WP 2 - Characterization of wood & bio-energy potentials

The overall objective is high throughput phenotyping of key wood and cell-wall chemical constituents that are potentially important for saccharification and or biofuel production. The specific objectives will be to (i) improve and develop high throughput phenotyping tools for QTL detection (sub-task 2.1) (ii) assess the impact of cell-wall constituents on saccharification and biofuel potentials (task 2.2) (iii) develop and apply micro-methods for phenotyping of transgenic wood tissues (task.2.3)

Task 2.1 Development and application of high throughput methodologies

The objective of this task is to replace costly, time consuming, and destructive phenotyping methods for poplar and eucalupts with high throughput, non-destructive technologies. We will select woood samples evenly distributed within the range of NIR spectral variations. These samples will be assessed for extractives, lignin, and polysaccharides composition by classical wet chemistry methods pursuant to established standards (P2, P3, P5, P9) and used further to develop high throughput methods such as NIR and or ATR-FTIR spectroscopy. The detailed analysis of the polysaccharides, including hemicellulose composition in terms of the hexosans/pentosans ratio, will be assessed by HPLC analysis of the hydrolysate released for lignin analysis (P5). Analytical pyrolysis will be used for lignin composition (P5, P9). Extractives composition with particular emphasis on non-structural carbohydrate will be determined by HPLC or enzymatic assays (P2, P5).

Task 2.2 Bioenergy potentials, saccharification, and bio-oil

Here, we will evaluate the saccharification and bio-oil potentials as well as the impact of key cell wall chemical constituents on these potentials. Selected contrasting samples of poplar and eucalyptus involved in wood chemical composition (including tension/normal wood) will be assessed for saccharification and its influence on bio-oil production and composition. Standardized steaming (from 1 g up to the 400 g scale) and organosolv will be tested as pre-treatments to improve the accessibility towards cellulolytic enzymes (P5, P6). After pre-treatments, the resulting material will be fermented to ethanol to determine the production potential (P6), and the residues from fermentation will be further assessed for bio-oil production (P5) to increase the efficiency of the process. Bio-oil potential will be tested at a micro scale

(20-50 mg) and at a lab-scale fluidized bed reactor, (minimum 100 g) real samples. Qualitative and quantitative analyses of the three fractions (oil, char, and gases) and mass balances by gravimetric analysis will be assessed in selected samples (P5).

Task 2.3 Micro-phenotyping of transgenic wood zones through microscopic and microspectroscopic investigation methods

The objectives are to i) assess the effect of CG expression on the structure and chemical composition of the cell walls in transgenic wood zones (WZs) or young transgenic poplar plants obtained in WP1, and ii) further quantify lignin and polysaccharides amounts in the most affected transgenic zones

As a first step in monitoring *in situ* changes in the structure and biochemical composition of transgenic WZs, we will use a combination of “mid throughput” microscopic techniques including wide-field microscopy combined with specific staining, spectral microscopy (P1, up to 200 samples), and mid-infrared microspectroscopy (P2: up to 400 transgenics lines, 200 per species). Detailed WZs architecture (especially structure, distribution of lignin within all three main wall layers of SW of various xylem cell types) of the most affected samples will be analysed in-depth by polarised light, cellular UV-microscopy (resolution 0.25µmx0.25µm), and transmission electron microscopy (TEM) (P5: up to 200 samples transgenic lines). Raman spectroscopy will be used to analyse mainly the cellulosic compound of the cell walls (P5: up to 200 transgenic lines). The combination of the microscopic techniques will allow identification of the most contrasting WZs changes in transgenic sectors which will be microdissected for chemical analysis by analytical pyrolysis to quantify polysaccharides and lignin amounts as well as lignin composition (S/G ratio).