# Open Topics

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## SEM and confocal Raman microscopic study of morphological and topochemical changes of poplar cell walls during alkali pretreatment

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Poplar is a fast growing feedstock with potential for biofuel production showing renewable and sustainable advantages. However, physical and chemical barriers caused by the recalcitrance of plant cell walls hinder the hydrolysis of polysaccharide fractions to fermentable sugars. Pretreatment is an essential step to reduce biomass recalcitrance for increasing the enzyme accessibility. Alkali pretreatment is attractive among the most promising pretreatment technologies that can effectively reduce the lignin content to enhance sugar release performance.

In this study, poplar cross-sections of 6 μm thickness were subjected to aqueous sodium hydroxide (2% w/v) pretreatment at 121 °C and analytical microscopy approaches were used to assess the impact of pretreatment upon morphology and topochemistry of plant cell walls.

SEM images revealed a great deal of pore formation on the surface of cell walls and structural disruption of biomass to some extent after pretreatment, especially in the areas adjacent to the cell corner middle lamella (CCML). The phenomenon was reasonably consistent with lignin removal during pretreatment (Figure 1.).

In order to track the topochemical changes of poplar, confocal Raman microscopy was used to acquire chemical images and spectra of main components within plant cell walls during pretreatment. As shown in Figure 2., before alkali pretreatment the highest level of lignification occurred in the CCML and the highest carbohydrate concentration was found in the secondary wall (S).

Comparison of Raman images of lignin distribution at various pretreatment time indicated that lignin concentration decreased significantly during pretreatment, especially within the first 60 min (Figure 2b.). It also indicated that cell wall swelling was mainly in the S and slight effect was observed on the CCML in terms of swelling, which was confirmed by the bright field images (Figure 2a-b.). These results suggested that it was easier for alkaline liquids to penetrate into cell walls from lumen. As a result of preferential swelling, the rate of delignification in the S was much faster than that in the CCML where there was a higher level of lignification.

As illustrated in the Figure 2c., carbohydrate dissolution in the S regions visibly occurred within the first 10 min, which was probably due to partial dissolution of hemicelluloses accompanied by delignification. Subsequently, reduction of carbohydrate concentration became slower for the S regions. It should be noted that significant dissolution of carbohydrate occurred again after about 90 min of pretreatment when a large amount of lignin was removed.

Further Raman spectral analysis allowed a semi-quantitative comparison of the chemical compositions within cell walls and showed a decrease of lignin Raman intensity to about 20% within the 10 min and to 82% under pretreated time of 180 min (data not shown).

Based on these findings, alkali pretreatment appeared to increase lignin solubilization, cell wall swelling and porosity of the biomass. These results were meaningful in bioconversion and utilization of renewable lignocellulosic biomass for providing valuable new insights toward the mechanism of alkali pretreatment. In this work, it also demonstrated that a combination of SEM and confocal Raman microscopy is capable of rapidly determining the morphological and topochemical changes of plant cell walls during alkali pretreatment.

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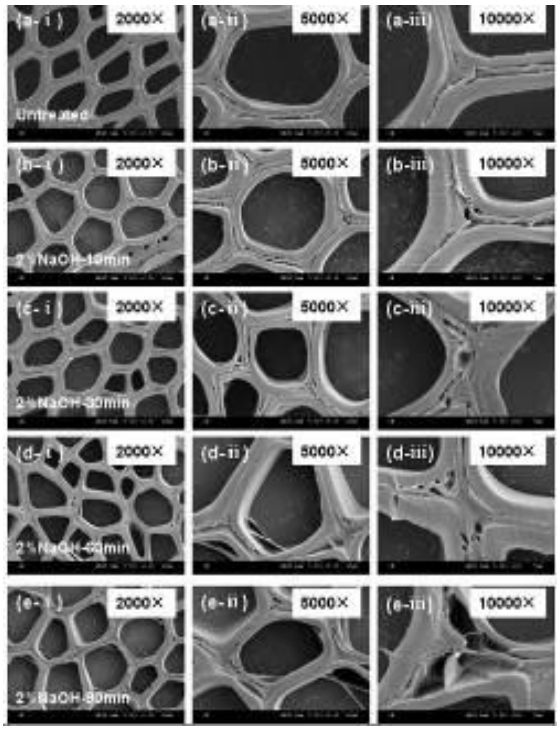


**Figure 1.** SEM images at various magnifications of poplar cross-sections at various pretreatment time of 0 min (a); 10 min (b); 30 min (c); 60 min (d); 90 min (e).

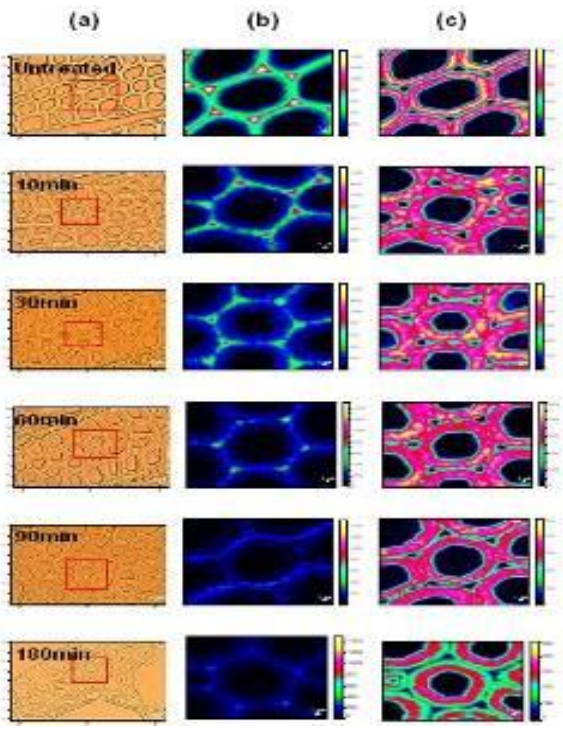


**Figure 2.** Bright field images of poplar cross-sections showing selected area (*red rectangle*) for Raman imaging (a); Raman images showing the distribution of main components within cell walls at various pretreatment time by integrating from 1570 to 1680 $cm^{-1}$ (b, lignin) and 2850-2920 $cm^{-1}$ (c, carbohydrate).