Structural variation of organosolv lignin isolated from tropical ramial chipped wood after their soil incorporation for sustainable agriculture

Rodrigue Daassi[1,2], Pierre Betu Kasangana[1], Damase P. Khasa[2], and Tatjana Stevanovic[1]

[1]Renewable Materials Research Center (CRMR), Université Laval, Quebec, QC, Canada G1V 0A6
[2]Centre for Forest Research Université Laval, Quebec, QC, Canada G1V 0A6
Presenting author email: tatjana.stevanovic@sbf.ulaval.ca

Introduction

Nowadays, due to the high soil degradation level over world, ecological and sustainable farming practices such as the use of cover crops, green manures, composting of waste residues or Ramial Chipped Wood (RCW) are promoted to restore soil fertility (Félix et al. 2018). RCW, referring to twigs, and undried branches with diameter lower than 7 cm subsequently fragmented, is an innovative technique which consists of application of lignocellulosic materials in soil to improve its fertility for sustainable agriculture. This technique can be used to valorize a large amount of waste twigs and branches obtained from pruning trees and shrub in several sub-Saharan areas. Several studies highlighted its beneficial effect on physico-chemical and biological properties of soil and on crop production (Félix et al. 2018). However, none of those studies has addressed the investigation of RCW lignin from different fast-growing or naturally regenerating tropical trees and shrub. In this study, we incorporated RCW from two tropical species (*Gmelina arborea* and *Sarcocephalus latifolius*) into soil in order to study in detail their potential characteristics for soil aggradation through lignin degradation. We compare the structural variation of organosolv lignin isolated from RCW of both species after their incorporation in soil. We also deal with the fate of these lignins in soil.

Materials and methods

RCW of *G. arborea* and *S. latifolius* were obtained by chipping branches of less than 7 cm diameter, without foliage and using a locally manufactured shredder in Benin. We put around 500g of RCW in nylon bag called litter bag which were incorporated in soil at a depth of 10 cm. RCW incorporated in soil were sampled per six months and were ground to particle size between 40 and 60 mesh prior to chemical analyses. Lignins from studied samples were isolated by patent organosolv process developed in our laboratory (Koumba-Yoya and Stevanovic 2016). The studied lignins were analyzed by HPLC, FTIR and ^{31}P and 2D HSQC NMR methods following the procedure described in the literature (Meng et al. 2019; Zhao et al. 2019).

Results and discussions

Prior to being incorporated into the soil, RCW (called RCW1) of both species were characterized, and the data presented in Table 1 show that the sample of *S. latifolius* had somewhat lower C/N ratio and were more richer in total lignin, nitrogen and mineral contents (P, K, Ca, Mg) than those of *G. arborea*. The RCW features of both species (Table 1) showed good properties relative to other studies to potentially improve soil properties (Félix et al. 2018) but *S. latifolius* samples appears to be more efficient.

Table 1. Lignin and mineral composition and C/N ratio of RCW from *G. arborea* (*G. a*) and *S. latifolius* (*S. l*)

Samples	C/N	Lignin	N	P	K	Ca	Mg
		%		mg/kg			
RCW1 (*G. a*)	94.3	24.6±0.2	0.5±0.1	537.2±4.0	5149.1±36.1	6059.9±30.7	2150.9±27.7
RCW1 (*S. l*)	82.7	35.0±1.1	0.6±0.0	1061.9±18.9	8852.6±14.4	7110.8±61.9	3360.1±36.3

Organosolv lignins were isolated from RCW collected after 6 and 12 months of residence into the soil in order to analyse its structural changes. The purity of all studied lignin samples, once isolated, was higher than 92 % (Klason lignin plus acid soluble lignin), confirming that organosolv biotechnology was the proper choice get access to representative lignins from the studied samples (Koumba-Yoya and Stevanovic 2016). For each species, FTIR spectra of studied lignins showed similarities features between initial RCW lignin (RCW1) and those sampled after 6 months (RCW2) and 12 months (RCW3). Lignin condensation indices from RCW1, RCW2, RCW3 samples of *G. arborea* were 0.87, 0.76 and 0.71, respectively and those from *S. latifolius* were 0.9, 0.8 and 0.73, respectively; showing gradual depolymerization of lignin from RCW incorporated into the soil. To investigate the changes of functional groups in each RCW lignin after incorporating into the soil, the quantitative ^{31}P NMR technique was applied (Figure 1**Erreur ! Source du renvoi introuvable.**).

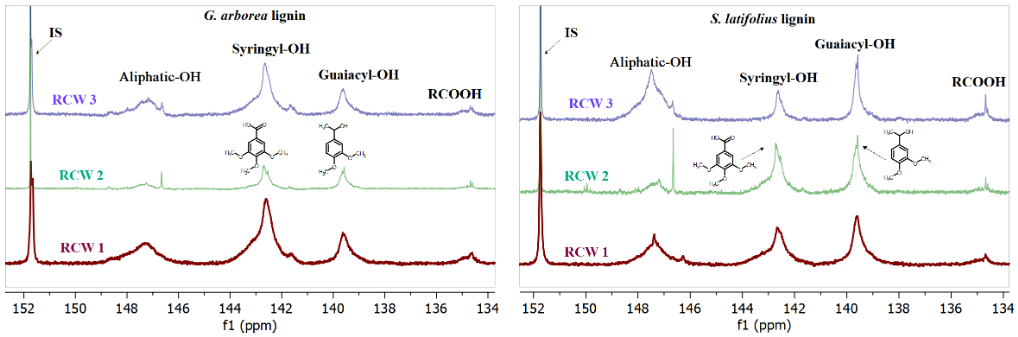

Figure 1. Quantitative ^{31}P NMR spectra and signal assignment of lignin from RCW of *G. arborea* and *S. latifolius* sampled after incorporating into the soil. The phosphitylation was carried out using 2-chloro-4,4,5,5-tetramethyl-1,2,3-dioxaphospholane (Meng et al. 2019).

For *S. latifolius* lignin samples, the total phenolic content (S+G) decreased with soil residence time (RCW1, RCW2, RCW3; 3.6, 3.5, 1.3 mmol/g, respectively). In *G. arborea* lignin samples, the total phenolic content varied little with soil residence time (RCW1, RCW2, RCW3; 3.8, 1.8, 2.4 mmol/g, respectively). The variation of phenolic content of studied lignins during retention time into soil could reveal that, RCW lignin release phenolic compounds which could be involved in humus formation (Thevenot et al. 2010). In addition, the S/G ratio in lignin determined by 2D-HSQC NMR (Table 2) increased with soil residence time of RCW from *G. arborea* whereas opposite trend was found for *S. latifolius* samples. This could indicate that decomposition process of the *G. arborea* sample is faster than that of *S. latifolius* in soil. The relative abundances of the main lignin interunit linkages are presented in Table 2. The main substructure present in all studied lignin were the β-O-4′ alkyl-aryl ether followed by β-β′ resinol substructures and β-5′ phenylcoumaran substructures with resides of p-Coumarates and Ferulates.

Table 2. Structural characteristics (lignin interunit linkages) and S/G ratio from integration of $^{13}C-^{1}H$ correlation signals in the HSQC Spectra of studied lignin

	G. arborea			*S. latifolius*		
	RCW1	RCW2	RCW3	RCW1	RCW2	RCW3
lignin interunit linkages (%)						
β -O-4′	66,4	84,5	65,6	65,7	67,7	23,8
β-β′	2,8	8,3	32,5	N/d	6,9	18,1
β-5	N/d	N/d	N/d	7,3	N/d	N/d
lignin aromatic units						
S/G	1,6	1,2	2,0	0,8	0,1	0,2

Conclusion

In this study, the properties of RCW determined, prior its incorporation into the soil, were potentially advantageous for soil amendment applications: higher mineral and lignin content and degradability indices but *S. latifolius* samples appears to be more efficient. RCW lignin had gradual depolymerization after 12 months residence into the soil. RCW lignin degradation of *G. arborea* is faster with soil residence time than that of *S. latifolius* as shown by their S/G ratios. All studied lignin were GS type and the main lignin substructures present include β-O-4′ alkyl-aryl ethers followed by β-β′ resinols and β-5′ phenyl- coumarans.

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