# Separation characteristics of lignin from *Eucalyptus camaldulensis* lignincelluloses for biomedical cellulose

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**Abstract**: The separation characteristics of *Eucalyptus camaldulensis* biomass cell wall were investigated and analyzed by FTIR, UV and XRD. The result showed that the crystallinity of untreated sample were lower than ones of treated samples at 10°C, 20°C and 30°C. Effect of temperature was very notable so as to use the lower temperature if cellulose would be kept natural structure during lignin separation from lignin cellulose. Treatment time and temperature had a notably significant effect on SLR of *E. camaldulensis* lignincellulose, and the SLR increased gradually with the temperature increment, and SLR were 73.35% at 10°C, 80.14% at 20°C, 83.73% at 30°C. The maximum of SLR increased with the rising of temperature 10°C, 20°C and 30°C. Consequently, the best separation conditions of lignin were 24h and 30°C. During hemicelluloses separation, the peak of C=O disappeared, and the peak of CO-OR, O-H and C-O abated, resulting that acetyl in hemicelluloses were mainly broken by the dehydration, heat decomposition reaction and mild thermal degradation. During lignin separation, the side chain and benzene ring of lignin of *E. urophynis* lignin cellulose reached the largest bond breaking characteristics under the temperature of 10°C at 7h, 20°C at 7h and 30°C at 24h.

**Keywords**: *Eucalyptus camaldulensis*, lignin, lignincelluloses, nature fibre.

## INTRODUCTION

Cellulose, which was made by all plants, was the most abundant organic compound on Earth. And cellulose had many routine uses. For example, cellulose could be used to make paper, explosives, film, plastics, clothes and diet. What's more, Cellulose also had many biomedical applications. In recent years, cellulose had been in the more and more field of biomedical materials including wound closure, drug delivery systems, novel vascular grafts, or scaffolds for *in vitro* or *in vivo* tissue engineering (Czaja *et al.*, 2007). Especially microbial cellulose, which was a form of cellulose that was produced by bacteria, had already been used quite successfully in wound-healing applications (Alvarez *et al,* 2004; Gisela *et al,* 2005). Microbial cellulose utilization were examined at successively higher levels of aggregation encompassing the structure and composition of cellulosic biomass, cellulase enzyme systems, taxonomic diversity, molecular biology of cellulase enzymes, physiology of cellulolytic microorganisms, ecological aspects of cellulase-degrading communities, and rate-limiting factors in nature (Lynd *et al.,* 2002). Besides artificial blood vessels, microbial cellulose was found to be very useful for nerve surgery, functioning as a protective cover of anastomosis (Dieter *et al.*, 2005). Biofill®, Bioprocess® and Gengiflex® were products of microbial cellulose that had wide applications in surgery and dental implants (Rainer *et al*, 1998). So cellulose would be used in biomedical field.

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The wood cell wall is mainly composed of hemicelluloses, cellulose and lignin. Cellulose could generate and natural fibre by hydrolysis reaction, hemicelluloses could generate biofuel such as furan by hydrolysis reaction, lignin could be prepared for biological diesel oil by thermal cracking reaction. Cellulose of wood has been succeeded in preparation for biomedical fibre. However, green and high efficient separation of cellulose from wood biomass was still a very difficult. *Eucalyptus camaldulensis* are abundant in south China and the fine natural biomedical fibre of wood biomass. Therefore, the separation characteristics of *E. camaldulensis* biomass cell wall were investigated and analyzed by FTIR, UV and XRD.

## MATERIALS AND METHODS

### *Materials and reagents*

The 18-year-old *E. camaldulensis* was collected from the Forest Farm of Central South University of Forestry and Technology, P. R. China. The sample chips were processed from fresh material, and dried to absolute dry with rotary evaporator in 55°C and negative 0.01MPa. About 200mesh powder was sieved out using AS200 Sieving Instrument (Made in America). Lignocellulosic biomass was obtained by benzene/ethanol, methanol and acetic ether extractions, and dried to absolute dry with rotary evaporator in 55°C and negative 0.01MPa. The benzene-ethanol solution was mixed according to $V_{ethanol}$ /$V_{benzene}$ 2 double. KOH, Acetic acid, 30% hydrogen peroxide (analytically pure grade) and deionized water were prepared for the subsequent experiments.

***Experiment methods***

*Hemicelluloses Separation*

The 5g above-mentioned powders of *E. camaldulensis* lignocellulosic biomass were treated in 100mL 17.5% KOH solution under 25°C for 3h, 6h, 9h, 12h, 16h, 20 h, 24 h, 36 h, respectively. After treated, the samples were filtered, then dried in the air, and dried to absolute dry in an oven, finally reserved in the dryer.

***Lignin separation***

Weighed the above-mentioned wood powders of *E. camaldulensis* lignincellulose, each was about 10g. According to 1g wood powder to 10mL AAHP (acetic acid/hydrogen peroxide solution), 50mL acetic acid was slowly added along the wall with even agitation, and then 50mL 30% hydrogen peroxide with even agitation. The samples were treated under the temperature10°C, 20°C and 30°C for 1h, 5h, 7h, 12h, 17h and 24h, respectively. After treated, the samples were filtered, then dried in the air, and dried to absolute dry in an oven, finally reserved in the dryer. The acetic acid / hydrogen peroxide solution was made by volumetric mixture ratio 1 to 1 of acetic acid and 30% hydrogen peroxide.

***FT-IR analysis***

KBr pellets of samples were recorded on a Thermo Nicolet FT-IR spectrometer (NEXUS 670FT-IR). The 0.1-0.3mg samples were mixed with 10-30mg KBr. The KBr pellets were prepared for measurement. Thirty-two scans were collected per sample at a spectral resolution of 4 $cm^{-1}$, and the collected spectra were ratio against air. Spectral range was from 4000 to 500 $cm^{-1}$.

***XRD analysis***

After sample preparation, the samples were measured by the XD-2 diffractometer(General analysis of Beijing General Instrument Co., Ltd.). X-ray tube was Cu tube, pipe pressure was 36kv, pipe flow was 20mA. Measurement method was 2 θ/θ continuous scanning, Use graphite crystal monochromator, Slit device DS=1° , SS=1°, RS=0.3mm. Requirement: Cu tube (X-ray wavelengths= 1.5406 nm), 36kV voltage, current 20 mA. Diffraction direction θ~2 θ linkage scanning system, rotary half cone angle 2θis from 5°to 42°, scanning velocity was 2°/min, Scan step angle was 0.01. Cellulose Crystallinity was calculated according to the formula (1).

$CrI=(I_{002}-I_{am})/I_{002}\times100\%$ (1)

Cr-ercentage of relative Crystallinity; $I_{002}$-ntensity of the peak at 002 of the crystal region; $I_{am}$-iffracted intensity of peak at 2θ=18° of amorphous region.

***UV analysis***

The sample solution were measured by UV-Vis SP-752 Spectrophotometer (Shanghai spectrum instrument Co., Ltd), respectively. Wavelength Range: 190-1100nm/ 00nm-1000nm. Optical system: Single beam auto collimation type optical path. acetylbromide method: 10~25mg of degreasing wood powder was weighed accurately, then placed in test tubes with 10ml of 25% acetyl bromide in acetic acid solution. The test tubes were kept in water bath for 30 min at 70°C, it does not need agitation at the beginning 15min, then every 3~5 min shake the test tubes, for 15 min, being aimed at making wood powder dissolve. Waiting the tubes cooling, then transferred the material in test tubes to the 100mL volumetric flask where have 9mL 2mol/LNaOH and 50 mL acetic acid; then flush the tubes with a small amount of acetic acid solution to make sure the transfer was complete, added 1mL 7.5mol/L hydroxylamine hydrochloride ($NH_2OH.HCL$) into the 100mL volumetric flask, Finally used acetic acid dilution this solution to scale. Absorbance of solution at 280nm was determined. Lignin content of the samples was calculated according to the formula (2)

lignin content=$100(A_s-A_b)\cdot V/amd-B(\%)$ (2)

$A_s$-absorbance of sample; $A_b$-sorbance of empty sample; V-olume of solution, L; A-tandard absorption coefficient of lignin,$lg^{-1}cm^{-1}$;m-ample quality, g; B-orrection factor; d-hickness of the cuvettes, cm.

## RESULTS

According to lignin content, SLR (separated lignin rate) were obtained and listed in table 1.

**Table 1**: SLR of *E. camaldulensis* lignin cellulose (%)

| Time (h) | *E. camaldulensis* | | |
|---|---|---|---|
| | 10°C | 20°C | 30°C |
| 1 | 67.88 | 79.27 | 82.98 |
| 5 | 71.67 | 77.69 | 82.74 |
| 7 | 71.93 | 76.97 | 83.26 |
| 12 | 74.92 | 82.12 | 83.78 |
| 17 | 74.72 | 82.22 | 84.07 |
| 24 | 78.98 | 82.56 | 85.54 |

The results of cellulose crystallinity in E. camaldulensis lignincellulose were showed in table 2.

## DISCUSSION

***Separation law of lignin from lignin cellulose***

On the Basis of the variance analysis of the data in table 1 at the level of 0.05, Treatment time and temperature had a notably significant effect on SLR of *E. camaldulensis* lignin cellulose ($F$=4.823> $F_{0.05}$ (5,10)=3.33; $F$=53.211> $F_{0.05}$ (2,10)=4.10) and the SLR of *E. camaldulensis* lignin cellulose increased gradually with the temperature increment, and SLR were 73.35% at 10°C, 80.14% at 20°C, 83.73% at 30°C. With the extension of treatment time, SLR gradually increased, namely SLR were 76.71%, 77.3%, 77.39%, 80.27%, 80.34%, 82.36%, when treatment time were 1, 5, 7, 12, 17 and 24h, respectively.

Table 1 showed that the SLR of *E. camaldulensis* lignin cellulose reached the maximum at 24h (78.98%), 24h (82.56%) and 24h (85.54%) under the temperature of

**Table 2**: Cellulose crystallinity of *E. camaldulensis* lignincellulose

| Time [h] | 10°C | | | 20°C | | | 30°C | | |
|---|---|---|---|---|---|---|---|---|---|
| | CrI [%] | O'KI | NO'KI | CrI [%] | O'KI | NO'KI | CrI [%] | O'KI | NO'KI |
| 0 | 44.39 | 2.39 | 1.33 | 44.39 | 2.39 | 1.33 | 44.39 | 2.39 | 1.33 |
| 1 | 56.64 | 1.83 | 1.23 | 59.45 | 2.25 | 1.26 | 60.95 | 1.99 | 1.33 |
| 5 | 60.83 | 2.24 | 1.37 | 62.36 | 2.16 | 1.23 | 64.87 | 1.98 | 1.27 |
| 7 | 62.55 | 2.08 | 1.12 | 54.04 | 2.25 | 1.29 | 52.21 | 1.95 | 1.25 |
| 12 | 60.97 | 2.09 | 1.26 | 61.51 | 2.20 | 1.30 | 63.78 | 2.01 | 1.30 |
| 17 | 62.97 | 2.08 | 1.25 | 63.68 | 2.26 | 1.24 | 57.91 | 1.88 | 1.27 |
| 24 | 53.50 | 2.40 | 1.21 | 64.77 | 2.07 | 1.25 | 60.15 | 1.62 | 1.29 |

**Table 3**: Group assignments of *E. camaldulensis* lignincellulose

| Peak [$cm^{-1}$] | Assignment |
|---|---|
| 3417 | O–H stretching in hydroxgl group |
| 1593 | Aromatic skeletal vibration plus C=O stretching |
| 1504 | Aromatic skeletal vibrations |
| 1420 | Aromatic skeletal vibration combined with C-H in plane deformation |
| 1330 | Syringyl ring plus guaiacyl ring condensed |
| 1230 | C–C, C–O and C=O stretch (G condensed > G etherified) |

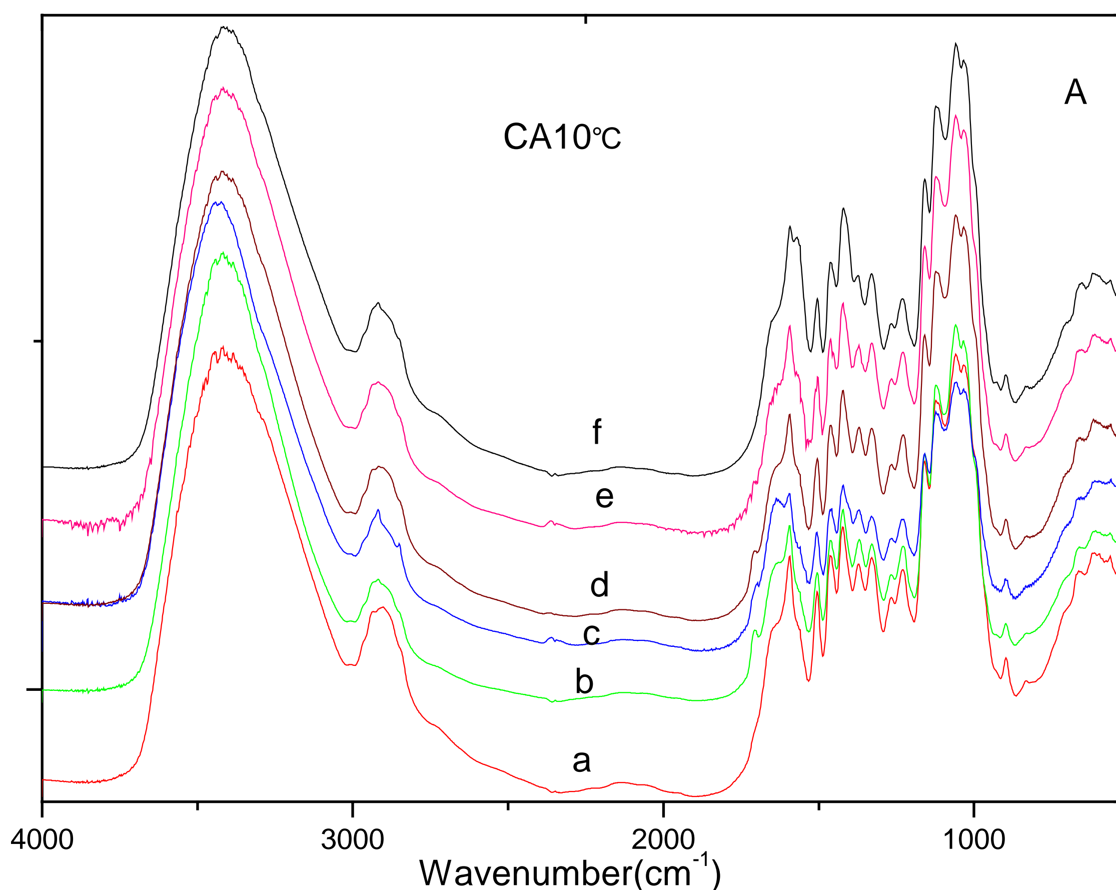


**Fig. 1**: FTIR spectra of sample of treated *E. camaldulensis* lignin cellulose at 10°C, 20°C, 30°C, respectively. (a)1h, (b)5h, (c)7h, (d)12h, (e)17h, (f)24h

10°C, 20°C and 30°C, respectively. The maximum of SLR of *E. camaldulensis* lignin cellulose increased with the rising of temperature 10°C, 20°C and 30°C. Consequently, the best separation condition of lignin were 24h and 30°C.

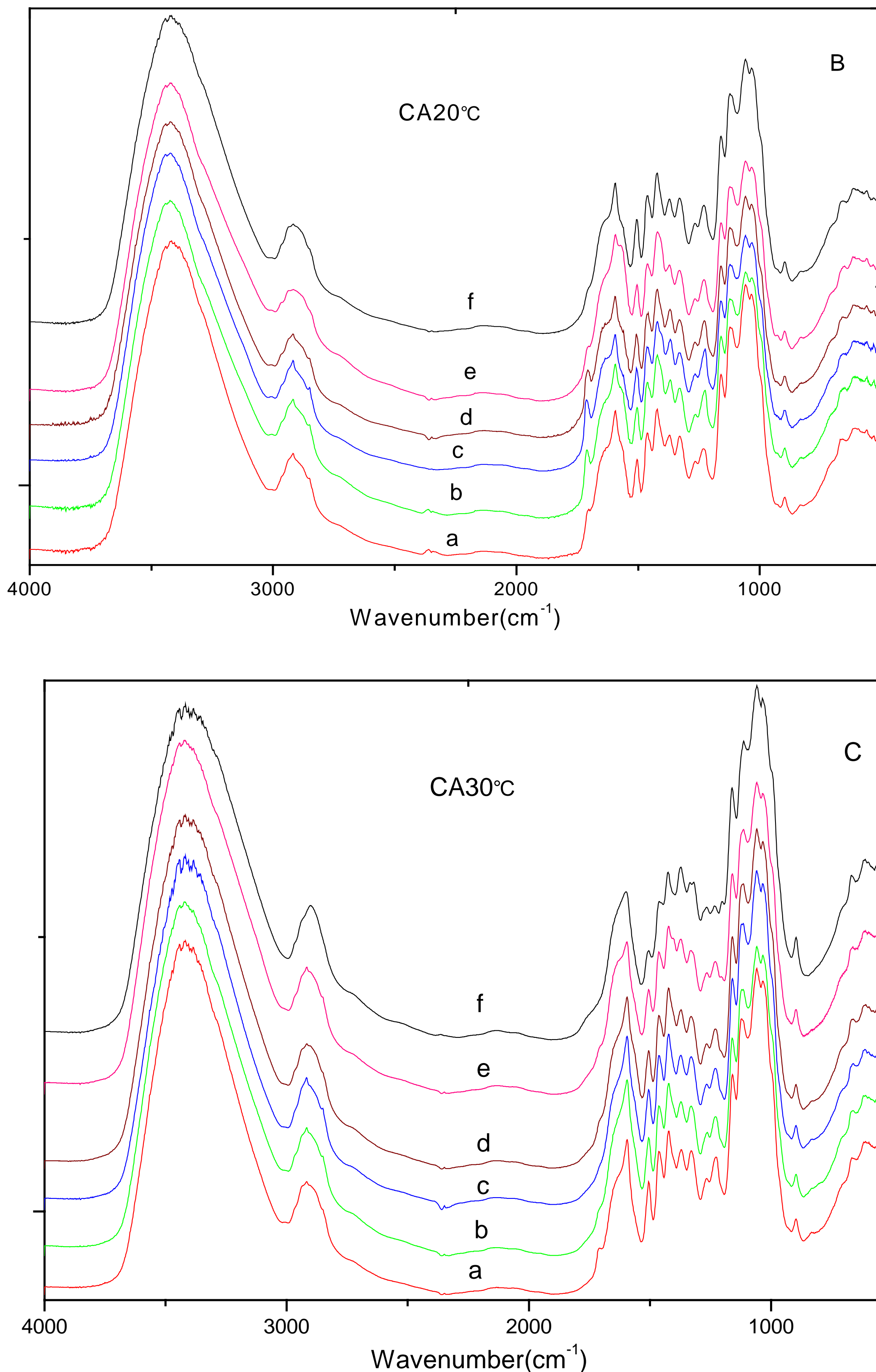


**Fig. 1**: FTIR spectra of sample of treated *E. camaldulensis* lignin cellulose at 10°C, 20°C, 30°C, respectively. (a)1h, (b)5h, (c)7h, (d)12h, (e)17h, (f)24h (continuation)

***Crystallinity changes during lignin separation from lignin cellulose***

According to Table 2, crystallinity of *E. camaldulensis* lignin cellulose firstly increased, then decreased, increased, and then decreased under the temperature of 10°C when the treatment time was prolonged, and reached the maximum 62.97% at 17h. Crystallinity of *E. camaldulensis* lignin cellulose firstly decreased, and then increased under the temperature of 20°C when the treatment time was prolonged, and reached the maximum 64.77% at 24h. Crystallinity of *E. camaldulensis* lignin cellulose firstly decreased and then increased under the temperature of 30°C when the treatment time was prolonged, and reached the maximum 64.87% at 5h. The crystallinity of untreated sample were lower than ones of treated sample at 10°C, 20°C and 30°C, suggesting that the cellulose of amorphous region could have oxidation reaction, or acid hydrolysis reaction when lignin was separated from *E. camaldulensis* lignin cellulose by AAHP method. O'KI and CrI changed similarly, but NO'KI changed inversely.

During lignin separation from lignin cellulose by AAHP method, Crystallinity of *E. camaldulensis* lignin cellulose increased by 20.48%, suggesting that cellulose of *E. camaldulensis* were the more destructive by AAHP. Effect of temperature was very notable so as to use the lower temperature if cellulose would be kept natural structure during lignin separation from lignin cellulose. O'KI and CrI could be chosen to analyze the crystallinity during lignin separation from lignin cellulose.

***Bond breaking characteristics during lignin separation from lignincellulose***

Based on FTIR spectra**,** the assignments were showed in table 3.

FTIR spectra of *E. camaldulensis* lignincellulose were showed in Fig.1. The most important O-H stretching in hydroxgl group was assigned to the 3100 -3650 $cm^{-1}$ region. The absorbance of a peak appeared at 3417 $cm^{-1}$ was the strongest. At 3417 $cm^{-1}$, 1593 $cm^{-1}$, 1504 $cm^{-1}$, 1420 $cm^{-1}$, 1330 $cm^{-1}$ and 1230 $cm^{-1}$ were O-H stretching in hydroxgl group, aromatic skeletal vibration plus C=O stretching, aromatic skeletal vibrations, aromatic skeletal vibration combined with C-H in plane deformation, syringl vibrations, benzene ring-hydrogen bond vibrations, respectively.

At 10°C, the absorbance of peaks at 3417 $cm^{-1}$ increased from 0.997 to 0.999 indicating that the O-H increased. The absorbance of peaks at 1593 $cm^{-1}$ reduced from 0.534 to 0.351 at 7h, the absorbance of peaks at 1504 $cm^{-1}$ reduced from 0.454 to 0.265 at 7h and the absorbance of peaks at 1420 $cm^{-1}$ reduced from 0.599 to 0.369 at 7h, resulting that the benzene ring of lignin reached the most destructive under the temperature of 10°C at 7h. The absorbance of peaks at 1330 $cm^{-1}$ disappeared at 24h, the absorbance of peaks at 1230 $cm^{-1}$ reduced from 0.504 to 0.294 at 7h, result that side chain of lignin also reached the most destructive under the temperature of 10°C at 7h.

At 20°C, the absorbance of peaks at 3417 $cm^{-1}$ increased slightly indicating that the O-H increased slightly. The absorbance of peaks at 1593 $cm^{-1}$ reduced from 0.465 to 0.429 at 7h, the absorbance of peaks at 1504 $cm^{-1}$ increased from 0.313 to 0.366 at 5h, and the absorbance of peaks at 1420 $cm^{-1}$ increased from 0.471 to 0.505 at 24h. The absorbance of peaks at 1330 $cm^{-1}$ reduced from 0.385 to 0.372 at 7h, the absorbance of peaks at 1230 $cm^{-1}$ reduced from 0.346 to 0.384 at 7h, result that side chain of lignin also reached the most destructive under the temperature of 20°C at 7h.

At 30°C, the absorbance of peaks at 3417 $cm^{-1}$ decreased from 0.997 to 0.948 indicating that the O-H changed slightly. The absorbance of peaks at 1593 $cm^{-1}$ reduced from 0.436 to 0.418 at 24h, the absorbance of peaks at 1504 $cm^{-1}$ reduced from 0.317 to 0.252 at 24h and the absorbance of peaks at 1420 $cm^{-1}$ increased from 0.462 to 0.495. Te absorbance of peaks at 1330 $cm^{-1}$ disappeared at 24h, the absorbance of peaks at 1230 $cm^{-1}$ reduced from 0.388 to 0.373 at 24h, result that side chain of lignin also reached the most destructive under the temperature of 30°C at 24h.

## CONCLUSION

*E. camaldulensis* lingocellulosic biomass changed in crystallinity and groups during lignin separation. The crystallinity of untreated sample were lower than ones of treated sample at 10°C, 20°C and 30°C, suggesting that the cellulose of amorphous region could had oxidation reaction, or acid hydrolysis reaction when lignin was separated from *E. camaldulensis* lignin cellulose by AAHP method. During lignin separation from lignincellulose by AAHP method, crystallinity increased by 20.48%, suggesting that cellulose of *E. camaldulensis* were the more destructive by AAHP. Effect of temperature was very notable so as to use the lower temperature if cellulose would be kept natural structure during lignin separation from lignin cellulose.

Treatment time and temperature had a notably significant effect on SLR of *E. camaldulensis* lignincellulose, and the SLR of *E. camaldulensis* lignin cellulose increased gradually with the temperature increment, and SLR were 73.35% at 10°C, 80.14% at 20°C and 83.73% at 30°C. The maximum of SLR of *E. camaldulensis* lignin cellulose increased with the rising of temperature 10°C, 20°C and 30°C. Consequently, the best separation conditions of lignin were 24h and 30°C.

During hemicelluloses separation, the peak of C=O disappeared, and the peak of CO-OR, O-H and C-O abated, resulting that acetyl in hemicelluloses were

mainly broken by the dehydration, heat decomposition reaction and mild thermal degradation. During lignin separation, the side chain and benzene ring of lignin of *E. urophynis* lignin cellulose reached the largest bond breaking characteristics under the temperature of 10°C at 7h, 20°C at 7h and 30°C at 24h.

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