



Figure 6. CLSM of a stoma from a portion of the untreated leaf sample (left) and the sample after the oxidative hydrolysis under conditions of Run #4 (right).

Images were taken for the untreated sample and obtained after runs #4, #6, and #S1. Mean fluorescence intensity was estimated from the images using the ImageJ software by averaging over the Z-axis converted to a 0-255 grayscale (8-bit). It was found that the mean intensity correlated well with the amount of lignin in the sample (**Error! Reference source not found.**). The fluorescence intensity of the samples decreased after the acid-oxidative hydrolysis, proportional to the severity factor. However, despite having the highest severity factor, the sample treated with sulphuric acid showed the highest lignin content and fluorescence intensity, indicating the low degree of delignification and partial dissolution of cellulose.

Table 3. Autofluorescence intensity as a function of the treatment severity.

Sample	Log (Ro)	% Lignin	Fluorescence Intensity
Untreated	-	40%	++++
Run #6	0.89	28%	++
Run #4	1.48	14%	+
Run S1	1.66	45%	+++

4.3. SEM imaging

The surface morphological characteristics of the untreated and treated LCW samples were analyzed by SEM, and representative results are presented in **Figure 4** and **Error! Reference source not found.** Leaves and stems structures have been observed in the SEM of the untreated sample (**Figure 4**). Stomata with guard cells are observed in the leaves

(**Figure 4** a-b), as previously reported (Pourkhabbaz et al., 2010). The untreated leaf samples exhibited a compact, non-porous, uniform appearance of surface structure. In contrast, the stems presented porous longitudinal arrangement of fibrils (**Figure 4** c-d), as observed for other LCW (Hernández-Hernández et al., 2014).