int_0^T \exp\left(-\frac{E}{RT}\right)\mathrm{d}T \tag{6}$$

Then, denote Eq. (6) with,

$$g(\alpha) = \int_0^\alpha \frac{\mathrm{d}\alpha}{f(\alpha)} = \frac{A}{\beta}\int_0^T \exp\left(-\frac{E}{RT}\right)\mathrm{d}T \tag{7}$$

where g(α) is the integral function of conversion.

Coats-Redfern used an asymptotic approximation for the resolution of Eq. (7), obtaining:

$$\ln\left(\frac{g(\alpha)}{T^2}\right) = \ln\left(\frac{AR}{\beta E}\right) - \frac{E}{RT} \tag{8}$$

Plotting in $\ln[g(\alpha)/T^2]$ versus $1/T$ should result in a straight line with a slope $-E/R$ and a intercept providing the values of E and A. There are different expressions of $g(\alpha)$ for the different solid state mechanisms. Assuming a reaction order of 1 (Orfão et al. 1999), the reaction mechanism function is f $(\alpha) = (1-\alpha)$. The integral equation can be written as,

$$\ln\left[\frac{-\ln(1-\alpha)}{T^2}\right] = \ln\left(\frac{AR}{\beta E}\right) - \frac{E}{RT} \tag{9}$$

and the integral equation (Eq. (9)) is used to describe the thermodegradation behavior of corn stover lignin pretreated and unpretreated by *I. lacteus* CD2. The results are given in Table 1.

Table 1. Kinetic Parameters of Biopretreated Lignin at the Heating Rate of 10°C min^{-1} in Air Atmosphere (Biopretreated for 15 days = corn stover lignin biopretreated by white rot fungi for 15 days, Biopretreated for 30 days = corn stover lignin biopretreated by white rot fungi for 30 days).

Temperature/^{o}C	Samples	E/(kJ/mol)	A/s^{-1}	R
162°C -220°C	Corn stover lignin	22.42	1.93×10^{9}	0.9914
	Biopretreated for 15 days	19.38	1.87×10^{7}	0.9984
	Biopretreated for 30 days	12.11	2.67×10^{6}	0.9940
220°C -447°C	Corn stover lignin	23.68	3.60×10^{11}	0.9931
	Biopretreated for 15 days	30.94	1.24×10^{7}	0.9994
	Biopretreated for 30 days	31.01	2.90×10^{5}	0.9992
447°C -736°C	Corn stover lignin	16.58	8.98×10^{16}	0.9780
	Biopretreated for 15 days	12.01	9.33×10^{19}	0.9613
	Biopretreated for 30 days	9.85	7.70×10^{20}	0.9605

Table 1 showed that, in the initiation stage of pyrolysis, the activation energy of biopretreated lignin was lower than that of the original lignin, and the value of activation energy was lower as the biopretreated time was longer (22.42 kJmol^{-1} for original corn stover lignin, 19.38 kJmol^{-1} for lignin biopretreated for 15 days, 12.11 kJmol^{-1} for lignin biopretreated for 30 days), indicating that by deconstructing the complex structure of lignin, white rot fungi can promote the initiation of the pyrolysis. In the third stage of the pyrolysis, the activation energy of biopretreated lignin was lower than the original lignin, making the reaction easier to maintain. The activation energy of the original lignin showed no significant differences during three pyrolysis stages, which was different from the biopretreated lignin. For biopretreated lignin, the activation energy decreased in the initiation and the third stage of pyrolysis, making the thermal degradation easier to begin and react.

Although the mechanism of pyrolysis is still not yet understood well, some probable explanations for the effect of biopretreatment can be given based on the current literature. Since the pyrolysis is a reaction of depolymerization (Chen et al. 1998), the depolymerization caused by biopretreatment can promote the pyrolysis reaction. Also, the production of carbonyl groups by biopretreatment might lead an increasing number of the cleavage of the carbon–oxygen bond at the temperature between 598–648 K (Demirba 2000), which is in the temperature range of the second stage of pyrolysis. Thus, it is probable that the increase of carbonyl groups might contribute to a somewhat higher activation energy in the second stage.

CONCLUSIONS

1. Structural analysis of biopretreated lignin showed that white rot fungi could destroy the aromatic rings of lignin and increase the C-H vibration in aliphatic carbons, converting the complex structure of lignin into relatively simple structures. By deconstructing the complicated structure of lignin and liberating cellulose and hemicellulose, biopretreatment can improve the feasibility of using biomass as a renewable and green energy source.
2. Differential thermal analysis (DTA) of biopretreated lignin showed that by biopretreatment, the process of lignin pyrolysis could release more heat than unpretreated lignin. The longer the white rot fungi pretreated, the more heat was released in lignin thermal degradation.
3. Thermal gravimetric analysis showed that biopretreatment could increase the thermal decomposition rate of lignin. The maximum rates of biopretreated lignin were much higher than for the control sample, and a shorter time may be required to reach the decomposition temperature for biopretreated samples.
4. Pyrolysis kinetic parameters showed that the activation energies of biopretreated were much lower than for unpretreated samples in the initiation and the end of the pyrolysis reaction in air atmosphere, making the pyrolysis reaction easier to initiate and maintain.

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