



Association of Mushroom Cultivation and Ozonolysis as Pretreatment for Enzymatic Saccharification of Sengon (*Falcataria moluccana*) Sawdust

Gabungan Pra-perlakuan Ozonolisis dan Budidaya Jamur Sebelum Sakarifikasi Enzimatis Serbuk Kayu Sengon (Falcataria moluccana)

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ABSTRACT

Wood industry based on sengon (*Falcataria moluccana*) material has developed rapidly and cause the huge wastes. Lignocellulosic materials, such as sengon sawdust, offer a great potential as cheap and abundant feedstock for biofuels production. Ozone oxidation pretreatment was carried out on sengon wastes to improve fermentable sugar production by enzymatic saccharification. Sengon wood (SW), sengon media (SM), and sengon spent media (SMM), that was waste of media after mushroom cultivation, treated with ozone for different treatment time were enzymatically saccharified. Then the decrease of hydrolysis weight, reducing sugar yield, and monosaccharide yields were determined. Ozone treatment resulted in Klason lignin degradation in the all samples, resulting in the improvement of subsequent enzymatic saccharification. Ozone treatment with the duration more than 30 min was not suitable for SMM sample. Glucose yield after saccharification from the ozone-treated sample for 60 min was the highest among all samples in SW and SM treated, but for SMM it was 30 min. Based on these results, we concluded that mushroom cultivation can reduce the ozone time treatment and it is an effective treatment to improve sugar yield by enzymatic saccharification of sengon wastes.

KATA KUNCI

sengon
 sisa media
 ozon
 sakarifikasi enzim
 gula pereduksi

INTISARI

Industri kayu berbahan baku kayu sengon (*Falcataria moluccana*) saat ini tengah berkembang dengan sangat pesat dan hal ini menyebabkan timbulnya banyak limbah kayu sengon. Materi lignosellulosa, seperti limbah serbuk kayu sengon, merupakan potensi yang sangat baik sebagai bahan baku *bio-fuel* karena murah dan banyak tersedia. Praperlakuan dengan oksidasi ozon pada limbah kayu sengon dilakukan untuk meningkatkan produksi gula reduksi melalui proses sakarifikasi enzimatis. Serbuk sengon (SW), media sengon (SM), dan limbah media sengon (SMM), yang merupakan sisa dari media budidaya jamur, diberi perlakuan dengan ozon pada berbagai lama waktu perlakuan. Sampel yang sudah diberi perlakuan ozon tersebut kemudian dianalisis kandungan kimianya dan di-sakarifikasi secara enzimatis. Selanjutnya diukur laju hidrolisis, kadar gula pereduksi, dan kadar monosakarida. Perlakuan dengan ozon menyebabkan degradasi kadar Klason lignin di semua sampel, sehingga mengakibatkan peningkatan kemampuan sakarifikasi dari enzim. Perlakuan ozon dengan lama waktu lebih dari 30 menit tidak sesuai untuk sampel SMM. Kadar glukosa hasil sakarifikasi yang tinggi diperoleh dari sampel SW dan SM yang diberi perlakuan ozon selama 60 menit, serta SMM yang diberi perlakuan 30 menit. Berdasarkan hasil ini, dapat disimpulkan bahwa perlakuan jamur dapat mengurangi lama waktu perlakuan ozon dan praperlakuan efektif untuk meningkatkan kadar gula reduksi yang dihasilkan dari sakarifikasi enzimatis limbah kayu sengon.

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Introduction

Sengon (*Falcataria moluccana*) is a fast-growing tree species generally used as raw material for pulp and paper manufacture. Indonesian wood industries are currently using sengon not only for pulpwood but also for light construction, furniture, and wood composite for construction (plywood and laminated veneer lumber). In the island of Java, sengon timber production reaches 2 million m³/year and causes timber industries based on sengon materials have developed rapidly. On the other hand, the industries also cause side effects such as waste sawdust especially from sengon. Most of the wastes of sawdust sengon are used by farmers to make cultivation media of wood mushrooms. However, after cultivation, residues of the media have also become a new problem. Media were dumped into the environments may decay and release methane gases that causes greenhouse gases to the atmosphere.

Lignocellulosic materials, such as sengon sawdust wastes, offer a great potential as cheap and abundant feedstock for biofuels production. Enzymatic hydrolysis of the cellulose fraction yields glucose that is fermented to obtain fuel-grade ethanol. The main disadvantage to produce economically ethanol from lignocellulosic materials is its low digestibility due to the complex chemical structure. Lignocellulosic materials are formed by cellulose, hemicellulose and lignin, closely linked. Lignin significantly impacts enzymatic hydrolysis of lignocellulosic biomass. Therefore, a pre-treatment step has to be considered because of the high crystallinity of the cellulose and the presence of lignin. The pre-treatment step should improve the accessibility of hydrolytic enzymes to cellulose. Moreover, the pretreatment should avoid degradation of hemicellulose and cellulose because sugar degradation not only decreases ethanol yield but also produces degradation products that are inhibitory to

the yeast used in the subsequent fermentation (García-Cubero et al. 2010).

One of the best studied pretreatment is ozonisation. Ozone is known to be a powerful oxidizing reagent and has a potential to degrade lignin without producing harmful compounds (Yokota et al. 2007). Ozone is highly reactive toward compounds with double bonds and functional groups (Sarkanen et al. 1992; Garcia-Cubero et al. 2009). In terms of pre-treating lignocellulose for subsequent enzymatic saccharification, the advantage of ozone treatment is highly selective to removal of lignin with slight degradation of hemicelluloses, whereas it hardly affects cellulose (Sun & Cheng 2002). Moreover, ozone reaction can be performed at room temperature under atmospheric pressure, leading to a low pretreatment cost.

The result of ozonisation is not complete mineralisation of such products, but rather formation of a variety of oxidised intermediates, whose concentrations dependent on the reaction time (Amat et al. 2003). The previous study of ozone oxidation pretreatment that was carried out on spent culture media (SCMs) of *Quercus serrata* after *Lentinula edodes* cultivation resulted that 1 h was an effective duration (Ueda et al. 2013). In addition, the type of material also influences the effectiveness of ozonation process (García-Cubero et al. 2010).

Sengon sawdust and mushroom cultivation media waste have different chemical contents. Mushroom cultivation media waste has a lower lignin content because the content of lignin was reduced during the growth of fungi (Irawati et al. 2012a). In this study, we observed the optimal duration of ozone treatment for sengon wood waste and sengon that was used for mushroom cultivation (spent media). Ozone-treated samples were enzymatically saccharified, and hydrolysis weight decrease, reducing sugar yield, and monosaccharide yields were determined. Based on the results obtained, the effects

of ozone treatment on enzymatic saccharification on sengon waste were discussed.

Materials and Methods

Biomass materials

Wood meals (in sawdust size about 9–80 mesh) of sengon (*Falcataria moluccana*) with 8.3% moisture content (MC) used in this study. The wood meal also was used for cultivation experiment of wood ear mushroom (*Auricularia polytricha*). After inoculation of *A. polytricha*, the media were cultured for 130 days in a culture room as previously described (Irawati et al. 2012b).

Ozone treatment

In ozone treatment, ozone was produced by an ozonizer (ED-OG-R6, EcoDesign, Japan) at a flow rate of 50 mL/min. Before ozone treatment, the samples were dried in the oven at 45°C. The dried sample (1 g) was put in a Kjeldahl flask (100 mL) and then MC of the sample was adjusted to 40 % with distilled water. Ozone treatment was done using 6 % concentration of ozone (0.26 g/h flow rate) for 15, 30, and 60 minutes.

Chemical component analysis

Before chemical analysis, the samples were ground by a rotary speed mill (P-14, Fritsch) and then sieved to collect samples in 40–80 mesh size. After that, the samples were dried in an oven at 45°C. The amounts of α -cellulose, Klason lignin, and acid-soluble lignin of the sample were determined by previous methods (Dence 1992; Anonymous 2000; Carrier et al. 2011). Chemical component analysis was conducted three times for each sample. Values of chemical components in ozone treated sengon wood (SWO) and ozone treated media (SMO) were adjusted to the values based on ozone un-treated samples using weight loss during ozone treatment. Ozone treated sengon spent media (SSMO) was adjusted to the values based on sengon media (SM) using weight

decrease ratio in a bag from SM to sengon spent media (SSM) and also weight loss during ozone treatment.

Enzymatic saccharification

A commercial enzyme, Meiselase (Meiji Seika), was used for the saccharification of each sample. Two hundred mg of oven-dried sample (40–80 mesh size) was saccharified by 50 mg of enzyme at 40°C for 48 h (Irawati et al. 2012a). Hydrolysis weight decrease (HWD) is the numbers of samples which was hydrolyzed, it is calculated by comparing the weight of hydrolyzed samples with the weight of original samples before treatment. Monosaccharides was quantified by using high-performance anion-exchange chromatography (DX 500, Dionex) (Irawati et al. 2013). Reducing sugar yield was determined by the dinitrosalicylic acid (DNS) method (Miller 1959). Enzymatic saccharification was conducted three times for each sample. Qualitative and quantitative analyses of the monosaccharides were carried out using a high-performance anion exchange chromatography (HPAE) system (DX500, Dionex, USA) equipped with a pulsed amperometric detector (ED40, Dionex). The analytical column was a CarboPac™ PA-1 column (4 x 250 mm, Dionex) connected with a CarboPac™ PA-1 guard column (4 x 50 mm, Dionex). Temperature of the column oven (Model 502, EYELA) was set at 30°C. The mobile phase consisted of ultrapure water (A), 100 mM sodium hydroxide (B), and 250 mM sodium hydroxide (C). Eluent flow was set at 1.0 mL/min. Galactose (Wako Pure Chemical Industries, Ltd.), glucose, and xylose (Wako Pure Chemical Industries, Ltd.) were used as standard monosaccharides. For monosaccharide analysis, a freeze-dried sample was dissolved in 10 mL distilled water. After that, the sample (1 mL) was filtered through Millex-L (0.20 μ m, Millipore, USA), and then 10 μ L of filtered sample was injected onto the HPAE system.

Results and Discussion

Effect of ozone treatment duration

Changes in chemical component contents during ozone treatment with different treatment times are shown in Table. 1. Chemical contents differences among SW and SM were caused by the addition of other ingredients in the media (such as bran and CaCO₃), while among SM and SSM were caused by the activity of fungi that degraded media during its growth. Differences in chemical contents which were showed among SW and SWO, SM and SMO, and SSM and SSMO occurred due to ozone treatment.

In this study, the Klason lignin content in ozone treated samples decreased with an increase in ozone treatment duration. In addition, holocellulose content also decreased gradually over 60 min of ozone treatment, while α -cellulose did not change significantly throughout the treatment. These results suggest that ozone treatment resulted in the degradation of lignin and hemicellulose in the samples. In general, ozone treatment mainly degrades lignin but also hemicellulose in lignocellulosic biomass, whereas cellulose is only slightly affected (Quesada et al. 1999; Sun & Cheng 2002).

Ethanol-toluene extracts content in the samples of ozone-treated increased start for the first 15 min, except in SSMO that slightly decreased. The increase in ethanol-toluene extracts in the sample of ozone-treated may have been caused by degradation of lignin and lignin–hemicellulose complexes during ozone treatment. Acid-soluble lignin content was stable or slightly decrease in the ozone-treated samples. In general, acid-soluble lignin is mainly composed of lignin degradation products, with the remainder composed of secondarily formed hydrophilic materials such as lignin–hemicellulose complexes (Yasuda et al. 2001). In contrast, Garcia-Cubero et al. (2010) reported that acid-soluble lignin content increased after ozone treatment in rye

straw, which probably caused by the differences of used biomass.

Effect of ozone treatment on enzymatic saccharification

The previous research pointed out that lignin inhibits enzymatic saccharification by limiting the enzyme accessibility to polysaccharides and by absorbing enzymes (Sun & Cheng 2002). Garcia-Cubero et al. (2010) reported a proportional increase in sugar production with lignin degradation by ozone pretreatment.

The effects of the duration of ozone treatment on HWD after saccharification are shown in Fig. 1. The largest HWD (74.3%) after saccharification was obtained after ozone treatment wood for 60 min. HWD increased along with the ozone treatment duration and the increase ratio of SSM from 30 to 60 min duration was the lowest one. These results showed that ozonisation for 60 min was not effective

for SSM and the optimum duration for SSM was 30 min.

Reducing sugar yield from the SW sample increased up to 60 min ozone treatment (Fig. 2). On the other hand, the reducing sugar yield of SM and SSM samples increased up to 30 min and then decreased. These results probably caused by holocellulose dan cellulose contents which are low wood carbohydrates in SSM. Even though *A. polytricha* is classified as Basidiomycetes which degrades lignin during their growth, the previous research in 5 Japanese species also showed that *A. polytricha* degraded cellulose dan holocellulose (Irawati et al. 2012a).

Furthermore, significant correlation was obtained between Klason lignin content and reducing sugar yield or hydrolysis weight decrease (Figure 3). Table 2 shows the monosaccharide yield after saccharification of the samples. After saccharification, glucose was the major monosaccharide, followed by

Table 1. Chemical components (%) of sengon wood, spent media, and ozone treated wood and spent media in several treatment times.

Tabel 1. Komponen kimia (%) dari kayu sengon, limbah media, kayu sengon setelah perlakuan ozon pada berbagai waktu perlakuan, dan limbah media setelah perlakuan ozon pada berbagai waktu perlakuan

Component	Klason lignin	Holo-cellulosa	α -Cellulose	ET-Extracts	Acid soluble lignin
	M \pm SD	M \pm SD	M \pm SD	M \pm SD	M \pm SD
Sengon wood ^{*)}	23.3 \pm 0.3	80.8 \pm 0.6	45.3 \pm 1.1	2.9 \pm 0.4	2.8 \pm 0.1
Ozone treated sengon wood:					
15 minutes	11.1 \pm 1.1	76.4 \pm 0.2	43.1 \pm 0.1	8.3 \pm 0.1	2.8 \pm 0.0
30 minutes	6.1 \pm 0.4	72.7 \pm 0.2	44.1 \pm 0.4	12.0 \pm 0.2	2.5 \pm 0.1
60 minutes	2.8 \pm 0.5	61.5 \pm 0.6	31.8 \pm 0.8	12.0 \pm 0.7	2.1 \pm 0.3
Sengon media ^{*)}	19.4 \pm 0.5	73.4 \pm 2.5	40.0 \pm 0.2	5.2 \pm 0.2	2.8 \pm 0.1
Ozone treated sengon media:					
15 minutes	9.3 \pm 0.3	71.1 \pm 0.6	41.2 \pm 0.3	6.5 \pm 0.3	2.7 \pm 0.0
30 minutes	7.5 \pm 0.7	64.8 \pm 0.3	37.4 \pm 0.3	8.4 \pm 0.1	2.8 \pm 0.0
60 minutes	7.0 \pm 0.2	53.0 \pm 0.4	30.1 \pm 1.7	9.5 \pm 1.0	2.5 \pm 0.0
Sengon spent media ^{*)}	5.5 \pm 0.1	41.0 \pm 4.2	16.9 \pm 0.8	2.6 \pm 0.0	2.5 \pm 0.0
Ozone treated sengon spent media:					
15 minutes	2.6 \pm 0.1	39.1 \pm 0.4	16.4 \pm 0.7	1.4 \pm 0.0	2.1 \pm 0.0
30 minutes	1.1 \pm 0.2	36.7 \pm 0.6	15.0 \pm 0.8	1.3 \pm 0.0	1.9 \pm 0.1
60 minutes	1.2 \pm 0.4	35.4 \pm 0.8	16.8 \pm 0.5	1.3 \pm 0.0	1.5 \pm 0.0

*). Irawati et al., 2013.

Note : M=mean, SD=standard deviation, ET=ethanol-toluene

Keterangan : M=rerata, SD=standar deviasi, ET=etanol-toluena

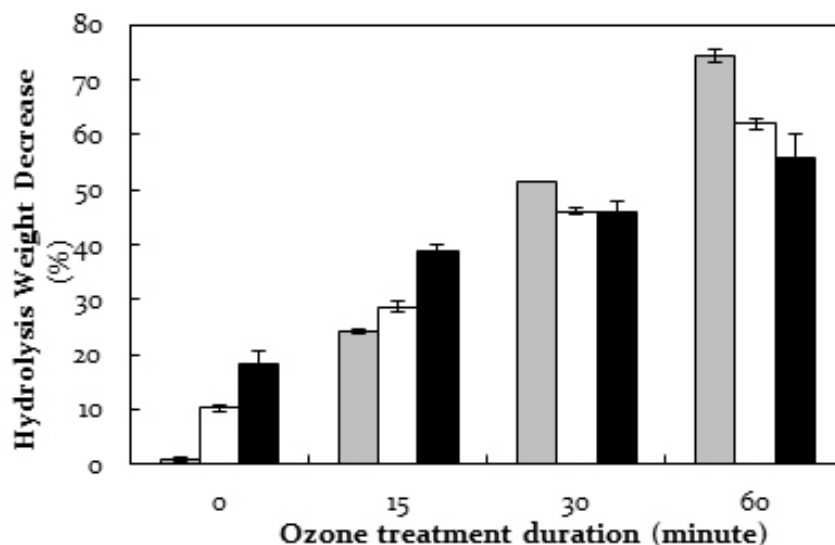


Figure 1. The histogram of hydrolysis weight decrease of the ozone treated samples in different duration treatment. Gray color is sengon wood. White color is sengon media. Black color is sengon spent media. Bars indicate the value of standard deviations.

Gambar 1. Histogram laju hidrolisis dari sampel yang diberi perlakuan ozon pada berbagai lama waktu perlakuan. Warna abu-abu adalah kayu sengon, putih adalah media, dan hitam adalah limbah media. Garis menunjukkan nilai standar deviasi.

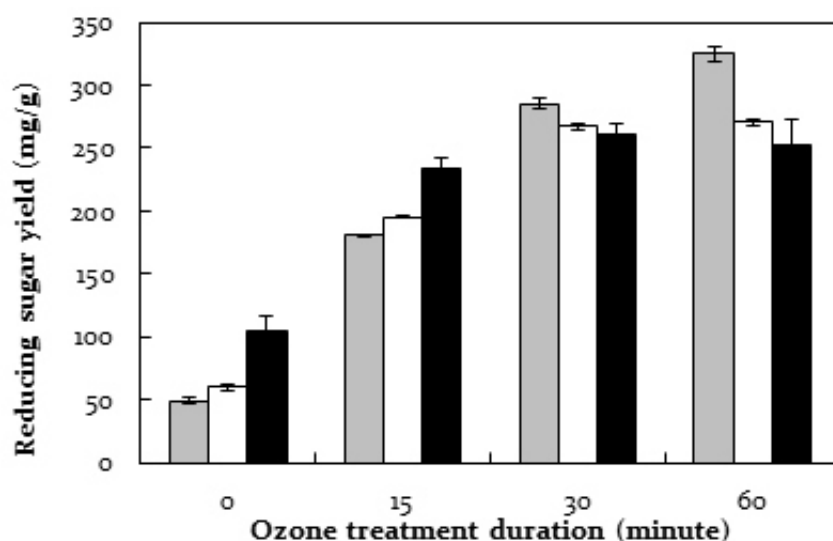


Figure 2. The histogram of reducing sugar yield of the ozone treated samples in different duration treatment. Bars indicate the value of standard deviations. Refer Fig 1. For the different color.

Gambar 2. Histogram rendemen gula reduksi dari sampel yang diberi perlakuan ozon pada berbagai lama waktu perlakuan. Garis menunjukkan nilai standar deviasi. Keterangan perbedaan warna histogram lihat Gambar 1.

xylose and galactose. In addition, glucose yield of SW and SM increased along with the ozone treatment duration, but not for SMM samples. Cellulose structure in SSM may have been degraded by enzymes of mushroom, so that long ozone treatment causes many glucose degradations. These results indicate that lignin degradation by ozone pretreatment for 60 min improved effectively the accessibility of the

enzyme to cellulose during the saccharification for SW sample. Further, it had caused too high degradation for monosaccharides and caused decreases of glucose and xylose yields. From these results, the optimal duration of ozone treatment to obtain the highest glucose yield from the SW or SM by enzymatic saccharification was 60 min, however for SMM was 30 min.

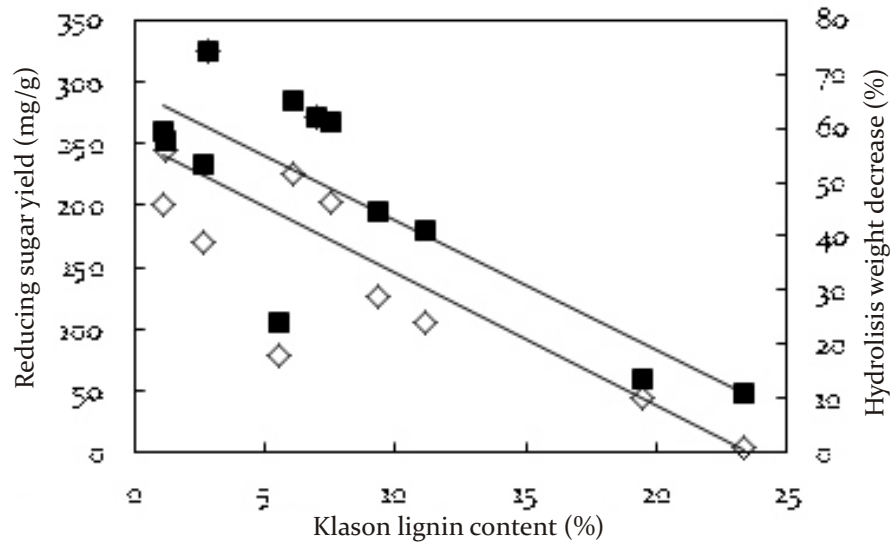


Figure 3. Correlations between Klason lignin content and reducing sugar yield or hydrolysis weight decrease in the ozone-treated and ozone untreated SW, SM, and SMM. Black square is reducing sugar yield ($r=-0.80$). White diamond is hydrolysis weight decrease ($r=-0.79$), $n = 12$.

Gambar 3. Hubungan antara kadar Klason lignin dan rendemen gula reduksi serta laju hidrolisis pada sampel SW, SM, dan SMM baik yang tanpa diberi perlakuan ozon maupun dengan perlakuan ozon. Bujur sangkar hitam adalah rendemen gula reduksi ($r=-0,80$). Belah ketupat putih laju hirolisis ($r=-0,79$), $n = 12$.

Table 2. Monosaccharide yield (g/100 g biomass) after saccharification of ozone treated samples

Tabel 2. Rendemen monosakarida (g/100 g biomasa) hasil sakarifikasi sampel yang diberi perlakuan ozon

Sample	Ozone treatment duration (min)	Glucose	Galactose	Xylose	Total
		M \pm SD	M \pm SD	M \pm SD	
Sengon wood	0	1.4 \pm 1.2	0.4 \pm 0.3	0.5 \pm 0.5	2.3
	15	12.0 \pm 0.4	0.2 \pm 0.1	3.7 \pm 0.7	15.8
	30	17.1 \pm 0.1	0.2 \pm 0.0	5.4 \pm 0.1	22.7
	60	18.5 \pm 2.2	0.1 \pm 0.0	7.0 \pm 0.3	25.6
Sengon media	0	3.1 \pm 0.0	0.3 \pm 0.0	1.7 \pm 0.0	5.0
	15	11.5 \pm 0.3	0.2 \pm 0.0	1.5 \pm 0.4	13.2
	30	13.8 \pm 0.3	0.3 \pm 0.1	4.7 \pm 0.1	18.8
	60	16.5 \pm 2.3	0.3 \pm 0.0	2.9 \pm 0.7	19.6
Sengon spent media	0	8.5 \pm 0.3	1.7 \pm 0.2	0.7 \pm 0.4	10.9
	15	13.0 \pm 0.7	0.3 \pm 0.0	3.7 \pm 0.2	16.9
	30	14.4 \pm 0.9	0.2 \pm 0.0	4.5 \pm 0.1	19.1
	60	13.2 \pm 1.6	0.3 \pm 0.0	2.3 \pm 1.2	15.9

Conclusions

The ozone treatment duration were determined for sengon wastes as reducing sugar feedstock. Ozone treatment resulted Klason lignin degradation in the all samples, resulting the improvement of subsequent enzymatic saccharification. Ozone treatment with the duration more than 30 min is not suitable for SMM sample. Glucose yield after saccharification from the ozone-treated sample for 60 min was the highest among all samples in SW and SM treated, but for SMM it was 30 min. Based on these results, we concluded

that mushroom cultivation can reduce the ozone time treatment and it is an effective treatment to improve sugar yield by enzymatic saccharification of sengon waste.

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