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Ozonization to Upgrade Waste-Derived Soluble Lignin-Like Substances to Higher Value Products

Enzo Montoneri,^{*[a]} Daniele Rosso,^[b] Giuseppe Bucci,^[c] Silvia Berto,^[d] Andrea Baglieri,^[e] Raniero Mendichi,^[f] Pierluigi Quagliotto,^[d] Matteo Francavilla,^[g] Davide Mainero^[b] and Michèle Negre^[c]

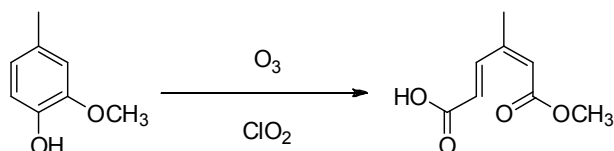
Abstract: Soluble biobased lignin-like polymeric substances (SBO) isolated from the alkaline hydrolysates of composted biowastes are promising chemical auxiliaries for multiple uses. They have good surfactant properties. Their black color spoils their performance in detergency and dyeing. Ozonization of SBO is reported now to yield bleached biosurfactants with improved performance. The work was performed, with SBO obtained from composted gardening residues, alone or mixed with kitchen wastes. They were dissolved in water at 3.3 % concentration. Oxygen containing 4 mol/mol % ozone was flown at 60 L h⁻¹ for 48 h through the SBO solution. The crude ozonized products in ca. 80 % yield were filtered through different molecular cut off membranes and characterized for chemical features, molecular weight, and surface tension in water. The ozonized fractions with 200-500 kDa molecular weights accounted for 12-29 % of the total ozonized fractions's organic matter. They lowered water surface tension to 48-52 mN m⁻¹. The lower molecular weight ozonized fractions had no surfactant activity. but their molecular features suggest other potentially valuable uses.

Introduction

Recently, soluble bio-based lignin-like substances (SBO) have been obtained by low temperature alkaline hydrolysis of the recalcitrant lignin fraction of urban biowastes.^[1] These substances have been shown to perform as ecofriendly chemical auxiliaries in diversified fields; e.g. in the formulation of detergents, textile dyeing baths, flocculants, dispersants and binding agents for ceramics manufacture,^[2] emulsifiers,^[3] auxiliaries for soil/water remediation^[4-6] and enhanced oil recovery,^[7] nanostructured materials for chemical^[8,9] and biochemical catalysis,^[10] plastic materials,^[11-14] soil fertilizers and plant biostimulants for agriculture,^[15,16] and animal feed supplements.^[17,18] The wide range of applications arises from the fact that SBO are a mix of polymeric molecules containing organic C and N distributed over a variety of aliphatic and aromatic C moieties substituted by acid and basic functional groups which are bonded to several mineral elements.^[1, 2] These chemical features represent the memories of the protein, fats, polysaccharide, and lignin proximates constituting the pristine biowaste. They are associated to the SBO properties as surfactants, agents for sequestering or carrying small molecules and mineral ions in solution, photosensitizers and reactive biopolymers. One main drawback of SBO is the black color. This causes critical fabric yellowing in surfactant assisted washing^[19] or dyeing^[20] operations performed in the presence of SBO. It, therefore, impairs the competitiveness of SBO, in relation to commercial surfactants.

Upgrading processes, properties and uses for the above SBO is worth more than just improving their surfactant properties for some specific uses. Indeed, the removal^[21-24] and conversion of lignin to added value products^[25, 26] is a critical point and a major issue for the valorization of dedicated or residual biomass as source of renewable fuels and chemicals. This is because lignin inhibits fermentation microorganisms, and is an insoluble recalcitrant material withstanding biochemical and chemical treatment. Lignin is the second most abundant organic component next to cellulose in the vegetable world. The emerging biomass refinery industry will inevitably generate an enormous amount of lignin. Development of selective biorefinery lignin-to-bioproducs conversion processes will play a pivotal role in significantly improving the economic feasibility and sustainability of biofuel production from renewable biomass.

Current biomass treatment technology,^[21, 27] mainly focused on the production of biofuel by fermentation, such as biogas and bioethanol, adopts a variety of biomass pretreatment methods to remove lignin from the fermentable fraction and/or processes the residual lignin fraction by combustion, pyrolysis, hydrocracking, or aerobic fermentation. These processes, respectively, convert the chemical energy to thermal and electric energy, produce hydrocarbons and other platform chemicals, and compost that is used for landscaping and/or soil fertilization.^[28] Yet, the biowaste lignin fraction has further potential^[29] which can be exploited by low temperature chemical technology. Under these circumstances, bleaching of SBO seemed to the authors of the present work a worthwhile research scope for upgrading the performance of SBO in order to be competitive with commercial surfactants. At the same time, the presence of the many C types and functional group^[1, 2] induced the authors to envisage that further C-C breaking and/or oxidation could transform SBO into other materials and/or platform chemicals useful in the chemical industry, thus widening the range of possible applications of waste-derived products.



Scheme 1. Oxidation of lignin phenolic ring.

As a contribution into this direction, the present work reports the ozonization of SBO in water. For the recalcitrant lignin-like moieties present in SBO, ozone seemed a suitable reagent due to its very strong oxidizing power. Research work performed in the paper pulp industry reports that ozone has very extensive delignifying and bleaching powers.^[26, 30] According to the authors of the cited reference,^[30] ozone or chlorine dioxide react with the lignin phenolic ring to form a muconic acid moiety, as exemplified in Scheme 1. The reaction makes lignin more hydrophilic. The ozone consumption in the oxidation of lignin is more efficient than the consumption of other oxidizing reagents, such as chlorine dioxide. The reaction is monitored by the decrease of the lignin UV absorbance at 457 nm during its progress. All lignin chromophores are destroyed in the case of ozone, whereas they are only partially removed in the reaction with chlorine dioxide. The reaction of lignin with oxygen under pressure in the presence of catalysts^[31] does not yield results, as good as by ozone. The ozonolysis of lignin destroys double bonds and aromatic rings, leaving the side chains intact in the form of carboxylic acids. The reaction converts lignin aromatic carbon to aliphatic carboxyl carbon.^[32, 33] Typical products are aliphatic polycarboxylic acid moieties, as in $-(\text{HOOC})\text{C}(\text{R})-(\text{R})\text{C}(\text{COOH})-$ (I), where R = H or C atom from other molecular fragment.

Ozonation of lignin rich solid fractions from corn stalks has also been performed.^[34] Different solid materials containing lignin were first obtained by steam explosion and organosolvolytic of corn stalks. These materials were further ozonized in order to observe the behavior of their constituent polymers. Particular attention was paid to lignin and the evolution of the water soluble reaction products during oxidation. Several small molecules were identified: e.g. glycolic, oxalic, malonic, glyoxylic, glyceric, *p*-hydroxybenzoic and malic acids, and *p*-hydroxybenzaldehyde and vanillin. Contrary to these findings, ozonation of pine Kraft lignin with 5500 kDa weight average molecular weight yielded products with higher molecular weight than the pristine lignin. The reaction was performed in alkaline solution with an ozone-air stream containing 2–2.5% of ozone.^[35] The results showed that pine Kraft lignin undergoes extensive oxidative cleavage of both side chains and aromatic moieties. The molecular weight of the ozonized product (up to 15000 kDa) results higher than that of the pristine material. The authors^[35] attributed this fact to dehydrogenative coupling of phenolic degraded fragments by active oxygen radical species, such as hydroperoxyl and hydroxyl radicals, formed by the decomposition of ozone in alkaline solution. Other authors^[36] have reported good surfactant properties by the ozonized lignin products.

To the authors of the present paper, the above literature review indicated that the ozonization of SBO had great potential to yield further valued added products, ranging from decolorized surfactants to commodity hydroxyl, carboxylic polymeric and small molecules. Hereinafter, the results of the ozonization of two different SBO are reported. The two SBO were obtained from two different biowaste composted materials from urban source. They differ for the content of aliphatic and aromatic C types, and of functional groups. The availability of these substances offered the opportunity to investigate also the possible effect of the pristine chemical nature on the nature of the ozonized products.

Predicting or explaining the behaviour of the SBO used in the present work was hard and intriguing, at the same time. The SBO had a very complex chemical composition.^[1] They consisted in heterogeneous mixtures of molecules with different chemical composition and molecular weights. Moreover, ozonization generated a huge range of diverse heterogeneous product mixtures. The determination of the nature and yield of the products was therefore very challenging. It required a significant amount of analytical work including product fractionation by filtration through membranes with different molecular cut off, NMR, IR and GC-MS spectroscopy, SEC-MALS molecular weight measurements, potentiometric titration, C and N microanalyses, and surface tension measurements.

The following Results and Discussion section is divided into several subsections, which are dedicated to the course of the ozonisation characterization, and to the characterization of the pristine SBO and the ozonized products by each of the above analytical techniques. Two specific subsections are also included to discuss (i) the complementarity of the above analytical techniques and (ii) the potential uses and economic sustainability of the soluble ozonized products, by comparison with commercial products exhibiting the same properties as the ozonized SBO. The authors of the present work are not aware of any previous work published on the ozonation of urban biowastes compost. The hereinafter-reported experimental work does not include any process optimization attempt. The scope was to carry out a preliminary exploration, in order to assess the nature and surfactant property of the products obtained, and to disclose further opportunities for the valorization of municipal biowaste as source of value-added products.

Results and Discussion

Origin and chemical nature of SBO

Sourcing matter and fractionation of SBO

The soluble lignin-like substances (SBO) for the ozonization were obtained from two different samples taken from the process streams of Acea Pinerolese Spa waste treatment plant located in Pinerolo (TO), Italy. The plant processes the biowaste through two integrated steps comprising anaerobic and aerobic digestion.^[2] Hereinafter, the SBO investigated in this work are named with the CV and CVD abbreviations, i.e. CV obtained from urban gardening vegetable residues composted for 230 days, and CVD obtained from urban gardening vegetable residues and food wastes digestate, which were mixed, respectively, in 2/1 w/w ratio and composted for

110 days. The CV and CVD SBO were obtained by hydrolysis of the pristine composts at pH 13, 60 °C and liquid/solid ratio of 4.^[1] These products are characterized by the data reported in Tables 1-2.

To understand the CV and CVD data, it must be considered first that the organic matter of these materials of biological origin is a complex mixture of molecules, differing for molecular weight and chemical nature.^[1] The sourcing food and gardening residues are constituted by the main proximates of vegetable and animal food species, i.e. cellulose, hemicelluloses, lignin, proteins, fats. The composts of these bio-wastes contain residual organic moieties of the pristine bio-waste proximates, which have survived the anaerobic and/or aerobic fermentation process. In the present work, the CV and CVD were separated into four fractions by sequential filtration through three membranes with different molecular cut off. The experimental details are described in the Experimental section of the Supporting Information (SI) file. These fractions were obtained from the retentates R35, R20 and R5, and from the permeates P5 of the membranes with 35, 20 and 5 kDa molecular cut off.

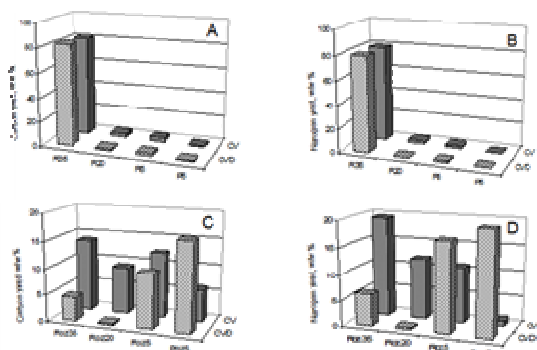


Figure 1. Carbon (A and C) and nitrogen (B and D) yields (% w/w), relative to carbon and nitrogen in the pristine CV and CVD materials, for fractions isolated from the retentates and permeates obtained by sequential membrane filtration. Yield data in Figure A and Figure B for R35, R20 and R5 retentates, and P5 permeate, from filtration of crude pristine CV and CVD through membranes with 35, 20 and 5 kDa cut off, respectively. Yield data in Figure C and Figure D for Roz35, Roz20 and Roz5 retentates, and Poz5 permeate, from filtration of soluble crude ozonized CV and CVD through the same above membranes, respectively.

The obtained products are listed in Tables 1-2 by their abbreviations R_xY and P_xY , where R and P stand for retentate and permeate, respectively, x indicates the crude sourcing CV or CVD, and Y stands for the membrane molecular cut off. Figure 1 shows that the fraction isolated from the retentates of the 35 kDa cut off membrane largely accounted for most (79-83%) of the C and N present in the crude pristine CVD and CV.

Chemical features for SBO fractions

The 35 kDa molecular weight fractions (R_{CVD35} and R_{CV35} in Tables 1-2) and the pristine CVD and CV materials were further analyzed for molecular weight by size-exclusion chromatography coupled to multi-angle light scattering detector,^[11-14] and for the content of volatile solids and the nature of the organic matter. For these products, the weight average molecular weight data (MW in Table 1) confirmed their polymeric nature. The volatile solid values (VS in Table 1) showed that both the crude pristine CVD and CV materials, and the R_{CVD35} and R_{CV35} fractions, were not entirely constituted by organic matter. They contained also about 33-37 % of mineral fraction. The organic matter was found to contain aliphatic and aromatic C moieties substituted by a variety of functional groups, as listed in Table 2. These moieties are structural memories of the proximates of the starting vegetable and animal biowastes. Specifically, the anomeric (OCO) and alkoxy (OR) C functional groups indicate the presence of polysaccharide matter, the aryl (Ar) C indicates the presence of lignin-like matter, and the carboxyl (COZ) and amino (NR) C groups indicate the presence of proteinaceous matter surviving the fermentation processes. It should however be considered that these moieties do not indicate separate proximates. They most likely arise from polysaccharide, proteins and lignin bonded to each other. The organic matter was further analyzed for the consistency of ¹³C NMR spectroscopy and potentiometric titration data.

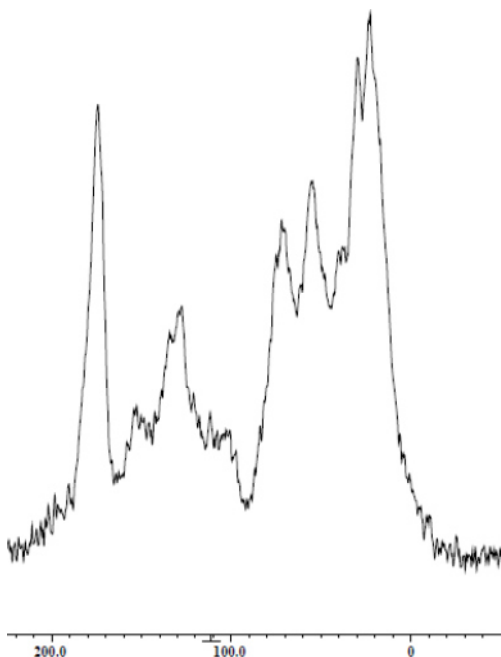


Figure 2. ^{13}C NMR spectrum of crude pristine CVD: numbers in abscissa as ppm from tetramethyl silane.

^{13}C NMR spectroscopy and potentiometric titration data.

The ^{13}C NMR spectra contained six broad bands. Figure 2 shows a typical spectrum, which was recorded for the crude pristine CVD. The spectra of the two pristine CV and CVD, and of their R35 fractions, were substantially similar, except for differences in relative band areas. Table 2 reports chemical shifts, assignments to C types and functional groups, and percent values of each band area, relative to the total band areas obtained by integrating each band area in the reported chemical shift ranges. Resonance bands' assignments are according to previous work carried out on the same crude CV and CVD materials.^[1] In evaluating this data, it should be considered that ^{13}C NMR band area distribution does not necessarily reflect^[37] the true molecular distribution of C types and functional groups. For instance, C in H poor moieties, such as polycyclic aromatic hydrocarbons, is underestimated due to poor polarization of ^{13}C far from ^1H nuclei. Vice versa, protonated C groups may be overestimated. Moreover, bands are very broad and, in most cases, do not allow a selective assignment. For instance, it is not possible to assess which type of carboxyl group is present.

To investigate further the nature of the carboxyl groups, independent measurements of the acid functional groups by potentiometric titration^[38] were carried out (see Experimental section in SI file). In these measurements, the pH vs titrant volume experimental plots allowed picking out two inflection points at pH about 8.5 and 5.3, respectively. These indicated the end points of the titration of two types of functional groups with different acid strength. To be consistent with the ^{13}C NMR spectroscopic data, the inflection point at pH 5 was assigned to the titration end point of the more acidic COOH groups in mono- and poly-carboxylic acids,^[39] and/or in amino acid C moieties. The inflection point at 8.5 was assigned to the titration end point of the weaker, other COOH groups in poly-carboxylic acid moieties, and/or of NH_3^+ and OH groups in amino acids^[40] and phenol^[41] moieties, respectively. Unfortunately, the differences in acid strength between the above functional groups are not enough to allow observing inflections points for each group under the experimental potentiometric titration conditions used in this work. For crude pristine CV, and for a CVD-like material obtained from a different compost, the titration curves containing the two inflection points were elaborated to fit different models accounting for up to three protogenic acid sites. The best fitting model was found the one accounting for three different mono- and di-protic acid sites with constants (K) in the 5-10 log K value range.^[42]

Table 2. Chemical features^[a] and/or water surface tension activity (γ) for crude pristine CVD and CV, crude soluble ozonized CVD and CV, crude insoluble ozonized CVD and CV, and fractions (R and P as in Table 1)^[b] of crude pristine CVD and CV, and of crude soluble ozonized CVD and CV.

Data from ^{13}C NMR spectroscopy: signal chemical shift (δ , ppm), assignment to C type and integrated relative band area % ^[a]	Af/Ar	Data (meq g ⁻¹) from potentiometric titration	γ ^[d]
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δ , ppm	0-53	53-63	63-95	95-110	110-160	160-185	and elemental analysis				
	Product/C type	Af	NR+OMe	OR	OCO	Ar	COZ	$\chi^{[c]}$	COOH ^[c]	N	
Crude pristine CVD	40.9	7.3	14.2	3.9	18.2	15.5	2.24	1.84	4.76	3.01	58.9
Crude soluble ozonized CVD	20.0	6.0	3.3	2.1	20.4	48.2	0.98	2.65	5.68	2.29	
Crude insoluble ozonized CVD	22.4	7.3	8.8	2.9	29.3	29.3	0.76				
R _{CV35}	22.5	9.3	8.8	3.4	33.1	22.9	0.68	0.55	3.11	3.21	59.8
R _{CVoz35}	62.9	5.5	8.6	4.9	5.2	12.9	12.1	1.63	3.18	3.53	51.9
R _{CVoz5}	31.5	9.3	21.1	4.7	4.5	28.9	6.95	0.77	2.92	3.81	66.6
P _{CVoz5}	16.6	5.5	15.8	2.4	2.4	57.3	6.90	1.48	7.32	1.36	70.5
Crude pristine CV	36.9	7.2	13.2	4.2	20.2	18.3	1.8	1.52	4.34	2.99	58.9
Crude soluble ozonized CV	17.3	6.0	6.8	3.3	22.4	44.2	0.77	2.61	5.42	2.34	
Crude insoluble ozonized CV	22.7	5.1	8.0	2.9	29.4	31.9	0.77				
R _{CV35}	26.7	8.3	8.1	2.6	31.7	22.6	0.84	3.61	6.90	2.93	59.2
R _{CVoz35}	49.9	10.1	13.5	3.1	5.3	18.1	9.47	0.58	3.57	2.93	48.5
R _{CVoz20}	21.5	11.4	17.7	3.1	4.1	42.2	5.30	2.01	5.00	1.68	63.2
R _{CVoz5}	17.9	9.6	19.5	2.9	1.9	48.2	9.4	2.27	5.35	1.09	70.5
P _{CVoz 5}						100		4.98	7.26	0.16	70.8

^[a]Data for aliphatic (Af), aromatic (Ar), methoxy (OMe), carboxyl (COZ, Z = OH, NW; W = H, alkyl or aryl), amine (NR), alkoxy (RO), anomeric (OCO) carbon.

^[b]Retentate (R) and permeate (P) by filtration through 35, 20, 5 and 5 kDa cut off membrane. ^[c]Data for acid groups content (meq g⁻¹) in virtual molecular fragment A-B-C, where A = NH₃⁺, COOH, B = aliphatic C moiety; A = OH, B = aromatic C moiety; C = COOH or other Medium (C = COOH) and weak (A) strength acids groups in aliphatic and/or aromatic C moieties (B). ^[d]Surface tension (γ , mN m⁻¹) in water containing 2 g L⁻¹ product.

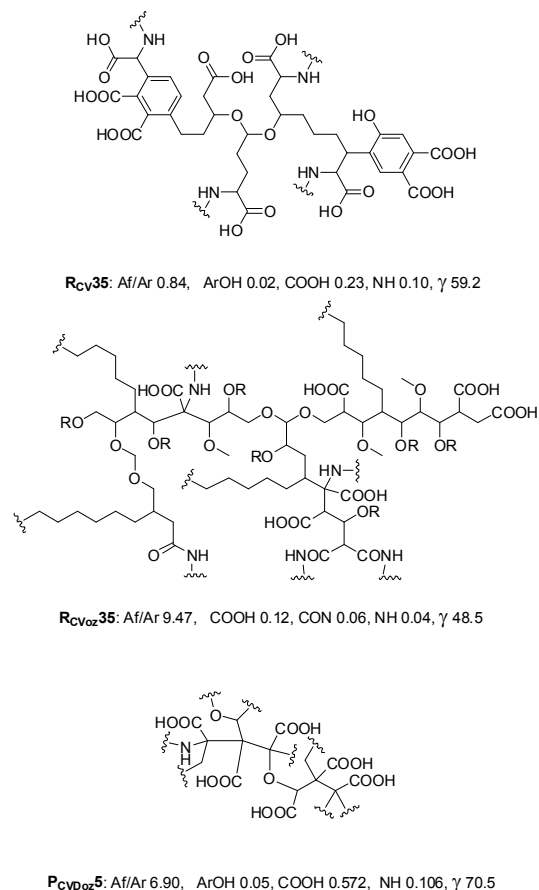


Figure 3. Examples of virtual molecular fragments fitting analytical data of products in Table 1 and 2: from top to bottom R_{Cv35}, R_{CvD35}, P_{CvD35}. Sinusoidal lines indicate bonds with C of other molecular fragments or with H; R = alkyl; aliphatic C with not indicated bonds may be bonded to H, OH and/or to aliphatic C of other molecular fragments through direct C-C bonds or C-O-C bonds. Data reported as follows: ArOH, COOH and NHR mol/mol ratio of functional group to total C atom; Af/Ar and γ as in Table 2.

Complementarity of ¹³C NMR spectroscopy and potentiometric titration

It is evident that, under the above circumstances, both ¹³C NMR spectroscopy and titration have limitations. Nevertheless, considering the complex chemical nature of the investigated products, the results by spectroscopy are consistent with those by titration. They are complementary and allow drawing some useful further information on the chemical nature of the above products. For instance, the ¹³C NMR spectroscopy data in Table 2 indicates that the two R_{CvD35} and R_{Cv35} fractions have nearly similar C types and functional groups composition. Moreover, the carboxyl groups (COZ) band area percent is about 23 for both fractions. The titration data however points that the two fractions have a rather different distribution of acid functional groups. A plausible explanation for the observed differences is that, for R_{Cv35}, the titrated COOH groups accounted for nearly all the COZ band area percent determined by ¹³C NMR spectroscopy. By comparison, the carboxyl groups of R_{CvD35} were composed by nearly equal amounts of COOH and peptide (CON) functional groups. The presence of these bonds is supported by the presence of elemental N in nearly equal relative amount as the titrated COOH groups. On this basis, one can reasonably presume that the elemental N concentration value, which is reported in Table 2 for R_{CvD35}, indicated the content of the CON functional groups. In this fashion, the sum of the concentration values for the COOH and for the presumed CON functional groups in R_{CvD35} (6.3 meq g⁻¹) equals the content of COOH functional groups in R_{Cv35}. Thus, in agreement with ¹³C NMR spectroscopy, the titration data confirms that the two products have the same content of total carboxyl groups. On the other hand, the titration data indicates that the R_{Cv35} fraction is likely to have higher content of weaker acid groups (X) than R_{CvD35} has. In essence, X (Table 2) equals 3.77 and 0.55 meq g⁻¹ for the former and the latter, respectively. The weaker acid groups (X) in R_{Cv35} are likely to be contributed mainly by amino acid moieties, as suggested by the presence of 2.34 N meq g⁻¹ versus 3.77 X meq g⁻¹. For R_{CvD35}, if the elemental N content is contributed entirely by CON functional groups, the 0.55 meq g⁻¹ content of the X groups is likely to be contributed mainly by the presence of phenol functional groups.

Overall, Table 2 data indicates that the two crude pristine CV and CVD materials, although having nearly the same contents of carboxyl C and elemental N, are different mainly for the distribution of the total carboxyl C and total N over carboxylic acid, peptide and amine functional groups. Figure 3 shows the virtual molecular fragment associated with the R_{CV35} product in Table 2. It represents the C types and functional groups, and their relative ratios, which are reported in Table 2. The fragment shows two aromatic rings, each substituted with one OH and two COOH groups. This is not necessarily true. The COOH groups might be substituents of aliphatic C atoms. Moreover, the two aromatic rings might be condensed to form a naphthalene moiety. This, in turn, might be condensed with the aromatic rings of other molecular fragments to constitute the chromophore moieties responsible of the black colour of the product. In addition, the product aromatic C atoms might be more than Table 2 reports, because ¹³C NMR spectroscopy likely underestimates quaternary C atoms.^[37]

Ozonization reaction and products color, solubility and surface activity

The ozonization of crude pristine CV and CVD in water at 3.3 % concentration was carried out for 48 h by flowing 60 L h⁻¹ oxygen with 4 mol/mol % ozone content. The starting solution pH values were 9.6 and 9.8, respectively. During the reaction, the pH tended to decrease, but it was kept at 9.0-9.5 by periodic addition of KOH. At the end of the reaction, the total amount of added KOH was 0.34 g per starting CV or CVD g, corresponding to the production of 0.21 and 0.31 acid eq per starting CVD and CV C mole, respectively. The total gas consumption was 129 moles, containing 5 ozone moles, corresponding to 3.7 O₃/CVD C and 8.9 O₃/CV C mol/mol consumption. The reaction was monitored by recording the UV spectrum of samples withdrawn at time intervals. The absorbance at 465 nm, relatively to that at 665 nm, decreased upon increasing the reaction time (see Figure 1S in SI file). The reaction was stopped when the absorbance ratio vs. time plot tended to become flat. Consistently with previous work on the ozonization of cellulosic fibers containing residual lignin,^[30] the decrease of the UV absorbance ratio depicted in Figure 1S and the KOH consumption during the reaction indicate destruction of double bonds and aromatic rings chromophore moieties, with consequent formation of carboxylic acids functional groups. Other workers^[43] have demonstrated the validity of the above UV absorbance ratio as a mean indicating the relative content of aromatic C moieties in the organic matter of different soils.

The reaction mixture was then centrifuged in order to separate the two crude ozonized insoluble and soluble products. The insoluble product was dried at 40 °C. The liquid phase containing the soluble product was freeze-dried and lyophilized to yield the crude soluble ozonized residue in solid form. The latter one was dissolved in water and processed by sequential filtration through the same three membranes with 35, 20 and 5 kDa molecular cut off, which were used also for the fractionation of crude pristine CV and CVD. These products are listed in Table 1 and 2 by their abbreviations R_{xoz}Y and P_{xoz}Y, where R and P stand for retentate and permeate, respectively, xoz indicates the crude soluble ozonized sourcing product obtained from crude pristine CV (x = CV) or CVD (x = CVD), and Y specifies the membrane molecular cut off. The ozonized product fractions isolated as above were soluble in the whole pH range, while crude pristine CV and CVD were soluble only at pH > 4.

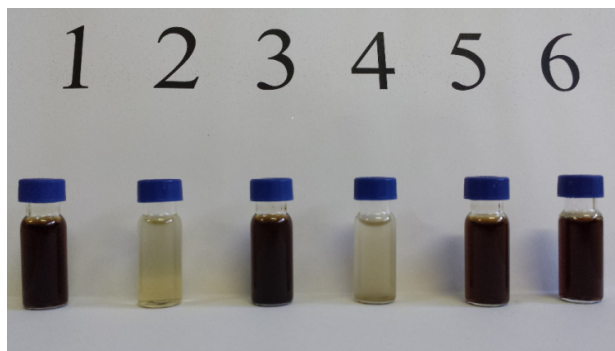


Figure 4. Aqueous solutions containing the following products at 1 g L⁻¹ concentration: from left to right crude pristine CV (1), R_{CVoz35} (2) 35 kDa fraction of crude soluble ozonized CV, crude pristine CVD (3), R_{CVDoz35} (4) 35 kDa fraction of crude soluble ozonized CVD, R_{CV35} (5) and R_{CVD35} (6) 35 kDa fractions of crude pristine CV and CVD; legends as in Table 1-2.

The soluble ozonized products were much lighter colored than crude pristine CV and CVD. The R_{CVoz35} and R_{CVDoz35} ozonized fractions, which were isolated from the retentates of the crude soluble ozonized product through the 35 kDa cut off membrane, were found the only two ozonized fractions able to decrease the water surface tension (Table 2), as well or better than crude pristine CV and CVD, respectively. The R_{CVoz35} and R_{CVDoz35} ozonized fractions accounted, respectively, for 29 and 11.5 % of the total organic matter recovered from the fractionation procedure. At 2 g L⁻¹ added product concentration in water, the surface tension values were 48.5 and 51.9 mN m⁻¹ for the R_{CVoz35} and R_{CVDoz35} solution, respectively. By comparison, the surface tension values for the solutions containing 2 g L⁻¹ pristine crude CV and CVD SBO, or R_{CV35} and R_{CVD35}, were about 59-60 mN m⁻¹. Figure 4 shows the color of the six aqueous solutions containing these materials, each one at 1 g L⁻¹ concentration. This is also the concentration value, at which the crude pristine CV and CVD materials had been investigated for their performance in fabric washing.^[19] The improved surface tension

and color features of the R_{CVoz35} and $R_{CVDoz35}$, compared to the pristine crude CV and CVD SBO, or R_{CV35} and R_{CVD35} fractions, are clearly evident.

Figure 3 compares the virtual fragments for R_{CV35} and R_{CVoz35} fitting the analytical data in Table 1 and 2. Aromatic carbon in the latter fragment is not shown, due to its relatively low content. Table 2 reports aromatic C content 5.3 % in R_{CVoz35} versus 31.7 % in R_{CV35} . Figure 3 depicts the compositional differences between the two products. Compared to R_{CV35} , R_{CVoz35} has much higher relative content of aliphatic carbon and lower content of acid functional groups. The chemical composition differences between the two products appear to be associated to the lighter color and higher surface activity by R_{CVoz35} . These features, respectively, are likely due to the presumed lower concentration of aromatic C chromophore moieties and to the more favorable hydrophilic/lipophilic balance of the molecular pool. The data demonstrates that bleaching of CV and CVD is achieved through destruction of chromophores^[30,31] by ozonization, while the water surface tension activity of the crude pristine materials is maintained or improved in the ozonized products. In addition, the solubility properties of the crude pristine CV and CVD, and of the ozonized products, prospect that the ozonized products are potentially applicable in a wider range of experimental conditions.

Ozonized products yields

The above results show that upgrading the performance of crude pristine CV and CVD, as surfactant, is technically feasible through ozonization. However, the R_{CVoz35} and $R_{CVDoz35}$ ozonated products exhibiting surfactant properties are not the only reaction products. To assess the full potential of the ozonization reaction carried out in the present work, the Experiment section (see SI file), Figure 2 and Table 1-2 report data on the reaction yield and on the characterization of the crude soluble and insoluble ozonized products, and all soluble ozonized product fractions. The calculation of product yield data was based on the amount of product dry matter, which was recovered at reaction end, and on its content of volatile solids (VS), and of organic C and N, relative to the data obtained for the starting pristine material (see Experimental section in SI file). The calculation of product C % yield accounted for the fact that, in oxidation reactions of organic matter, CO_2 is the thermodynamically most favored product. As ozonization of CV and CVD was carried out at alkaline pH (see Ozonization reaction subsection), any produced CO_2 remained in the raw products as carbonate. The inorganic C content was therefore determined (see Experimental section in SI file). The CO_2 C yield was found about 3 % of the starting organic C. Net organic C % yield values were then calculated, based on total C determined by microanalysis, after subtracting the determined CO_2 C.

Yield values of crude soluble and insoluble ozonized products

The Experimental section (see SI file) reports raw dry matter, VS, C and N data relative to the recovery of the soluble and insoluble ozonized products. From the raw VS content in the pristine starting material and in the reaction products, and the total added KOH amount at reaction end (see Ozonization reaction subsection), the % ash recovery with the reaction products was calculated, relative to the total starting amount of ash in the pristine starting material and to the amount contributed by the added KOH. The ash recovery values resulted about 108 % in both the CVD and CV reactions. Assuming a reasonable 8 % relative error stemming from the ash content analysis and from the calculation of the ash content contributed by added KOH during the reaction, the ash recovery with the reaction products turns out quantitative. This fact validates the reliability of the sampling and analytical procedure adopted in this work to calculate the reaction mass balance. Moreover, the data reported in the Experimental section (see SI file) showed that the crude soluble and insoluble ozonized products were characterized by very different C/N and VS/(C + N). These were C/N 5 for the soluble crude ozonized product, C/N 11 for the crude insoluble ozonized product, and VS/(C + N) 1.8-1.9 for the soluble crude ozonized product and 2.6-2.8 for the crude insoluble ozonized product. These figures indicate that, compared to the crude insoluble ozonized product, the crude soluble ozonized product had relatively more organic N moieties, but less oxidized C.

Contrary to the quantitative ash recoveries, the VS, C and N % recoveries were not quantitative. Volatile solids (VS) recovery values with the reaction products were 73 % and 66 % in the CVD and CV reactions, respectively. The C recovery values for the crude soluble and insoluble ozonized products were, respectively, 42.7 and 16.6 % in the CVD reaction, and 35.8 and 13.5 % in the CV reaction. The N recovery for the crude soluble and insoluble ozonized products were, respectively, 65.7 and 12.1 % in the CVD reaction, and 65.3 and 10.4 % in the CV reaction. The data shows that the crude ozonized soluble products account for about 72-73 % C and 84-86 % N, relatively to the total recovered C and N calculated from the sum of values obtained for the soluble and insoluble ozonized crude products. In addition, it results that the crude soluble and insoluble ozonized products together account for most of the crude pristine CVD and CV C and N amounts. The missing C and N amounts may represent volatile low molecular products, which are lost during the products drying or lyophilization. Due to their water solubility property and higher reaction yield, the crude soluble ozonized products were further investigated for the content, chemical nature and surfactant activity of their fractions separated by membrane filtration (see Ozonization reaction subsection). The insoluble ozonized products were not investigated further. They were considered secondary products, offering less interesting perspectives of potential uses, compared to the soluble ozonized products.

Yield values of soluble ozonized product fractions

For the fractions obtained by membrane filtration of the crude soluble ozonized products, Figure 1 reports carbon and nitrogen recovery yields (% w/w), relative to carbon and nitrogen in the pristine CV and CVD materials before ozonization. These were calculated from the weights and the C and N content of the recovered products (see Table 1 and Experimental section in SI file). The

total C, N and/or volatile solids (VS) recoveries do not account for all C, N and VS amounts present in the pristine crude soluble ozonized material. The missing C, N and/or VS amounts may represent volatile low molecular products, which are lost during the products drying or lyophilization. Moreover, during the membrane ultrafiltration, some further material loss also occurred, due to absorption of material on the membranes and leaching through the ultrafiltration cell. Nevertheless, the data reported in Figure 1 shows that the product obtained from crude pristine CVD had a very different composition from the product obtained from crude pristine CV. For the former crude soluble ozonated product, the major fraction was isolated from the permeate through the 5 kDa cut off membrane; i.e. the P_{CVDoz5} fraction. For the latter product, the major fraction was isolated from the retentate of the 35 kDa membrane filtration; i.e. the P_{CVoz35} fraction. In the order of decreasing C % yield, the ozonized fractions rank as follows: from crude soluble ozonized CVD, $P_{CVDoz5} > R_{CVDoz5} > R_{CVDoz35} > R_{CVDoz20}$; from crude soluble ozonized CV, $R_{CVoz35} > R_{CVoz5} > R_{CVoz20} > P_{CVoz5}$.

Ozonized products characterization

Size exclusion chromatography

The size-exclusion chromatograms of crude pristine CVD and CV before ozonization showed a unimodal molecular weight distribution. The chromatograms of their main fractions isolated from the retentates of the membrane with 35 kDa cut off (R_{CVD35} and R_{CV35}) and of the fractions of the crude ozonized soluble products indicate the presence of one or more main components with different molecular weight and different relative composition. The chromatograms of the fractions isolated from the crude ozonized soluble product exhibit two or three bands. Some typical examples are reported in Figure 2S and 3S (see SI file). Table 1 reports the weight average molecular weights (MW), the molecular weight dispersity index, and the recovered mass from the size-exclusion chromatographic columns relatively to the starting amount of the analyzed sample. The mass recoveries range from 71 to 99 %. Hence, the reported molecular weight values are well representative of the analyzed sample.

From the yield data in Figure 1, and the MW data in Table 1, it is interesting to observe that the crude pristine CVD and CV contained 80 % or more C contributed by the R_{CVD35} and R_{CV35} fractions with 279 and 18.5 kDa weight average molecular weight, respectively. By comparison, the crude soluble ozonized products contain a more even distribution of fractions with higher and lower molecular weight than crude pristine CVD and CV. These are the $R_{CVDoz35}$ and R_{CVoz35} fractions with 487 and 223 kDa molecular weight, and the other ozonized product fractions with 4.6-65.1 kDa molecular weight. Based on the C % recoveries reported in Figure 1, the ozonized product fractions were obtained with the following relative C yield distributions: from crude pristine CVD, 14 % $R_{CVDoz35}$, 0.9 % $R_{CVDoz20}$, 32 % R_{CVDoz5} and 52 % P_{CVDoz5} ; from crude pristine CV, 33 % R_{CVoz35} , 22 % R_{CVoz20} , 30 % R_{CVoz5} and 15 % P_{CVoz5} . Thus, the highest molecular weight $R_{CVDoz35}$ and R_{CVoz35} fractions, respectively, represent 14 and 33 % of the total C recovered with all the ozonized fractions. By comparison, the C recoveries with the lower 4.6-65.1 kDa molecular weight fractions range from the lowest 0.9 % to the highest 52 % values, respectively achieved with $R_{CVDoz20}$ and P_{CVDoz5} .

The formation of lower and higher molecular weight molecules in the ozonized products than the molecules in the pristine CV and CVD lignin-like materials is consistent with previous findings reported in the ozonization of pine Kraft lignin under similar conditions^[35] as those used in the present work. This fact was attributed^[35] to dehydrogenative coupling of phenolic degraded fragments by active oxygen radical species, such as hydroperoxyl and hydroxyl radicals, formed by the decomposition of ozone in alkaline solution. This reaction may also explain the formation of the insoluble ozonized product (see Ozonized products yields section). The product insolubility may be the result of extensive cross-linking between ozonized molecules.

¹³C NMR spectroscopy and potentiometric titration

The data reported in Table 2 contribute further information on the chemical nature of the ozonized products. Both the insoluble and soluble ozonized products have higher content of total carboxyl (COZ) groups, compared to the crude pristine CV and CVD materials. The alkali consumption during the ozonization reaction (see Ozonization reaction subsection) corresponded to the production of 0.21 and 0.31 acid eq per starting crude CV and CVD C mole. Thus, a great part of the total carboxyl groups in the ozonized products must be in carboxylate (COO⁻) form.

Potentiometric titration demonstrated the presence of at least two types of acid groups with different strength in the soluble ozonized products, as well as in the crude pristine CV and CVD. These groups are reported in Table 2 as X and COOH, where X may be an amino and/or phenol, and/or a second COOH group, which are weaker than the primary stronger COOH group, as in amino acids, hydroxyacids, and/or polycarboxylic acid moieties.

The data in Table 2 shows the content of both acid groups determined by potentiometric titration, along with the content of total carboxyl (COZ) groups determined by ^{13}C NMR spectroscopy. Both the titrated acid groups and the carboxyl COZ groups tend to increase, upon decreasing the molecular weight of the ozonized fraction. The other relevant feature is evidenced from the ^{13}C NMR spectra of the ozonized products (Figure 5), compared to the spectra of the crude pristine CV or CVD materials, which are exemplified in Figure 2. It may be observed that the relative contribution of the aliphatic and aromatic C atoms resonances bands with chemical shift below 160 ppm is significantly reduced in the spectra of the ozonized products (Figure 5), compared to the spectral pattern (Figure 2) for the crude pristine CV and CVD. In addition, the contribution of the aromatic C signals at 110-160 ppm, relatively to that of the aliphatic C signals at 0-53 ppm, seems much lower in the ozonized product fractions, relatively to that in the main fractions ($R_{\text{CVD}35}$ and $R_{\text{CV}35}$) of the crude pristine CV and CVD. These features may also be appreciated by comparing the band areas % values for the above resonances reported in Table 2; i.e. the total carboxyl (COZ) band area % for the ozonized products versus the crude pristine CV and CVD, and the aliphatic to aromatic C ratio (Af/Ar) for the ozonized product fractions versus the main $R_{\text{CVD}35}$ and $R_{\text{CV}35}$ fractions of the crude pristine CV and CVD.

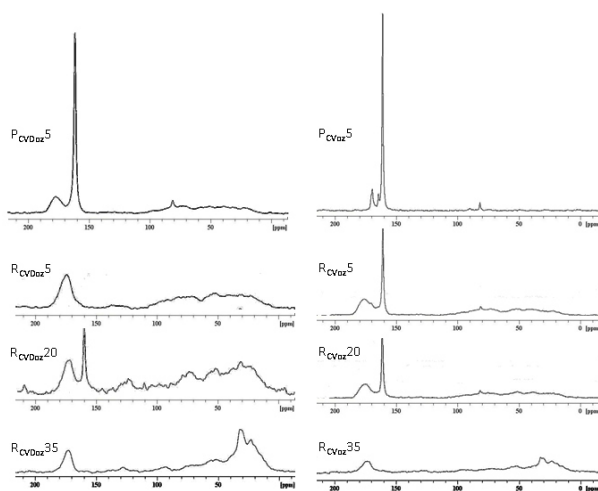


Figure 5. ^{13}C NMR spectra of fractions obtained from ozonized CVD and CV SBO; legends as in Table 1-2

The above results are consistent with literature data on the ozonization of lignin,^[32-35] reporting that oxidation by ozonization occurs primarily at the aromatic C, and it produces aliphatic carboxylic acid moieties. Particularly relevant in these respects are the spectra of the $P_{\text{CVD}oz5}$ and $P_{\text{CV}oz5}$, which are the lowest molecular weight fractions of the crude soluble ozonated CV and CVD products, respectively. The spectral patterns of these products' fractions exhibit two strong bands arising from carboxyl groups. In these spectra, signals below 160 ppm, arising from other C atoms, can hardly be detected. The potentiometric titration data (Table 2) indicates that the two products have nearly the same content of COOH groups. They differ for the amounts of X groups and elemental N. The $P_{\text{CVD}oz5}$ fraction has nearly equal amounts of X and elemental N. This may suggest that the X groups in $P_{\text{CVD}oz5}$ are mainly contributed by amino acid moieties. The $P_{\text{CV}oz5}$ fraction contains $4.98 \text{ X meq g}^{-1}$ and only $0.16 \text{ N meq g}^{-1}$. Thus, for this fraction, the X group can only indicate the presence of weak COOH functional groups. The presence of two COOH groups with different acid strength is consistent with the presence of the relative ratio and the chemical shifts of the two ^{13}C NMR signals at 170-185 ppm in Figure 5. The product made by only two COOH groups is oxalic acid. However, this assignment is not consistent with the 4.26 kDa MW value reported for the $P_{\text{CV}oz5}$ fraction in Table 1. To account for these results, the most likely explanation is that the $P_{\text{CV}oz5}$ fraction contained mainly aliphatic polycarboxylic and/or poly hydroxyl carboxylic acid macromolecules, such as polymalic acid^[44] and polymaleic and polyfumaric acid.^[45]

Other work^[46-48] reports that various dicarboxylic acids and hydroxyacids, such as muconic, maleic, and succinic acids, fumaric, 2,3 buten 1,4 dicarboxylic acid, butan 2 hydroxy 1,4 dicarboxylic acid, are obtained by oxidation of lignin. The polycarboxylic and/or poly hydroxyl carboxylic acid macromolecules in the low molecular weight ozonized product fractions obtained in the present work are likely constituted by repeating units of the above monomers containing quaternary C atoms, as in the molecular fragment (I). Quaternary C atoms may be underestimated or go undetected by ^{13}C NMR spectroscopy.^[37] This seems particularly true for $P_{\text{CV}oz5}$. The ^{13}C spectrum of this fraction (Figure 5) in the 160-185 ppm range is similar to that reported for poly(maleic acid)^[43] in the same chemical shift range. The spectrum of this polymer exhibits two distinct broad resonance signals for carboxyl carbon atoms, respectively falling at 173-174 and at 175-176.5 ppm. In each signal, four resonances can be discerned. The resonances of the carboxyl carbon atoms are assigned to configurations of heptad sequences along the polymer chain. The spectral pattern is a result of differences in the direct surroundings of the carbon atoms.

Figure 5 shows that several ^{13}C NMR signals between 161 and 176 ppm, arising from carboxyl C atoms, may also be picked out in the spectra of the lower molecular weight fractions of the ozonized products isolated from the retentate and permeate of the membranes with molecular cut off ≤ 20 . By comparison, the spectra of the higher molecular weight fractions $R_{\text{CVD}oz35}$ and $R_{\text{CV}oz35}$

exhibit one main carboxyl broad band at 173 ppm. These features suggest that the lower molecular weight fractions of the ozonized products are likely to be largely constituted by aliphatic polycarboxylic moieties. Figure 3 shows an example of virtual fragment for the P_{CVDoz5} fraction fitting the analytical data of Table 1 and 2. The anomeric (OCO) and aromatic (Ar) carbon types reported in Table 2 for $PCVDoz5$ account for only 4.8 % of the total C content. Thus, these carbon types have not been included in the P_{CVDoz5} fragment represented in Figure 3.

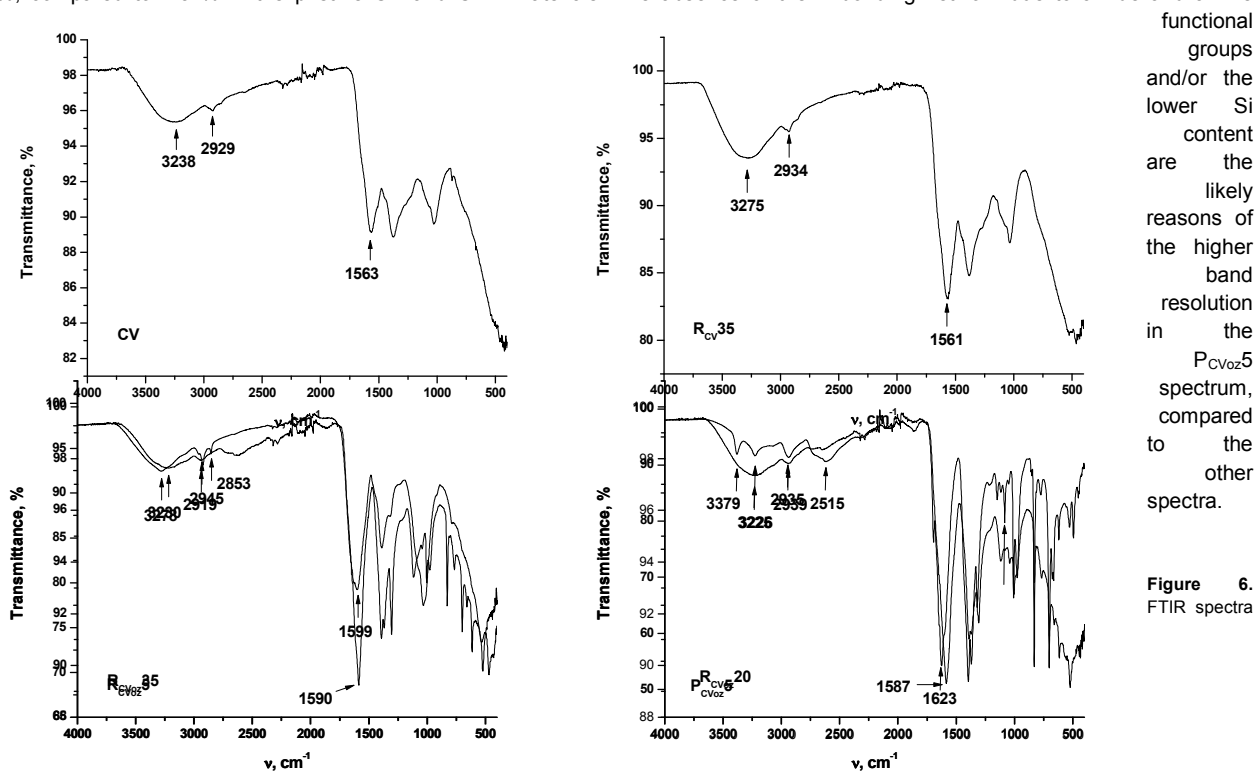
FTIR spectroscopy

The presence of aliphatic and/or aromatic C-H and C-C bonds in Table 1 and 2 products was confirmed by FTIR spectroscopy. Bands arising from these bonds vibrations fall in several regions of the infrared spectrum. However, for a correct interpretation of the spectra of these products, several other infrared absorptions must be taken in consideration.

Table 1 shows that crude pristine CV and CVD contain 33-37 % mineral matter. According to previous work,^[1] the organic matter of these materials is bonded to several mineral elements, including Si, Ca, Mg, Al, Fe, Na and K. These elements are presumably bonded to and/or complexed by the acid functional groups of the organic matter reported in Table 2. Metal-oxygen bonds vibration yield rather broad IR absorptions, covering most of the region below 1300 cm^{-1} . Silicon, representing over 50 % of the total mineral elements present in crude pristine CV and CVD, yields a broad IR absorption covering the region from 2000 to 850 cm^{-1} , with the peak absorbance at about 1050 cm^{-1} . The band arises from Si-O bonds vibration.^[49] All Table 2 products yield alkaline pH, when dissolved in water. Thus, no free COOH absorptions, but only COO⁻ absorption should be considered. The other possible carboxyl absorptions are those arising from amide functional groups. In addition, based on Table 2 data, absorptions arising from amine N-H and phenol O-H vibrations should be considered. Here in after, assignments of bands arising from vibrations of bonds in the organic matter are based on dedicated literature^[50] (see SI file).

Figure 6 and 7 report the FTIR spectra of the products listed in Table 2. All spectra contain broad bands above 2000 cm^{-1} . They arise from O-H and N-H stretching vibrations of functional groups engaged in H-bonding interactions. All spectra contain one or two weak absorptions, superimposed over the broad O-H and N-H absorption pattern. The weak absorptions confirm the presence of aliphatic hydrocarbon moieties. The differences of major diagnostic values are observed in the spectral region below 2000 cm^{-1} . It may be observed that, for crude pristine CV and CVD, their 35 kDa fractions R_{CV35} and R_{CVD35} , and the ozonized R_{CVoz35} and $R_{CVDoz35}$ fractions, three broad bands dominate the spectral region below 2000 cm^{-1} . The broad band at 1030 cm^{-1} is of little diagnostic value, since it is likely to arise mostly from metal-oxygen bonds vibration. The absorptions of these bands are likely to contribute^[49] background broadness over the entire spectral region below 2000 cm^{-1} . The presence and shape of the 1030 cm^{-1} band in the spectra of the R_{CV35} , R_{CVD35} , R_{CVoz35} , and $R_{CVDoz35}$ fractions indicates that Si is intimately bonded into a molecular assembly with molecular weight above 35 kDa. The two bands falling in the 1630-1375 cm^{-1} region are mainly due to the presence of carboxylates, amide and/or amine functional groups. The absorptions arising from these functional groups are also relatively broad, due to significant H-bonding interactions, which result in the presence of a broad range of C=O and N-H bond lengths. These functional groups are present in the exemplified virtual molecular fragments associated to the above products, which are shown in Figure 3. The C atoms bonded to these groups account for 16-28 % of the total fragment C atoms.

For the lower molecular weight ozonized fractions, the FTIR spectra (Figure 6 and 7) are more resolved. Sharp strong bands arising from paraffin and aromatic hydrocarbon moieties are well evident. Mostly relevant for these features is the P_{CVoz5} product. Due to the relatively low N content (Table 2), the contribution of amide and amine functional groups in the spectral pattern of P_{CVoz5} (Figure 6) is likely negligible. The same is true for the contribution of Si-O bonds. The Si content in P_{CVoz5} and P_{CVDoz5} was found 0.05 %, compared to 2.5 % in the pristine CV and CVD materials. The absence of the H-bonding network due to amide and amine



of fractions obtained from crude soluble ozonized CV by membrane filtration; abbreviations as in Table 1-2.

Based on the above chemical and spectral features, the bands below 1500 cm^{-1} in $P_{\text{CVOz}5}$ spectrum (Figure 6) can be reasonably assigned to C-H and C-C vibrations of hydrocarbon moieties. For instance, the strong doublet at 1395 and 1378 cm^{-1} is typical of C-H bending vibrations in isopropyl and tert-butyl functional groups.^[50] The sharp bands pattern in the $675\text{--}823\text{ cm}^{-1}$ region, is likely to arise from aromatic hydrocarbon moieties.^[50] These bands, and the broad strong band at 1623 cm^{-1} , are consistent with the presence of polycarboxylic acid salts. The $P_{\text{CVOz}5}$ fragment represented in Figure 3 depicts this situation. The fragment contains short chain C3-C5 atoms polycarboxylic moieties. The fragment implies also the presence of longer carbon chains, which may be visualized upon substituting H in a protonated aliphatic carbon atom with aliphatic C belonging to other molecular fragments. The FTIR spectra, compared to the results obtained from the ^{13}C NMR spectra (Table 2), confirm that these C moieties are present in relatively higher concentration in the lowest molecular weight ozonized fractions, but are poorly detected by ^{13}C NMR spectroscopy, due the fact they are largely constituted by highly substituted and/or quaternary C atoms.

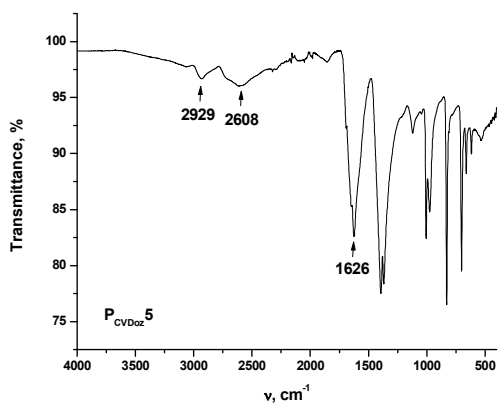
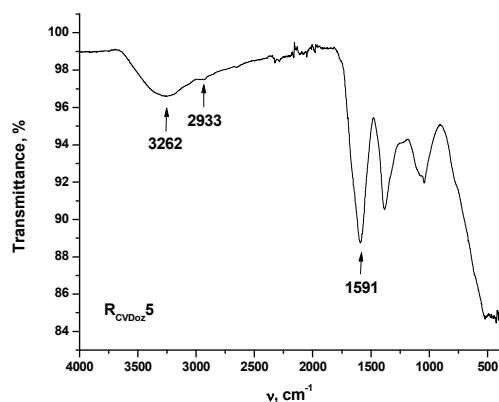
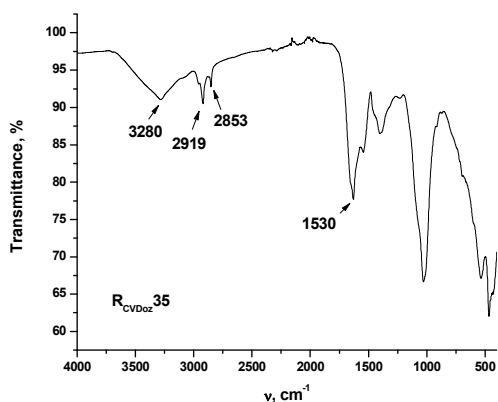
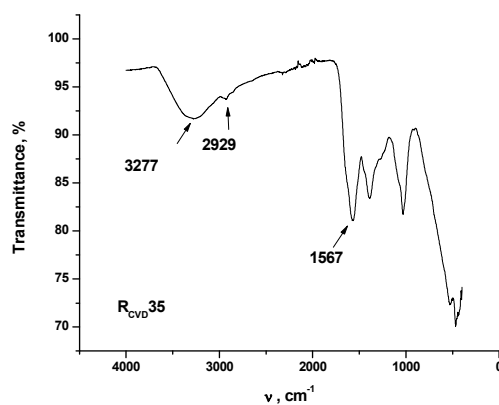
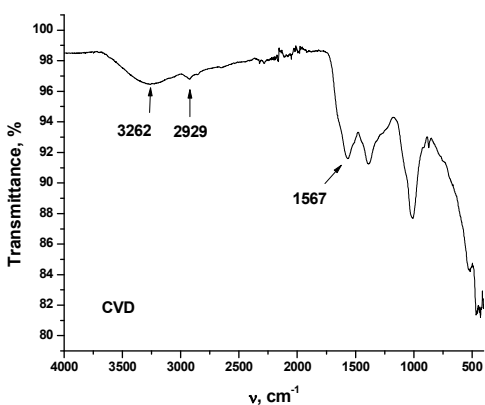


Figure 7. FTIR spectra of fractions obtained from crude soluble ozonized CVD by membrane filtration; abbreviations as in Table 1-2

1H NMR spectroscopy and GC-Mass spectrometry

The spectra of the soluble ozonized CVD and CV were recorded in D₂O solutions containing 20 % dry matter sample. There was no significant difference in the spectra of the ozonized fractions of CV and CVD isolated from membrane filtration with equal molecular cut off. The spectra are reported in Figure 4S (see SI file). All spectra exhibit low intensity patterns. This indicates that the products contain molecules made by C chains with low H content. Signals arising from protons bonded to aliphatic C appear in the 0-5.5 ppm chemical shift range. No signals are present at higher chemical shift, which could arise from protons bonded to aromatic C. The aliphatic proton signals appear as sharp singlets and unresolved multiplets. The singlets, mostly falling above 2 ppm, arise from protons not coupled to neighboring protons. Broad unresolved multiplets, mostly falling below 2 ppm, arise from protons coupled to other neighboring protons, such as in protonated long C chain.^[50] The spectra in Figure 4S show that the relative contribution of the multiplet signals to the spectral pattern decreases, upon decreasing the product molecular weight. Yet, the broad unresolved multiplets are still evident in the 5 kD permeate P_{CVoz5} fraction.

In order to obtain further insight into the nature of the lowest molecular weight ozonized fractions, the P_{CVoz5} and P_{CVDoz5} fractions were methylated at room temperature, and at 100 °C in the presence of HCl by microwave heating for 40 minutes. The methylated products were extracted in chloroform, and the solvent was evaporated to obtain a viscous liquid residue, which was weighed and then analyzed by gas chromatography (GC)-mass spectrometry (MS), as reported in the Experimental section of the SI file. The liquid residue obtained from the room temperature methylation amounted to 0.5 % of the starting product weight. It contained only methyl stearate. The liquid residue obtained from microwave assisted acid methylation at 100 °C amounted to about 85 % of the starting product weight.

Table 1S in the SI file reports the products and relative % peak areas, which were determined by GC-MS analysis of the viscous liquid obtained from the microwave assisted acid methylation of the P_{CVoz5} and P_{CVDoz5} fractions at 100 °C. The data shows that the identified products account for 69-73 % of the total chromatogram peaks' area. The identified products are grouped into five categories and listed in the order of decreasing total groups peak area %, relative to the sum of all peaks' areas measured in the chromatogram. The 85 % total yield of these products, relative to the weight of the P_{CVoz5} and P_{CVDoz5} fractions before methylation, is consistent with the 81 % recovered mass reported in Table 1, which was obtained in the molecular weight measurements of the P_{CVDoz5} and P_{CVoz5} fractions by size-exclusion chromatography. The fact that the products listed in Table 1S are obtained only by P_{CVDoz5} and P_{CVoz5} prolonged microwave assisted methylation in acid medium at 100 °C, and not by room temperature methylation, confirms that Table 1S compounds are products of the methanolysis and/or hydrolysis of the complex polymeric P_{CVDoz5} and P_{CVoz5} fractions, carried out under drastic conditions.

The methylated products, which are listed in Table 1S, confirm that the P_{CVDoz5} and P_{CVoz5} fractions contain highly substituted aliphatic and aromatic C acid moieties, together with long aliphatic C chains. To account for the P_{CVDoz5} and P_{CVoz5} molecular weight reported in Table 1, it must be admitted that Table 1S compounds represented the memories of the C moieties bonded to each other in the macromolecular pools with 14.8 and 4.3 kDa weight average molecular weights, which are reported for the P_{CVDoz5} and P_{CVoz5} fractions, respectively. The exemplified P_{CVDoz5} virtual fragment shown in Figure 4 accounts for the presence, and somehow for the relative abundance, of the carbon moieties that may have originated Table 1S compounds upon methylation.

Consistency and complementarity of collected product characterization data

The low intensity of the ¹H NMR spectra (Figure 4S) is consistent with the observations made on basis of the ¹³C NMR spectra (Figure 5). In essence, both ¹H and ¹³C NMR spectra indicate that the ozonized products are likely to be largely constituted by molecules containing condensed aromatic rings and quaternary aliphatic C atoms moieties. On the other hand, the ¹H NMR spectra (Figure 4S) and the GC-MS data (Table 1S) are well consistent with the results obtained by FTIR spectroscopy. They confirm that the P_{CVDoz5} and P_{CVoz5} fractions contain also protonated C chains moieties. The potentiometric titration data show that these C moieties contain acid groups of different strength. The data obtained by size exclusion chromatography coupled to multi-angle laser light scattering measurements (Table 1) demonstrate that the above C moieties belong to macromolecules with weight average molecular weights ranging from 4.3 to 487 kDa.

Potential uses and economic sustainability of soluble ozonized products

A major expectation from the present work was bleaching crude pristine CV and CVD by ozonization. The aim was obtaining products that, compared to the pristine materials, were lighter colored, but exhibited similar or improved surfactant properties. The results of the work show that all ozonized product soluble fractions appeared lighter colored than crude pristine CV and CVD. Thus, all ozonized soluble products were tested for their capacity to decrease the surface tension of water, in comparison with crude pristine CV and CVD. Measuring the water surface tension in the presence of added product concentration is the first step to qualify a product as surfactant.

For crude pristine CV and CVD, the water surface tension versus added product concentration plots were available from previous work.^[4] The plots allowed assessing the critical micellar concentration of the two crude pristine CV and CVD occurring at added product concentration of about 2 g L⁻¹. Thus, in the present work, crude pristine CV and CVD, their R35 fractions, and the fractions obtained from the crude soluble ozonized products, were tested for their surface

tension activity at 2 g L⁻¹ concentration. At this product concentration, all solutions had pH near or above 8. The experimental values of the surface tension measured under these conditions are reported in Table 2. The data shows that only the R_{CVoz35} and R_{CVDoz35} fractions give lower surface tension values than crude pristine CV and CVD. The surface tension value of the solutions containing the ozonized fractions with lower molecular weight is nearly equal to the value for plain water. The results are well consistent with the chemical nature of the ozonized products fractions. The less oxidized R_{CVoz35} and R_{CVDoz35} exhibit good surfactant properties, by virtue of the presence of lipophilic and hydrophilic C moieties in proper ratio. The lower molecular weight ozonized fractions, having higher content of hydrophilic acid groups and lower content of aliphatic and aromatic C moieties, do not exhibit surfactant properties. Nevertheless, due to their high content of aliphatic polycarboxylic acids, and/or hydroxy- or amino-carboxylic acid polymeric moieties, the above ozonized fractions with lower molecular weight may represent a valuable source of soluble oxidized organic matter deserving further research work to assess its potential valorization.

While being attracted by this research scenario, one should also wonder about the economical sustainability of the proposed processes and products. The production cost of the crude pristine soluble CVD, obtained by hydrolysis of the sourcing compost, has been estimated 0.1-0.5 € product dry matter kg⁻¹.^[2] Much work has been carried out to assess performance in multiple uses^[3-18] and economic sustainability for this product. Two very recent papers have assessed interesting economic revenue from investing in the compost hydrolysis facility to produce the soluble CVD for use in agriculture^[16] and in ecofriendly biowastes' anaerobic fermentation processes.^[51] A business model with reduced entrepreneurial risk has also been proposed, which comprises a virtuous bio-waste cycle to produce biogas without ammonia and speciality bio-based chemicals.

For the ozonization reaction, a most recent paper^[52] reports a cost figure of 0.135 € per kg of generated ozone; ozone generator equipment maintenance costs not included. The cost of the ozonisation of the crude pristine SBO may be estimated based on the relevant reaction and product data. For example, for CVD these are: (i) the 3.7 O₃/crude pristine CVD C mol/mol ozone consumption (see Ozonization reaction section), (ii) the 42.7 % C/C mol/mol yield of crude ozonized soluble CVD, relatively to crude pristine soluble CVD, and (iii) the 16.6 % C content in the crude ozonized soluble CVD dry matter (see Experimental section in SI file). From this data, the cost of ozone consumption, referred to the produced crude ozonized soluble CVD, results 4.7 € per kg of product carbon and about 0.8 € per kg of product dry matter (Table 3).

The above cost figures for the production of the ozonized SBO do not mean much, unless they were compared with the potential market value of the product. Data is available for products with features similar to those represented in Figure 3. Table 3 reports also cost figures for some selected commercial biobased products, which can presumably be comparable with the ozonized products in Figure 3 for chemical features and properties. For example, the potential market value for phenol derivatives obtained from lignin-based products is reported in the 1.1-2.5 € per kg range.^[53] Some special compounds can be sold at much higher prices. Syringol is commonly used in the flavor and fragrance industry and sold for about 25-30 € per kg.

Table 3. Crude ozonized soluble CVD cost, based on ozone consumption experimental data (see Ozonization reaction section and Experimental section in SI file) and market price for selected most comparable commercial biobased products.

O ₃ generation cost, ^[52] € kg ⁻¹	0.135
O ₃ w/w consumption, relative to crude ozonized soluble CVD C	34.6
O ₃ consumption cost per crude ozonized soluble CVD C, € kg ⁻¹	4.7
O ₃ consumption cost per crude ozonized soluble CVD dry matter, € kg ⁻¹	0.8
Potential market value for phenol derivatives obtained from lignin-based products, ^[53] € kg ⁻¹	1.1-2.5
Syringol market price, ^[53] € kg ⁻¹	25-30
Rhamnolipid biosurfactants, ^[55] € kg ⁻¹	30-150
Rhamnolipid biosurfactants, ^[56] € kg ⁻¹	30-150

Market prices per product kg may be even much higher for some high performance biodegradable surfactant molecules, such as rhamnolipids.^[54] These products are a class of glycolipids produced by *Pseudomonas aeruginosa*, amongst other organisms, frequently cited as the best characterized of the bacterial surfactants. The four most important rhamnolipids comprise either one or two rhamnose molecules attached to one or two β-hydroxydecanoic

acids. Figure 8 represents the structure of a rhamnolipid with two rhamnose and two β -hydroxydecanoic acid units. These wander molecules lower the surface tension of water from 70 down to 28 mN m⁻¹ at the critical micellar concentration of 0.8-2 g L⁻¹. Based on 2007 prices, the cost of the surfactant in a washing solution at the critical micellar concentration was calculated to vary from 0.02 to 0.23 £ per liter of solution,^[55] which correspond to about 30 to 150 € per kg of surfactant at the 1.3 € £⁻¹ exchange rate. In 2014, the current market price of rhamnolipids was reported \$227/10 mg (Sigma-Aldrich) and \$200/10 mg (AGAE technologies, USA).^[54] A most recent 2016 quotation is US \$ 650-800 per 50 grams,^[56] which is equivalent to 586-720 € per 50 grams based on 0.90 € \$⁻¹ exchange rate.

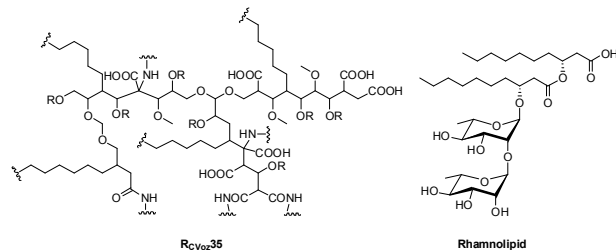


Figure 8. Example of Rhamnolipid structure compared to the ozonized R_{CVoz}35 fraction in Figure 3.

The cost figures and the list of biobased products reported in Table 3 are not exhaustive. Commercially relevant quotes for these materials are certainly available from dedicated publications and sources of chemical pricing. Given the preliminary exploration nature of the experimental work reported here, a detailed accurate cost and potential market price comparison of the soluble ozonized products with commercial biobased products was not the main scope of the present work. Although uncomprehensive, the data in Table 3 points out that the product sustainability depends much on its market value, and thus on its performance in the intended application. It is obvious that adding value adds costs. In the case of the crude pristine soluble CVD and CV, their conversion to upgraded biosurfactants, as the R_{CVoz}35 product fraction represented next to the rhamnolipid high performance surfactant in Figure 8, requires ozonization, and thus adds further product costs. Ozone generation is an important drawback considering two factors: its current production cost and the large amount that the process needs. Technological advances are steadily reducing ozone production costs.^[52] For the specific case of the products obtained in this work, at the current stage of research it is not possible assessing definitely process/product sustainability. First, no optimization of the ozonization reaction has been attempted. Secondly, the ozonized products' uses, performance and market value need to be established. Nevertheless, based on the market value of the above commercial phenol and bacterial surfactants, the ozonization cost for the production of Table 2 products appears sustainable, provided that the ozonized products matched the performance of the commercial products.

The vis a vis comparison of the rhamnolipid structure and the R_{CVoz}35 virtual molecular fragment (Figure 8) shows that the two products have most C moieties in common. These are the long aliphatic C chains, the carboxylic groups, and the anomeric C. The anomeric (OCO) C and the OR carbon in R_{CVoz}35 may indicate the presence of carbohydrate moieties. According to Table 2 data, the OR/OCO C ratio for R_{CVoz}35 is 4, which might indicate the presence of substituted pentose and/or hexose moieties. However, based on Table 2 data, R_{CVoz}35, compared to the rhamnolipid in Figure 8, has from 2 to 3 fold more carboxyl groups per total C atom, and much less possible carbohydrate moieties. The different hydrophilic/lipophilic balance between the two products is likely to be responsible of the different surface activity properties. Indeed the surface tension values of the rhamnolipid^[57] and R_{CVoz}35 (Table 2) at the same 2 g L⁻¹ concentration in water are 28 and 48.5 mN m⁻¹, respectively. The comparison of the above cost figures, market value, chemical features and surface activity properties between the two products justifies further research efforts for the development of biosurfactants by chemical hydrolysis and/or ozonization of as collected or previously fermented biowastes. A real breakthrough would be improving the surface activity properties of the high molecular weight ozonized R_{CVoz}35 and/or R_{CVoz}35 to match the rhamnolipid biosurfactant properties. There is a long way to go. Nevertheless, the authors feel that it is worth exploring it.

Conclusions

The analytical work carried out and the production of fractions with improved surfactant properties is the main novelty achieved in the present work. The concept of ozonization is not new (see Introduction section). However, ozonolysis to valorize lignin-like material recovered from composted and chemically hydrolyzed municipal biowastes is reported here for the first time. The subject is highly relevant, upon considering the large worldwide easy availability of municipal biowastes as source of valuable organic matter. The authors are aware that the feedstock used in the present work has a very complex nature and its composition varies significantly across locations and seasons. Moreover, ozonization generates a huge range of diverse products. These features are common to all renewable feedstocks and/or to most

oxidation reactions. They represent important criticalities for scaling up research results to commercial level. All processes intended to valorize renewable sources of products must cope with them. On the other hand, if properly exploited, the feedstock and products variability may represent a great opportunity to widen the range of marketable biobased products for use in place of synthetic chemicals.

It is shown here that a wide range of bio-polymeric products can be obtained by ozonization of two materials sourced from two different hydrolyzed municipal biowaste composts, i.e. the crude pristine CV and CVD compost hydrolysates. These have been obtained from as collected urban biowastes in three steps. In the first step, the biochemical oxidation of the solid biowaste yields a compost. In the second step, the compost is hydrolyzed in alkaline water. In the third step, the compost soluble hydrolysate is ozonated. In the first composting step, the biodegradable proximates of the as collected biowaste are mostly mineralized. The recalcitrant lignin is likely to be partly oxidized. However, the aromatic moieties are recalcitrant to biochemical oxidation, and the product is black. The compost hydrolysis is not likely to affect the chromophore moieties. It breaks hydrolysable bonds between aromatic moieties and yields some soluble high molecular fragments, which are still black. The chemical oxidation by ozone destroys the aromatic chromophore moieties, and yields lighter colored and/or uncolored products.

The results (Figure 1 and Table 2) point out that the nature of pristine biowaste material affects the yield and chemical composition of the ozonized products. The data also shows that the ozonized product soluble fractions, which are obtained from the two crude pristine CV and CVD materials through membrane filtration with the same molecular cut off, have different chemical distribution of acid functional groups. These findings prospect that, through integration of biochemical and low temperature chemical technology applied on different biowastes, the native lignin present in the biowaste may be converted to a wide variety of potentially value added products. The ozonization of the soluble hydrolysate obtained in the above step 2 has to be optimized, in order to minimize ozone consumption and to improve the reaction selectivity to the desired product. Another interesting research approach would be performing the hydrolysis and ozonization reactions in one step, both on the biowaste before and after composting. Certainly, much more work is necessary to assess the sustainability of the reported processes and products, and to explore their full development potential. The authors feel that, within its limitations, the present work on the ozonisation process to upgrade SBO is a step forward in this direction.

Supporting Information summary

The Supporting Information file contains experimental section, literature data for FTIR bands assignments, Figure 1S through 4S, and Table 1S and 2S.

Abbreviations

Af Aliphatic carbon

Ar Aromatic carbon

COY carboxyl carbon

CV Soluble biobased substances isolated from the alkaline hydrolysate of composted home gardening and park trimming residues

CVD Soluble biobased substances isolated from the alkaline hydrolysate of a composted mix of home gardening and park trimming residues and digestate of the anaerobic fermentation of the municipal waste humid organic fraction

δ Chemical shift of nuclear magnetic resonance signal

γ Water surface tension

GC-MS Gas chromatography-mass spectrometry

MN and MW Number and weight average molecular weights, respectively

NMR Nuclear magnetic resonance

NR Amine carbon

OCO Anomeric carbon

OMe Methoxy carbon

PCVD5 and PCV5 fractions isolated from the permeates obtained by ultrafiltration of crude pristine CVD and CV through membrane 5 kDa off, respectively

PCVDoz5 and PCVoz5 fractions isolated from the permeates obtained by ultrafiltration of crude soluble ozonized CVD through membrane 5 kDa cut off, respectively

RCVD35, RCVD20, RCVD5 fractions isolated from the retentates obtained by ultrafiltration of crude pristine CVD through membranes with 35, 20 and 5 kDa cut off, respectively

RCV35, RCV20, RCV5 fractions isolated from the retentates obtained by ultrafiltration of crude pristine CV through membranes with 35, 20 and 5 kDa cut off, respectively

RCVDoz35, RCVDoz20, RCVDoz5 fractions isolated from the retentates obtained by ultrafiltration of crude soluble ozonized CVD through membranes with 35, 20 and 5 kDa cut off, respectively

RCVoz35, RCVoz20, RCVoz5 fractions isolated from the retentates obtained by ultrafiltration of crude soluble ozonized CV through membranes with 35, 20 and 5 kDa cut off, respectively

RO Alkoxy carbon

SBO soluble bio-based substances

SEC-MALS Size exclusion chromatography-multiangle laser light scattering

VS Volatile solids

X Weak acid functional groups as identified in Table 2.

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