URBAN WASTES AS SOURCES OF VALUABLE CHEMICALS FOR SUSTAINABLE DEVELOPMENT: SURFACTANTS, DISPERSING POLYMERS AND POLYELECTROLYTES OF BIOLOGICAL ORIGIN

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ABSTRACT

Following previous work indicating urban wastes to be sources of valuable products with remarkable surface activity properties, a new humic acid-like (HAL) substance (cHAL3) was isolated from green residues composted for 7 days. This material was characterized for its chemical composition by elemental analysis, nuclear magnetic resonance and infrared spectroscopy, and potentiometric titration of carboxylic and phenol groups, for its surface activity properties in aqueous solution at pH 7 and for its molecular weight. The results indicate that cHAL3 has good surface activity properties mostly due to molecules with molecular weight up to 1 kg/mol; it also contains larger ionic polymeric molecules or aggregates with molecular weight up to 267 kg/mol. In the attempt to obtain evidence for possible source–structure–properties relationships, cHAL3 was compared with other previously reported HAL substances (i.e. cHAL2) isolated from the same green residues before composting and cHAL isolated from a mix of kitchen and green residues composted for 15 days. The results show that cHAL3 and cHAL2 have slightly different chemical compositions but similar surface activity properties in aqueous solution at pH 7 (i.e. critical micellar concentration (cmc) 0.91–0.97 g/L and surface tension at the cmc (γ_{cmc}) 38 mN/m). By comparison, the cHAL biosurfactant is less polar and less hydrophilic and exhibits enhanced surface activity (i.e. cmc = 0.40 g/L and γ_{cmc} = 36 mN/m). Thus, composting in the 0–7-day range does not appear to result in chemical composition changes in the isolated HAL substances, which can significantly affect surface tension properties. However, changing both the biomass nature and the composting time seems to affect significantly the surface activity and the content of ionic functional groups in the humic-like isolates. The results offer scope for a long-range process and product development study aiming to the valorization of biomass wastes as source of high-value chemical auxiliaries.
Keywords: compost, humic acid, surfactants, wastes recycling.

1 INTRODUCTION

At present, the valorization of lignocellulosic materials (LCM) constitutes a new frontier of economically sustainable and environmentally friendly processes for the production of fuels and chemicals [1–3]. The environmental advantages of using LCM in place of fossil sources of organic carbon are readily envisioned in the lowering of CO_2 emission. The economic sustainability of using these materials needs, however, the solution of a number of problems. Biomasses as a source of fuel and chemicals may be available as dedicated crops (DC) and lignocellulosic wastes (LCW). The cultivation of plants for energy and chemical uses raises socioeconomic and moral concerns due to the subtraction of land for food supply, the expected price increase in agriculture and the rather uneven food distribution throughout the world [4]. On the contrary, the use of wastes, such as urban organic humid and agricultural residues, is looked upon as a promising approach to the struggle for a cleaner environment as well as for rational use of space. Both LCW and DC, however, share the problem of being high entropy sources of organic C due to the high amount of water content and/or the low spatial mass density. One very recent trend to face cost competition from fossil sources is the development

of biorefineries [2, 3]. These installations fed with LCM are conceived based on the same principle and/or technology of oil and coal refineries, i.e. to produce multiple products, including high-value chemicals as well as fuels and power, by use of enzymatic processes or thermochemical treatments such as pyrolysis and/or gasification to syngas. Although fascinating, these approaches must overcome several critical points related to low product yield and/or to process high energy consumption. In this context, composted urban wastes have rather peculiar features. They are low entropy systems and contain high concentrations of humic-like substances (HLS) with interesting surfactant properties [5, 6]. Furthermore, HLS can be extracted using rather mild process conditions. In our previous work [7], we have pointed out that composts are available in confined spaces and contain less water (35–55%) and more organic matter (26–50%) than the fresh starting LCW. Furthermore, composting does not demand any external energy supply and is carried out nowadays in public and private facilities throughout the world. We have also reported very interesting surfactant properties for two humic-like materials, cHAL [5] and cHAL2 [6], which were respectively, isolated in 10–12% yield from a food kitchen–urban green residues mix composted for 15 days and from sole urban green wastes before composting. These products have been shown to perform as well as major commercial synthetic surfactants in a few important chemical technological applications such as textile dyeing [7], fabric washing [8] and soil remediation [9]. Since composting is likely to yield a wide range of different products depending on the initial wastes mix and on the composting time [10, 11], we have undertaken a long-range process and product development project funded by Italian Regione Piemonte. The project aims to isolate products from biomass wastes and to establish source–structure–properties relationships for products to be recycled to the market as valuable chemical auxiliaries. This paper investigates the chemical nature and the surface activity of a new biosurfactant hereinafter named cHAL3. This material was isolated from urban green wastes after 7-day composting. The product is compared to the previously reported cHAL and cHAL2 isolates that were extracted from biomasses differing in composition and/or composting time. Hence, the comparison between the above three materials can provide hints for understanding changes of chemical composition and/or structure for HLS isolated from different biomasses.

2 EXPERIMENTAL PROCEDURE

All reagents were Aldrich products, unless otherwise indicated. Ground urban green wastes from separate source collection in the city of Torino (Italy), consisting of public park trimmings and private yard gardening residues, were collected after 7 days composting and extracted according to a known procedure [5] to yield the humic acid-like (HAL) material (cHAL3) investigated in this work. The above compost was treated for 24 h at 65°C under N_2 with aqueous 0.1 mol/L NaOH and 0.1 mol/L $Na_4P_2O_7$ at 1:50 w/v compost/solution ratio. The resulting suspension was cooled to room temperature and centrifuged at 6000 rpm for 20 min. The supernatant solution was separated. The solid residue was washed repeatedly with distilled water until the supernatant liquid phase was clear. All collected liquid fractions were mixed and acidified with 50% sulfuric acid to pH < 1.5. The precipitated cHAL3 fraction was separated by centrifugation as above, washed with water until the final washing had neutral pH, vacuum dried at 60°C and weighed. The extraction yield for the final cHAL3 product was 12% of compost dry matter. This material was found to contain 6.3% water, 91.7% volatile solids and 1.9% ash by the weight losses measured after heating first at 105°C and then at 800°C. Further characterization for cHAL3 was performed by elemental analysis, solid state ^{13}C and solution ^1H nuclear magnetic resonance (NMR) spectroscopy, infrared (IR) spectroscopy and surface tension measurements as previously reported [5, 6]. The determination of the proportion of free phenol and carboxylic acid groups (Table 1) was accomplished by potentiometric titration according to a previously reported procedure [6, 12]. Under our experimental conditions, deionized water was boiled under

Table 1: Data[a] for HAL matter isolated from urban wastes under different composting conditions: cHAL3 isolated in this work and cHAL2 [6] and cHAL [5] from previous work.

| Compost | Substance | C (w/w %) | Empirical formula | | | | | pH | COOH (meq/g) | ArOH (meq/g) |
			C	H	N	O	S			
Kitchen–green wastes after 15 days	cHAL [5]	59.9	10	13.4	0.86	3.4	0.036	4.00	1.10	1.90
Green wastes at start	cHAL2 [6]	57.9	10	12.6	0.63	3.0	0.018	3.96	2.91	0.87
Green wastes after 7 days	cHAL3	56.5	10	11.0	0.78	2.9	0.026	4.00	3.00	2.50

[a]C content (w/w %) in ash-free dry matter, elements/atoms in empirical formula, pH of a water suspension (12 mg/30 mL) containing 0.01 N NaCl, free carboxylic (COOH) and phenol (ArOH) groups by potentiometric titration.

nitrogen to remove dissolved CO_2. This water was used to make the required sample and reagent solutions. The cHAL3 sample was dissolved at 0.6 g/L concentration in 1 N KOH. The total alkali content in the solution was in excess relative to the total cHAL3 carboxyl and phenoxide content (Table 2) determined by ^{13}C NMR spectroscopy. This solution with pH about 13.8 was titrated with standardized 1 N aqueous HCl. Similar titration was performed on a blank solution containing the same amount of alkali as the above sample solution, but no cHAL3. The titrations were performed at 25°C using an automatic Cryson Compact Titrator with a resolution of 1 μL of titrant in a thermostated glass cell under nitrogen blanketing to prevent dissolution of atmospheric CO_2 in the sample. ^1H NMR spectra in 3 M NaOD-D_2O solution were recorded on a JEOL EX 400 (B0 = 9.4 T, ^1H operating frequency = 399.78 MHz) spectrometer. A standard presaturation sequence was used in order to delete the proton signal arising from the water protons. Solid-state ^{13}C-NMR spectra were acquired at 67.9 MHz on a JEOL GSE 270 spectrometer equipped with a Doty probe. The cross-polarization magic angle spinning (CPMAS) technique was employed and for each spectrum about 10^4 free induction decays were accumulated. The pulse repetition rate was set at 0.5 s, the contact time at 1 ms, the sweep width was 35 KHz and MAS was performed at 5 kHz. Under these conditions, the NMR technique provides quantitative integration values in the different spectral regions [13]. Chemical shifts of NMR resonances are referred to tetramethylsilane. The HAL materials were characterized for their distribution of organic C and functional groups that were obtained according to the assumption underlying the following equations: PhOR = PhO- – PhOH (PhO- calculated from Table 2, PhOH from Table 2, R = alkyl); CON = COX – COOH, (COX calculated for carboxyl in Table 2 COOH from Table 1); N-alkyl = N – CON (N from Table 1 and CON as above); OCH_3 = Z – N-alkyl (Z calculated from total methoxy and N-alkyl carbon in Table 2).

Surface tension (γ, mN/m) measurements of aqueous solutions containing cHAL3 at variable concentration (Cw = 0.005–2 g/L) were carried out at 25°C with a Kruss K100 automatic tensiometer as described in previous work [5]. IR spectra were recorded on a cHAL3 sample-KBr pellet using a FTIR-8400 Shimadzu (Shimadzu, Japan). Dynamic light scattering (DLS) measurements were obtained with a ZetaSizer® (Malvern, UK), which has a detection window included between about 0.6 nm and 5 μm. The measurements were conducted at pH 7.0 and 25°C after filtering the cHAL3 sample solution on a cellulose acetate disk (Schleicher & Schuell) with a size cut-off of 0.8 μm. The

Table 2: Assignments by chemical shift (δ, ppm) ranges and relative area of ^{13}C CPMAS NMR bands and C distribution per molecular fragment containing two aromatic rings.

| Assignment | Aliphatic C bonded to other aliphatic chain or to H | | Total aliphatic C | O-CH$_3$ or N-alkyl C | O-Alkyl C | Di-O-alkyl C | Aromatic C | Phenoxide (PhO-), C | Carboxyl C | Keto C |
	Short chain	Long chain								
Band δ range (ppm)	0–32	32–53	0–53	53–63	63–95	95–110	110–140	140–160	160–185	185–215
cHAL										
Band relative area (%)	32.3	12.9	45.2	8.4	9.2	3.5	14.8	6.8	11.5	0.6
C distribution for two aromatic rings	18.3	7.3	25.6	4.7	5.2	2.0	12.0		6.5	0.3
cHAL2										
Band relative area (%)	36.0	13.6	49.5	6.5	11.5	3.6	9.1	7.4	4.6	5.1
C distribution for two aromatic rings	30.4	9.5	39.9	5.5	9.7	3.0	12.0		6.2	3.9
cHAL3										
Band relative area (%)	28.0	14.2	42.2	7.9	13.2	4.0	12.3	5.1	9.5	4.0
C distribution for two aromatic rings	20.0	10.1	30.1	5.6	9.4	3.0	12.0		6.8	2.8

CONTIN method [14] was used to analyze the DLS data for calculating the hydrodynamic diameter (D_H) of the molecules or aggregates in solution. Electrospray ionization measurements mass spectrometry (ESI-MS) analyses were performed using a LTQ Orbitrap high-resolution spectrometer (Thermo, Rodano, Italy), with electrospray interface and ion trap as mass analyzer. The flow injection effluent was delivered into the ion source using nitrogen as sheath and auxiliary gas (flow rate 10 µL/min). The source voltage was set at 4.0 kV in the negative ion mode. The heated capillary was maintained at 270°C. The tuning parameters adopted for the ESI source were the following: source current 100 µA, capillary voltage ±9 V and tube lens ±100 V. Mass spectra were collected in full-scan positive and negative mode in different ranges between 200 and 1500 m/z. Molecular investigation was performed also through fractionation and characterization by a multi-angle light scattering (MALS) detector on-line to a size exclusion chromatography (SEC) system. The SEC-MALS system consisted of an Alliance 2690 separation module, a 2414 differential refractometer (DRI) from Waters (Milford, MA, USA) and a MALS Dawn DSP-F photometer from Wyatt (Santa Barbara, CA, USA). This multi-detector SEC-MALS system was described in detail previously [15]. The wavelength of the MALS laser was 632.8 nm. The light scattering signal was detected simultaneously at 15 scattering angles ranging from 14.5° to 151.3°. The calibration constant was calculated using toluene as standard, assuming a Rayleigh factor of 1.406×10^{-5}/cm. The angular normalization was performed by measuring the scattering intensity of a concentrated solution of bovine serum albumin globular protein in the mobile phase assumed to act as an isotropic scatterer. The refractive index increment, dn/dc, with respect to the solvent was measured by a KMX-16 DRI from LDC Milton Roy (Riviera Beach, FL, USA). The dn/dc value for cHAL3 was 0.214 mL/g. The HAL substance dissolution procedure and the SEC experimental conditions were quite similar to those previously reported [16]. The starting sample solution contained cHAL3 at 1 g/L concentration in 0.01 M K_2HPO_4–0.01 M KH_2PO_4 aqueous buffer (pH 7.0) containing 10% methanol. The same solvent was used as SEC mobile phase under the following conditions: single aqueous Shodex OHpak KB805 column from Showa Denko (Tokyo, Japan), 35°C temperature, 0.8 mL/min flow rate and 100 µL sample injection volume.

3 RESULTS AND DISCUSSION

In this paper, we report the data obtained for a new HAL substance, cHAL3, next to the data obtained for previously reported HAL substances, cHAL [5] and cHAL2 [6]. To understand compositional and structural differences between materials isolated from composted wastes, one should first consider that composting is an aerobic biodegradation process of refuse biomass, leading to some mineralization of the original organic C and N and chemical modifications of the remaining organic residue [6]. Compared to the starting biomass waste, the composted waste is generally characterized by a lower content of polysaccharides and a relatively higher concentration of lignin-like material. Changes also involve chemical identities. Native lignin is modified to lignin–humus. The latter material, although not well defined, is usually characterized by the aliphatic/aromatic C ratio and by the content of carboxylic and phenolic functional groups. The HAL organic fraction is separated from the polysaccharide, protein, fats and other humic-like matter by extraction of the starting biomass with alkali and precipitation of HAL at pH<1.5. Compost products are affected by process parameters such as the nature and source of the starting biomass and the composting time. In the case of the HAL substances investigated in this work, composting was carried out by making 300 m³ piles of food residues and/or urban green wastes, which were turned over once a week to guarantee biomass aeration. A pile of this size allows the microbial degradation to occur during the thermophilic phase where the heat generated by the biochemical reactions causes the pile temperature to raise up to about 65–70°C and then to decrease back to the ambient temperature during 4 weeks. Whereas this

time lag is normally sufficient to achieve an environmentally acceptable biochemical stability and volume reduction of the organic wastes, from our point of view, the optimum composting time had to be defined only in relation to the products that could be expected and used for the intended application.

In this work, the cHAL2 and cHAL3 substances originate from the same green urban waste sampled before (i.e. at the time the composting pile was made) and after 7 days composting. The comparison of these two materials allows determining the effect of the composting time on the chemical nature and properties of the HAL substance contained in the urban green biomass waste. The cHAL material originates from a 1:1 w/w mix of urban organic humid kitchen wastes and green residues after 15 days of composting. The comparison of this material with the other two materials (cHAL2 and cHAL3) does not allow to separate the effect of the nature of the biomass waste from that of the composting time. Nevertheless, it helps to indicate what type and level of changes should be expected in the HAL isolate from changing both source and process conditions. Indeed, for real commercialization of products of biological origin, it is most important not only to determine their properties and level of performance in the proposed applications but also to assess the sensitivity of properties and performance to the changes in chemical composition caused by the source and the process variability.

Many plausible virtual molecular models are reported in literature to aid understanding source–structure–properties relationships for polymeric HLS. These models contain several organic moieties that represent the memory of the main molecular constituents of the starting biomass left over by the biodegradation of polysaccharides, proteins, lipids and lignin. Many of these models are based on the monomer concept of Flag [5] using an oxyphenylpropyl (OPhPr) unit as a building block. Figure 1 reports one model specifically proposed for our previously reported cHAL material [5]. Although accounting satisfactorily for the experimental analytical data reported in Tables 1 and 2 for cHAL, this model remains virtual. In reality, several questions remain unsolved for HLS, a major one being the rather wide molecular weight distribution. Thus, a range of different molecular fragments and molecules with greater and lower molecular weight than that of the fragment represented in Fig. 1 is likely to compose cHAL. Also, other representations combining the same organic moieties in several other ways could certainly be drawn and fit the experimental data as well. Nevertheless, we like to
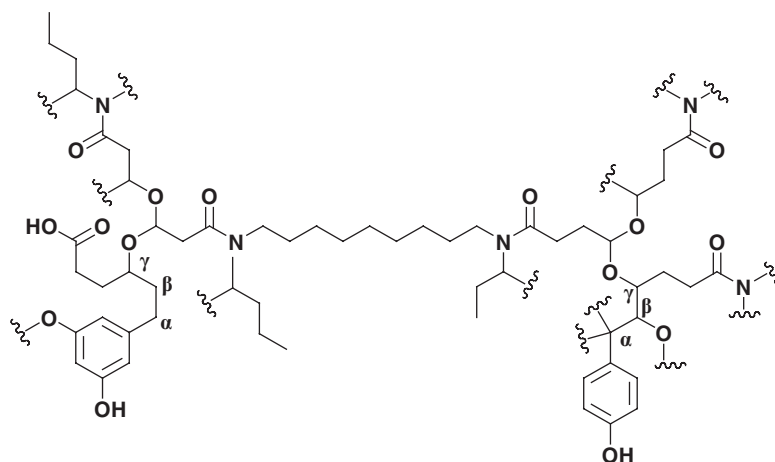

Figure 1: Proposed molecular fragment for cHAL [5]. H bonded to C omitted; sinusoidal bold lines indicate other fragments (C, O, N atoms).

show here again the model in Fig. 1 because we feel that the conversion of a set of numbers as in Tables 1 and 2 into a graphical, although virtual, representation helps the reader to have a longer memory of the nature of the investigated materials and their differences.

Previous works [10, 11] report significant changes in HAL matter isolated from compost upon increasing the composting time over 4 weeks, i.e. a decrease of the H/C, O/C, C=O/Ar, alkyl/Ar, OMe/Ar, OX/Ar, X = carbohydrate and/or protein residue. This suggests that HAL tends to become less polar and more aromatic. In our case, this trend is evidenced by comparing cHAL2 and cHAL3 isolated from the same green urban biomass waste, but at the start and after 7 days of the composting process. For these two isolates, the elemental composition data reported in Table 1 confirm the decrease of the H/C ratio upon increasing the composting time. Coherently, the concentration data for the organic moieties reported in Table 2, as measured by ^{13}C NMR, show a large decrease (25%) of the alkyl/aryl C ratio for cHAL3 compared with cHAL2.

The relative decrease of the aliphatic moieties was also supported by IR spectroscopy. In the IR spectra of the two materials, two bands were identified, one with its peak absorbance centered at 2920 cm^{-1} and arising from the aliphatic C–H stretching vibration, the other centered at 1383 cm^{-1} and arising from the aromatic ring skeletal vibrations [17]. For cHAL3, the ratio of the area of the former band to that of the latter band turned out to be nearly half that for cHAL2. Aside from the decrease of the aliphatic to aromatic C ratio, no other significant differences involving the other organic moieties or functional groups were evident by comparing the cHAL2 and cHAL3 isolates originating from the same green waste composted for 0 and 7 days, respectively. The data in Table 2, however, show that compared to the cHAL matter obtained from the kitchen–green wastes mix composted for 15 days, cHAL2 and cHAL3 have almost double the content of O-alkyl C and 50% higher content of di-O-alkyl C. This suggests a quite higher content of carbohydrate [18] and/or oxyalkyl moieties in the two HAL isolates from the biomass waste containing green residues only. This observation is supported also by the relatively higher intensities of the methine and/or methylene proton resonance signals at 2.9–4.3 ppm reported in Fig. 2 for cHAL2 and cHAL3 compared to cHAL. In addition to the higher content of C–O and O–C–O functional groups, the data show that cHAL2 and cHAL3 contain more free carboxylic acid and less amide functional groups than cHAL.

Figure 3 is a graphical representation for the distribution of organic moieties and functional groups in the three HAL isolates being compared. Aside from the difference in aliphatic C content, no other great differences are observed between cHAL3 and cHAL2. On the contrary, cHAL differs from cHAL3 and cHAL2 mostly for the lower alkyl/aryl C ratio, the lower content of C–O and COOH functional groups and the higher content of amide functional groups. Thus, the most apparent difference between these materials is that cHAL3 and cHAL2 are expected to be more ionic and hydrophilic than cHAL.

As previous work on cHAL and cHAL2 indicated [6–9] that the potential technological appeal of the HAL isolates from biomass wastes might lie on their surface activity properties in solution, we characterize all our HAL isolates by their surface tension versus concentration plots such as those shown in Fig. 4. Surfactant properties for the cHAL isolates are expected to be based on their chemical structure, i.e. presence of hydrophobic C chains and hydrophilic polar groups. Typical synthetic surfactants belong to several categories depending on their chemical structural parameters, e.g. the non-ionic polyoxyethylenes, alkanolamides, sugar esters, alkyl polyglycosides, alkyl glucosamides and the anionic surfactants comprising a carboxylate moiety or sulfate and sulfonate groups.

To understand the plots in Fig. 4, it should be considered that when surfactants are in solution as single molecules, these lie at the air–water interface to expose the lowest possible hydrophobic surface to water. At higher concentration, when the air–water interface is saturated, the excess surfactant molecules aggregate forming micelles in the bulk water phase. In this form, several molecules are held together by intermolecular forces to yield spherical or quasi-spherical clusters where hydrophobic
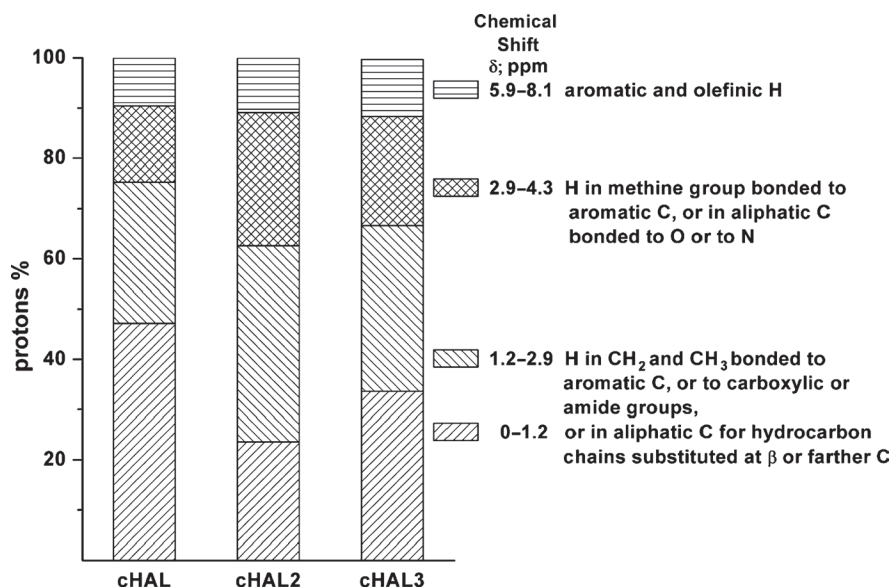
Figure 2: Distribution of the H atoms in cHAL, cHAL2 and cHAL3 as calculated by ^{1}H NMR. The spectra were measured on samples dissolved in 3 M NaOD in D_2O. The distributions were normalized without taking into account the phenolic and carboxylic acid protons that exchange with the solvent deuterium atoms.

surfaces stay in the inner micellar core and polar heads are directed toward the water phase. Such arrangement is ideal to host hydrophobic molecules in the inner micellar core, thus enhancing their water solubility. In this fashion, micelle formation is responsible for the technological performance of surfactants in many applications. As to the effects of these events on the surface tension of water, for simple surfactant molecules with one polar head and a relatively long hydrophobic C tail, one should in principle expect a surface tension (γ) versus the logarithm of the concentration (C) plot, where two clear linear regimes, premicellar and postmicellar, are evidenced. The intersection of the two straight lines gives the critical micellar concentration (cmc) at which the formation of molecular aggregates occurs. Oligomeric surfactants, such as the gemini surfactants [5], show a gradual transition between the two regimes, whose extension may be very large. These surfactants are made of molecules in which two or more polar groups are connected by lipophilic chains of variable length. Their oligomeric or polymeric nature is a key point in determining their capacity to micellize .The same could be expected for the HAL substances studied in this work, which according to Table 2 and Fig. 3 have most of the above-cited polar groups. Indeed, the γ–C plots shown in Fig. 4 for cHAL3 and cHAL2 show three regions that can be distinguished by the different value of the curve slope. The region at C ≥ 0.91 g/L, where the slope is almost horizontal, can be assigned to the presence of micellar aggregates [18]. In the region at lower concentration, a second change of slope is observed, i.e. at ca. 0.2 g/L for cHAL2 and ca. 0.6 g/L for cHAL3. This second change of slope may correspond to the concentration at which premicellar aggregates start forming in the aqueous solution, as already reported for other sugar-based surfactants [18]. The values for the cmc and the surface tension at the cmc (γ_{cmc}) of the three HAL isolates are reported in Table 3. It may be observed that all three substances have rather strong surface activity inasmuch as they are capable of lowering by nearly 50% the water
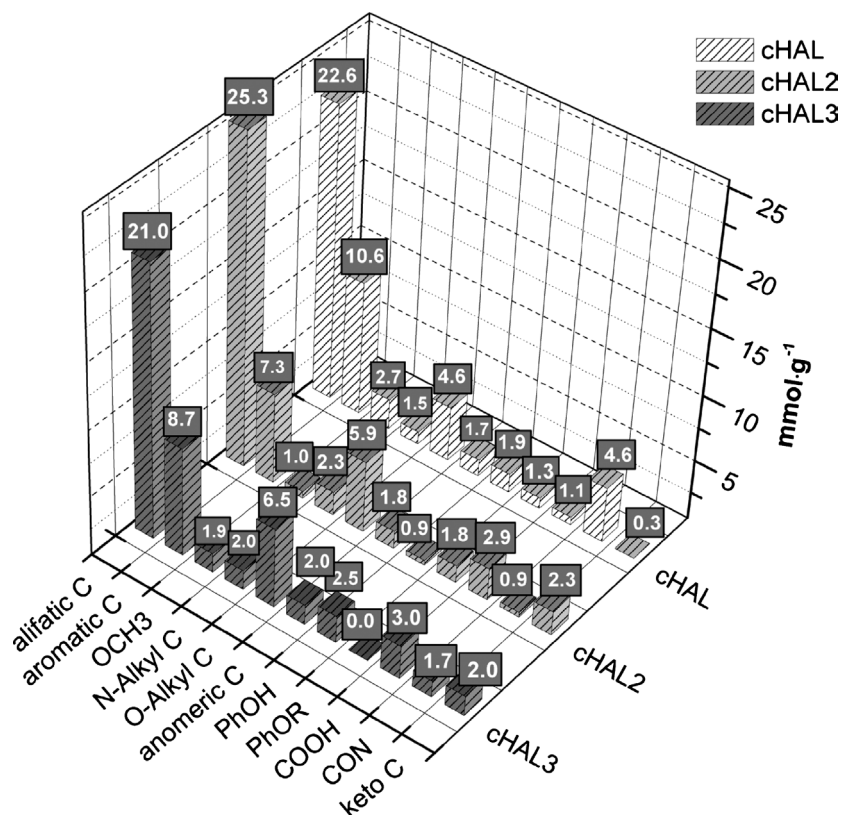
Figure 3: Distribution of organic C and functional groups in cHAL, cHAL2 and cHAL3. Concentration values (mmol/g of humic-like material) calculated as reported in Section 2.

surface tension at their cmc value. For this behavior and for their biological origin, the three substances may be referred to as biosurfactants. The two biosurfactants isolated from the same green residue biomass before (cHAL2) and after (cHAL3) composting have rather similar values for both the cmc and γ_{cmc}, and these are rather higher than the corresponding values for cHAL. This overall behavior seems correlated to the chemical composition data discussed above for the materials under consideration. The higher degree of hydrophilicity expected for cHAL2 and cHAL3 is likely to hinder micelles formation [18, 19] from these substances compared to cHAL.

One structural feature of HAL material which is currently a matter of dispute is its molecular weight [20]. It has not been established if HAL substances are polymeric molecules containing covalent bonds only or aggregates of small molecules linked by hydrogen bonds and hydrophobic interactions. The dispute over the molecular weight is not only academic but also has relevance in relation to the performance of these materials as surfactants [7–9]. Indeed, small molecules need to micellize for best performance, whereas polymeric molecules with carboxylic functional groups may act as dispersants of hydrophobic molecules and therefore may perform satisfactorily at concentrations lower than that expected based on the cmc value. Assessing the size of the molecules would therefore make possible to establish structure–properties relationships and then to use these in order to exploit the full potential of the material for specific technological applications.
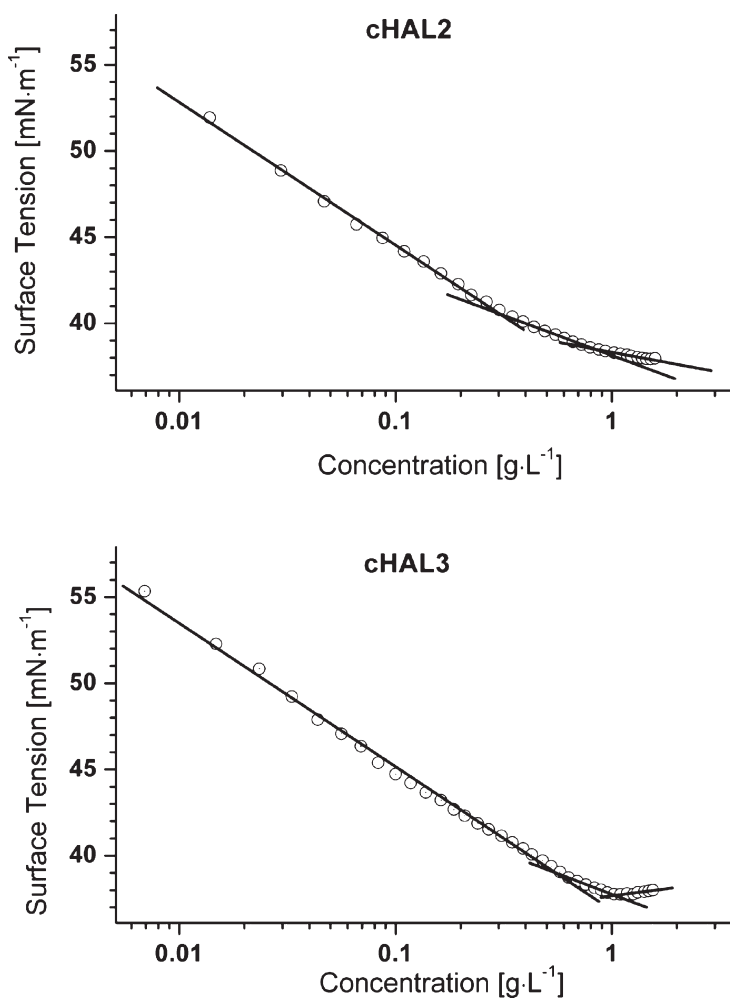
Figure 4: Surface tension versus concentration for cHAL2 and cHAL3 water solution at pH 7.

Table 3: Critical micellar concentration (cmc) and surface tension at the cmc (γ_{cmc}) for cHAL3 and the other two previously reported biosurfactants.

Biosurfactant	cmc (g/L)	γ_{cmc} (mN/m)
cHAL	0.40	36.1
cHAL2	0.97	37.8
cHAL3	0.91	38.4

In our previous work [6], the size and mass distributions of cHAL2 were analyzed by three different techniques, i.e. ESI-MS, SEC-MALS and DLS. This analysis suggested that cHAL2 consists of both macromolecules and small molecules. However, only the latter ones are able to form micelles when dissolved above a certain concentration. A similar analysis has been carried out for cHAL3 and the results are compared here to the data obtained for cHAL2.

DLS is a well-established technique for measuring particle size over the 10^{-10} to 10^{-6} m range [21]. DLS measures the velocity at which particles within a solvent diffuse due to Brownian motion. This is done by monitoring the fluctuation in intensity of the scattered light over the time. Indeed, the scattered intensity due to the phase addition of the moving particles is constantly evolving, depending on the particle size. The Brownian diffusion velocity is inversely proportional to the particles size, expressed as hydrodynamic diameter (D_H). As this technique allows the determination of particle size distributions in solution at variable concentration, and therefore allows the calculation of the hydrodynamic diameter of the molecules or aggregates in solution, we performed DLS measurements for cHAL3 in aqueous solution at pH ~ 7 and two concentration values, 3 and 0.3 g/L, which are, respectively, comprised in the region above the 0.91 g/L cmc value and in the region below the 0.6 g/L concentration value at which premicellar aggregates form (see Fig. 4 and Table 3). A similar analysis performed for sodium dodecylbenzenesulfonate [6], a well-known commercial anionic surfactant, gave D_H values of 2–3 nm above the cmc value and no significant value below the cmc value of the surfactant. Analogously, for cHAL3 we were expecting a larger hydrodynamic diameter at 3 g/L than at 0.3 g/L. From the plots in Fig. 5, it may however be observed that, contrary to the expectations, no significant change of D_H with cHAL3 concentration is evidenced.

The results suggest that cHAL3 consists of particles with sizes included between 10 and 100 nm and that this particle size distribution does not change upon changing the sample concentration. An average D_H of 28 nm was extrapolated from both the distributions presented in Fig. 5. Very similar results were reported also in the case of cHAL2; a 24-nm D_H value was measured regardless of concentration being above or below the biosurfactant cmc value [6]. The D_H values obtained for cHAL3 and cHAL2 are well comparable to the particle size found by the same DLS measurements on a reference humic acid sample isolated from Suwannee River and supplied by the International Humic Substance Society (St Paul, USA), which was analyzed in solution at pH 7 and 0.04 g/L concentration [22]. The sizes measured for cHAL3 and cHAL2 do not imply the absence of smaller particles. In fact, scattering intensity is inversely proportional to the sixth power of the scatter radii. Therefore, large particles may mask the presence of smaller ones in the sample. The lack of change of D_H with the concentration of our biosurfactants, and therefore the absence of correlation between DLS and surface tension data, suggests that the large particles observed by DLS are not responsible for the surface tension dependence on concentration shown in Fig. 4, as their size seems unchanged over a very wide concentration range. We therefore sought evidence for the presence of both small molecules and macromolecules by the other two techniques, SEC-MALS and ESI-MS.

In SEC-MALS measurements, the optimization of the solubilization procedure and SEC experimental conditions for HAL substances is not trivial. It is well known that HAL substances in aqueous solution are strongly aggregated and may yield aggregates of various sizes. Furthermore, the aggregation extent may be significantly affected by pH, ionic strength and type of counterion of the used solvent [23]. In addition, the SEC fractionation for these substances is problematic because column non-steric separation could occur. Often, upon using non-optimized SEC conditions, multimodal and/or asymmetrical chromatograms with very long tails are obtained. Under our SEC experimental conditions, i.e. using the pH 7 buffered water and 10% methanol solvent, chromatograms without long tails were obtained. Figure 6 shows the comparison of the concentration detector signal (DRI chromatogram) for the cHAL2 and cHAL3 samples. Clearly, each chromatogram is composed of two major polymeric
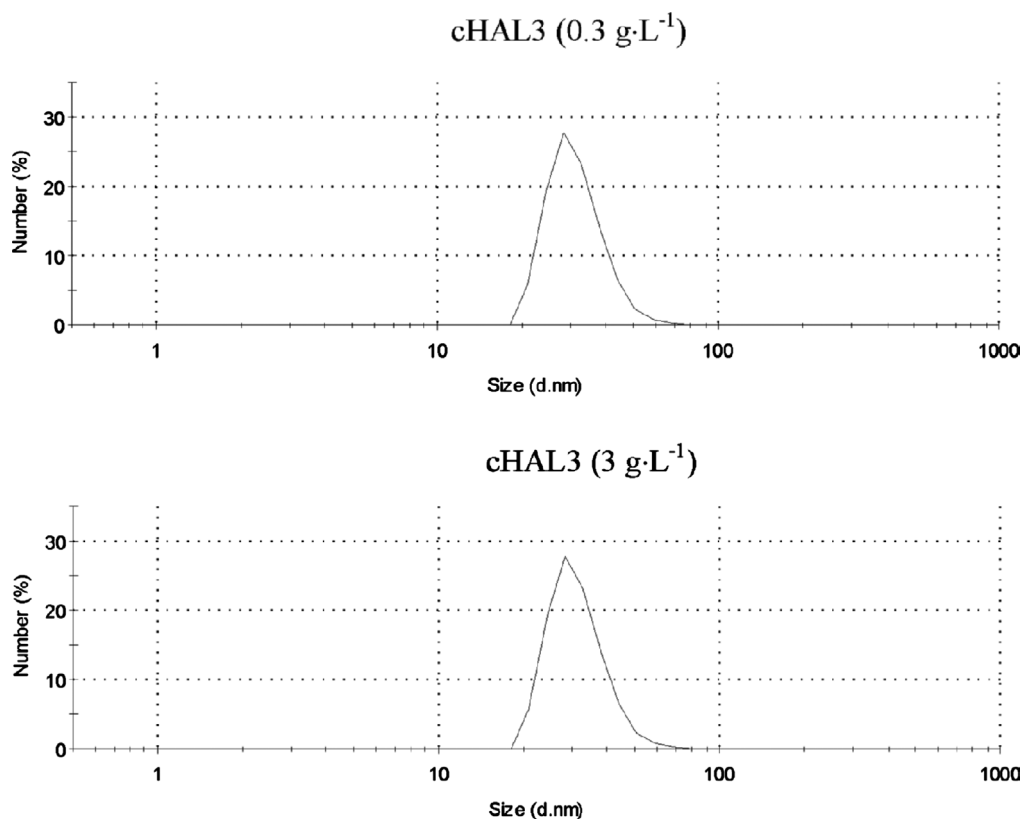
cHAL3 (0.3 g·L^{-1})

cHAL3 (3 g·L^{-1})

Figure 5: Distribution of particle size as indicated by the hydrodynamic diameter (D_H, nm) measured by dynamic laser scattering in two aqueous solution at pH 7 containing cHAL3 at concentration below (0.3 g/L) and above (3 g/L) its 0.91 g/L cmc value.

peaks and one initial 'shoulder'. Consequently, for convenience, each chromatogram was divided in three elution volume bands, as clearly depicted in Fig. 6, and the relative amount of each fraction (Table 4) was estimated based on band areas.

The on-line MALS detector is able to measure both the molar mass (M) of the eluting fractions and the dimension of macromolecules which is generally expressed as radius of gyration (R_g). The latter parameter is measured from the angular variation of the scattering intensity. Two experimental functions, $R_g = f(V)$ and $M = f(V)$ (where V denotes the elution volume), of the cHAL3 sample from SEC-MALS are reported in Fig. 7.

It is well known that the $M = f(V)$ curve, i.e. the classical SEC calibration, is used to calculate the molar mass distribution (MMD) of the polymer. Unfortunately, SEC fractionation of HAL molecules is not simple and the calibration is not the classical monotone decreasing curve in molar mass. Assuming a pure steric fractionation, the hypothetical cHAL3 'clusters' are fractionated (sorted) on the basis of decreasing size. Really, looking to Fig. 7, one can see a regularly decreasing in size $R_g = f(V)$ curve. On the contrary, the $M = f(V)$ experimental function does not regularly decrease upon increasing the elution volume. The molar mass of each HAL fraction was measured directly from the on-line MALS detector, and the unusual course of the $M = f(V)$ experimental function
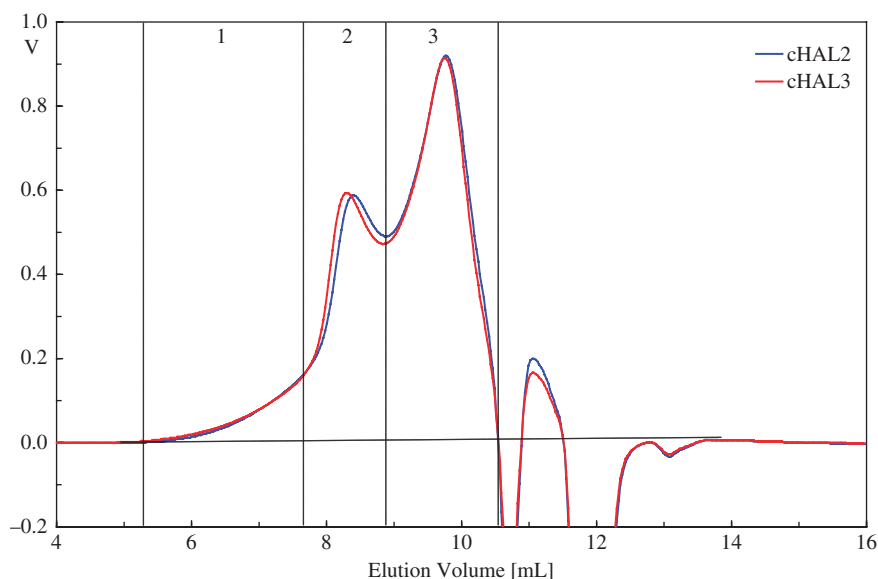
Figure 6: Comparison of DRI signals of cHAL2 and cHAL3 samples. Lines indicate the elution volume range of three chromatographic 'peaks', i.e. three HAL macrofractions.

Table 4: Area (%) of chromatographic band and approximate molecular weight (Mp) associated with each band peak in order of increasing elution volume (see Fig. 6) and recovered mass.

	Peak 1[a]		Peak 2		Peak 3		Recovered mass (%)
Sample	M_p (kg/mol)	Area (%)	M_p (kg/mol)	Area (%)	M_p (kg/mol)	Area (%)	
cHAL2	–	7.6	97	31.0	200	61.4	86.0
cHAL3	–	7.3	186	32.6	267	60.1	87.0

[a]Peak associated with initial shoulder of peak 2 band.

means that the cHAL3 aggregates are not homogeneous but more or less compact depending on the eluting fraction. In other words, based on the $M = f(V)$ experimental function depicted in Fig. 7, one can state that the cHAL3 solutions contain very compact aggregates that correspond to the tail of the chromatogram where the $M = f(V)$ curve suddenly increases. These aggregates are small in size and very high in molar mass. Usually these fractions in polymers are defined as 'microgel'. The elution pattern shown in Fig. 7 suggests that for HAL substances the usual MMD concept of a polymer does not make sense, and probably it is more useful to quantify the approximate molar mass of each chromatographic peak (M_p) and the relative area of each peak as shown in Fig. 6. For both cHAL2 and cHAL3, Table 4 reports the total recovered mass calculated from the whole DRI area after an accurate calibration of the concentration detector together with the M_p value associated to each peak.
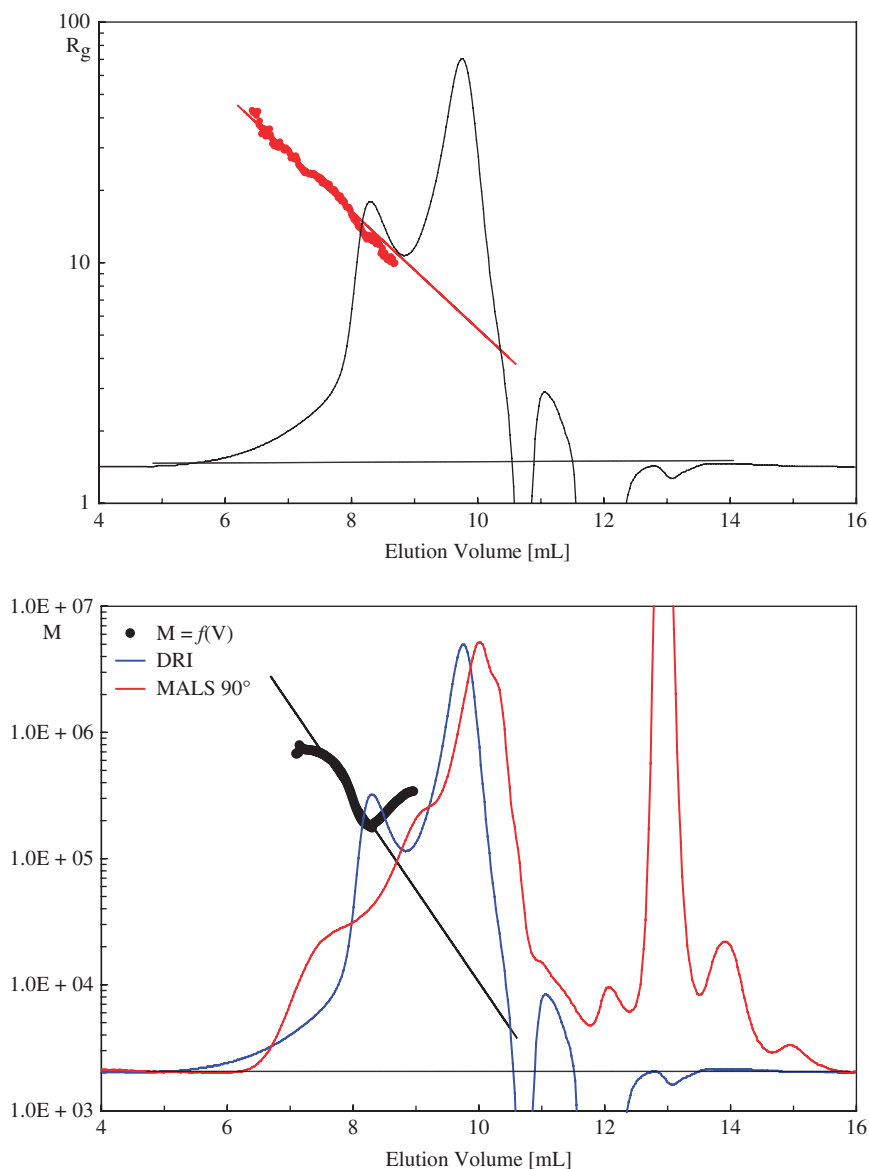
Figure 7: $R_g = f(V)$ (upper panel) and $M = f(V)$ (lower panel) experimental functions from SEC-MALS of the cHAL3 sample.

It may be observed that the recovered mass associated to the whole chromatographic peak accounts for 86–87% (very high) of the initial injected mass of each polymer. Considering the remarkable fractionation problems, the M_p molar mass values shown in Table 4 could be representative of the MMD of each sample. In general, it is evident that the two substances – cHAL2 and cHAL3 – have quite similar molar masses and sizes.

Figure 8 shows the $R_g = f(M)$ experimental function, generally known as conformation plot, for cHAL3 as obtained from the SEC-MALS measurements. From the estimated slope of this plot
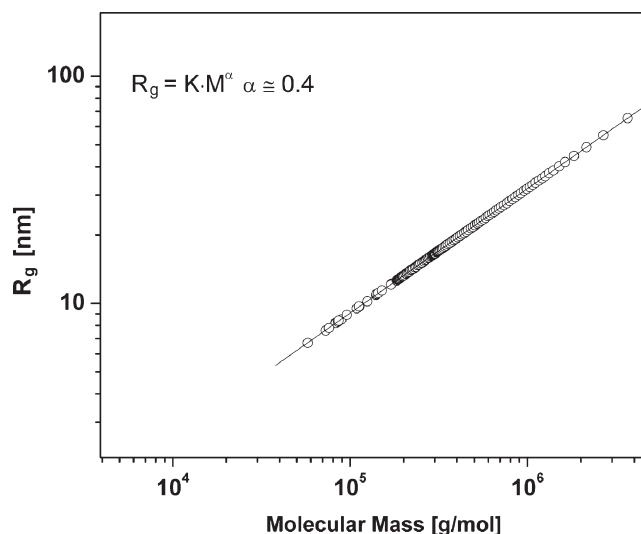
Figure 8: Conformation plot from SEC-MALS of the cHAL3 sample: radius of gyration (R_g) as a function of the molar mass.

(approximately 0.40–0.42) and from the very similar one previously published for cHAL2 [6], a compact quasi-spherical conformation for both substances may be inferred. The 10–25 nm R_g values measured by SEC-MALS are consistent with the average hydrodynamic diameter (D_H = 24–28 nm) measured by DLS. By comparison, the molecular radius of naturally occurring humic acids has been reported to increase from 9.8 to 17.8 Å as the molar mass increases from 5,000 to 30,000 kg/mol [22]. Similar to DLS measurements, the SEC-MALS do not provide evidence of the presence of small molecules in any of the samples analyzed here.

Soft desorption ionization techniques, such as ESI, volatilize large ions for identification via MS, providing ion mass/charge distributions that may represent the mass distribution of molecules within humic fractions [8]. By this technique, compounds may be analyzed from aqueous or aqueous/organic solutions at variable concentrations, similar to the case of the above DSL measurements. As solvent evaporation occurs, the droplet shrinks until it reaches the point that the surface tension can no longer sustain the charge (the Rayleigh limit). At this point, a 'Coulombic explosion' occurs and the droplet is ripped apart. This event produces smaller droplets that can repeat the process as well as naked singly or multiply charged analyte molecules. High-resolution MS makes it possible to assign the charge value to an ESI signal originated by an ionized molecule through the examination of the signal isotopic peak intensity. Our ESI-MS measurements were performed on the same cHAL3 solutions, which were analyzed by DLS, i.e. the 0.3 and 3 g/L cHAL3 solutions. The ESI-MS spectra for these solutions collected in the negative mode exhibited signals for molar masses associated with singly charged anions ranging from 340 to 590 m/z. The spectral pattern was the same for both solutions.

The results obtained by the above three techniques suggest the presence of small molecules together with large macromolecules in cHAL3. The largest particles are probably not responsible for the surface tension dependence on concentration, because their size, as shown by DLS measurements, seems unchanged over a wide concentration range. Hence, the small molecules, detected by ESI-MS, are likely to be the main contributors to the surface tension versus concentration profile observed in Fig. 4. The fact that the two solutions in the premicellar and postmicellar cHAL3 concentration

ranges give similar ESI-MS spectra may indicate that the micellar aggregates do not withstand the conditions of the ionization process. These results are consistent with those already reported for cHAL2. For their wide molecular weight distribution, surface tension properties and the content of ionic and/or polar carboxylic, phenol and amino groups, the humic-like materials reported in this work have potential to perform as surfactants, dispersing polymers and/or polyelectrolytes, and therefore offer scope for being tested in a wide range of technological applications.

4 CONCLUSIONS

Two HAL substances (cHAL2 and cHAL3) isolated from the same green biomass waste before and after composting for 7 days, respectively, have been shown to have different chemical composition, mostly due to lowering of the aliphatic to aromatic C ratio upon composting. These materials also contain several other functional polar groups but do not exhibit much difference in the content of these groups. The chemical compositional changes between cHAL2 and cHAL3 are not shown to bring a significant change in the surface activity properties of these substances. The comparison of the two cHAL materials with a third HAL material isolated from food and green residues composted for 15 days shows rather significant and large effects in both chemical composition and surface activity properties presumably ascribed to the different biomass and/or to the increase of the composting time to 15 days. We are aware that a more powerful experimental design is necessary to isolate the effects of the composting parameters such as ingredients nature and time on product structure, properties and performance in technological applications. Work in this direction is in progress in our laboratories. Indeed, our long-term purpose is to build a data inventory which at some point will make it possible not only to understand how the parameters characterizing the waste nature and the composting process influence the structure and surfactant properties of the isolated HLS, but also to assess how much the source and structural difference of these substances affect their performance as auxiliaries for chemical technological uses.

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