Biobased Surfactant-Like Molecules from Organic Wastes: The Effect of Waste Composition and Composting Process on Surfactant Properties and on the Ability to Solubilize Tetrachloroethene (PCE)

GIORGIA QUADRI,[†] XIAOSONG CHEN,[‡]
JAMES W. JAWITZ,[‡] FULVIA TAMBONE,[†]
PIERLUIGI GENEVINI,[†] FRANCO FAORO,[§]
AND FABRIZIO ADANI*,[†]

Dipartimento di Produzione Vegetale, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy, Soil and Water Science Department, University of Florida 2169 McCarty Hall Gainesville, Florida 32611, and Istituto di Patologia Vegetale, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy

In this work, four surfactant-like humic acids (HAs) obtained from garden lignocellulose wastes and kitchen food wastes mixed with garden-lignocellulose wastes, both before and after composting, were tested for surfactant properties and the ability to solubilize tetrachloroethene (PCE). The waste-derived HAs showed good surfactant properties, lowering the water surface tension from 74 mN m^{-1} to 45.4 ± 4.4 mN m^{-1}, with a critical micelle concentration (CMC) of 1.54 ± 1.68 g L^{-1}, which is lower than many synthetic ionic surfactants. CMC was affected by both waste origin and composting processes. The addition of food waste and composting reduced CMC by adding alkyl-C (measured by CP MAS ^{13}C NMR) and N- and S-HA contents (amide molecules), so that a multistep regression was found [CMC = 24.6 − 0.189 alkyl C − 2.64 (N + S); R^2 = 0.77, $P < 0.10$, $n = 6$]. The four HAs solubilized PCE at the rate of 0.18–0.47 g PCE/g aqueous biosurfactant. These results were much higher than those reported in the literature for a commercial HA (0.026 g/g), but they were in line with those measured in this work for nonionic surfactants such as Tween-80 (0.69 g/g) and Triton X-100 (1.08 g/g).

Introduction

Surfactants are molecules characterized by a polar headgroup and a nonpolar hydrocarbon tail so that molecules assume amphiphilic characteristics. At low concentration, surfactants exist as monomers, but these monomers concentrate at interfaces (e.g., water–air) as surfactant concentration increases [1]. At higher concentrations, the free energy of the system can be reduced by the aggregation of the surfactants molecules into micelles [2] with the hydrophobic group

located in the center (hydrophobic core) of the cluster and hydrophilic headgroup toward the aqueous solvent (hydrophilic shell) [3]. This hydrophobic core gives micelles the ability to enhance the aqueous solubility of hydrophobic organic compounds increasing their apparent solubility [1].

The concentration at which the micelle formation occurs is defined as the critical micelle concentration (CMC). The CMC is an important parameter for pollutant solubilization as a lower CMC equates to a lower concentration required to initiate solubilization of hydrophobic compounds because of micelle formation.

Synthetic surfactants are a diverse group of petroleum-derived chemicals that are designed to have cleaning or solubilization properties [3]. Because of their synthetic origin, surfactants can be resistant to biodegradation, persist in the environment, and affect organisms in the environment [3].

On the other hand, the development of surfactants taken directly from a natural source (called biobased surfactants or natural surfactants) [4] is fascinating and allows the development of environmentally friendly surfactant-based technologies [5]. Some natural substances can have surfactant properties, and they are designated natural (or biobased)-surfactants [4]. Natural surfactants, such as fatty acid esters of sugars and fatty acid esters or amides of amino acids, have been proposed for different applications [4, 6]. Rhamnolipids produced by *Pseudomonas* sp. have shown good surfactant properties and have been used to enhance the solubility of tetrachloroethene (PCE) [7] and other organic pollutants [8].

Tetrachloroethene (PCE) is used in a variety of commercial and industrial applications and represents one of the most commonly identified organic contaminants in groundwater [9]. This solvent has been associated with both acute and chronic human-health problems [9, 10]. In addition, natural biotic and abiotic transformations of this solvent lead to the production of toxic intermediates [11]. Despite the extent of this contamination problem, consistently successful remediation techniques have not been developed [12].

Tetrachloroethene is denser than water (density of 1.67 kg m^{-3}) and an aqueous phase liquid (DNAPL) that is a persistent groundwater contaminant due to its low aqueous solubility (PCE water solubility of 0.15 g L^{-1}) and slow rate of dissolution [13]. Because surfactants are able to increase the solubility of DNAPLs they have the potential to enhance PCE remediation technologies [12]. Surfactant-enhanced aquifer remediation (SEAR) of PCE is a technology that is becoming based on a variety of water treatment and acquifer/soil remediation processes [5].

Humic acids (HA), widespread in water and soil, are natural amphiphilic molecules with hydrophilic portions composed of both ionic groups (R—COO$^-$) and nonionic groups (R—OH), and hydrophobic portions composed of aliphatic chains and aromatic rings. HAs have shown surfactant activity and have been used to solubilize a variety of organic compounds [14, 15], although data relative to PCE solubilization are very scarce. PCE has been reported to have a solubility of 0.026 g for each gram of commercial-HAs (Aldrich), but this value is 2−30 times lower than those obtained by using synthetic surfactants [16].

To our knowledge, all previous reports of solubilization of organic compounds with HAs have used commercial HAs (Aldrich) or HAs extracted from soil and water organic matter. However, the cost of the commercial products, the low capability to solubilize PCE, and the very low yields (mg of HAs extracted per g of parent material dry matter) of HAs extracted from natural sources limit their potential use in SEAR technologies. However, HAs with surfactant properties

* Corresponding author e-mail: fabrizio.adani@unimi.it.
[†] Dipartimento di Produzione Vegetale, Università degli Studi di Milano.
[‡] University of Florida.
[§] Istituto di Patologia Vegetale, Università degli Studi di Milano.

also can be obtained from composted agro-industrial and municipal organic wastes (17). This prospect is attractive as organic wastes have no-cost, are widely available, and the HA yield may be 10s greater than those from natural sources. In our previous research, we isolated a HA from composted organic waste at a yield of 12% of waste dry matter (dm) (17). No investigation was made into a possible environmental application of this HA, but the low CMC detected 1−4 orders of magnitude lower than those of synthetic anionic surfactants (17) that suggested further investigation into the application of this new class of biobased-surfactants in environmental technologies.

Waste-derived HAs have characteristics that depend on the raw materials used. Moreover composting processes modify HAs as a consequence of both degradation and humification of the organic matter contained in the waste (18). Thus, our approach was to study two different wastes: (1) garden lignocellulose wastes, and (2) garden lignocellulose wastes combined with kitchen food waste. The addition of food waste was expected to increase the total content of the alkyl carbon and protinaceous material of HA because of their high contents in the parent material. On the other hand, composting processes were expected to reduce the content of the O-alkyl fractions concentrating both aromatic fractions because of degradation processes (18). All these modifications could affect the surfactant properties of HAs.

The objective of this work is to describe for the first time the effects of both waste composition and composting process on the surfactant properties of waste-derived HAs. Moreover the ability of extracted HAs to solubilize PCE was studied in comparison with synthetic surfactant.

Experimental Section

Composting Process. The raw material from source separate collection, garden lignocellulose (designated L), and garden lignocellulose plus kitchen food wastes (designated LF) were sampled from a facility located in Cologno Monzese urban area, near Milano (North Italy). Macromolecular compositions of raw materials before and after composting are reported in Supporting Information Table S1. Ten kilograms of wet weight of the two raw materials were composted for 15 days by using a self-heating 30 L laboratory-scale composter (19).

Isolation of Humic Acid. Humic acids were extracted from the two organic wastes, before and after composting, with aqueous 0.1 mol L^{-1} NaOH and 0.1 mol L^{-1} Na$_4$P$_2$O$_7$, using 1:50 w/v organic wastes/solution ratio and successively precipitated at pH <1.5 as previously described (17).

Chemical Characterization of HAs. Micro analytical data (C, H, N, S, and O contents) were obtained by C. Erba (Rodano, Milan, Italy) NA-2100 elemental analyzer. Oxygen was calculated by difference to 100. Molecular weight measurements were carried out by high pressure size exclusion cromatography (HPSEC) using a Waters Protein Pak 125 column (porous silica, 7.8 mm × 30 cm), a Waters 1500 series HPLC pump, a Waters 2487 dual λ absorbance detector at 254 nm, and TRIS (tris-hydroxymethylaminomethane)-phosphate 75 mmol L^{-1} eluent at pH 7.2, 1 mL min^{-1} flow rate. The following materials were used as calibration standards: polystyrene sodium sulfonates with 70 000–35 000–18 000–4600 Da. The HAs were dissolved in water containing 0.01 mol L^{-1} NaOH e 150 mmol L^{-1} TRIS to yield a 2 mg mL^{-1} HAs solution which (20 μL) was injected into the silica column. The ^{1}H NMR spectrum for HAs in 3 mol L^{-1} NaOD D$_2$O solution was recorded on a Bruker AMX 300 (B_0 = 9.4 T, ^{1}H operating frequency 399.78 MHz). CP MAS ^{13}C NMR spectra were acquired at 10 MHz on a Bruker AMX 600 spectrometer. The pulse repetition rate was set at 0.5 s and the contact time at 1 ms.

HAs Surface Tension Measurements. Surface tension (γ, mN m^{-1}) measurements of aqueous solutions containing HAs at variable concentration (HAs = 0.005–15 g L^{-1}) at pH 7 corrected by the addition of 0.1 mol L^{-1} NaOH, were carried out at 25 °C with a du Nouy ring tensiometer (model 21 Fisher Tensiomat, Fisher Scientific, Pittsburgh, PA) (20). Each experiment was performed in quadruplicate. The surface area occupied by a HA molecule was determined by using the Gibbs equation for a dilute solution (21).

PCE Solubility Measurements. One mL of pure PCE (99% pure, density 1670 kg m^{-3}) was equilibrated for 72 h in 5 mL vials with solutions at concentrations in the (1–10 g L^{-1}) of Tween-80, Triton X-100, and HA surfactants. HA solutions were prepared at pH 7 by the addition of the proper amount of 0.1 mol L^{-1} NaOH. Separate tests showed that 72 h was sufficient for equilibrium of the batch systems to be reached and confirmed also that volatilization in the batch reactors were negligible. All samples were prepared in triplicate. The supernatant was sampled (1 mL), and diluted with isopropyl alcohol (1 mL), an aliquot of the mixture was analyzed by GC-FID (Perkin-Elmer, Norwalk, CT) with initial temperature at 60 °C for 2 min, then increased to 160 at 20 °C min^{-1} and remained there for 1 min.

Transmission Electronic Microscopy (TEM). TEM- 400 mesh copper grids were coated with collodion film and a thin layer of carbon film (<10 nm), deposited by evaporation (22). Grids were then ionized by glow discharge and immediately layered onto a drop of HA suspension, deposited on parafilm for 30–60 s. The suspension was drained out from the grids with filter paper, without allowing them to dry. Staining was performed adding a drop of 5% ammonium molibdate, pH 7, in 2% trehalose water solution, or, alternatively, of 2% Uranyl acetate in water, and draining out the excess of staining, as above. Grids were examined with a Jeol 100 SX TEM, operating at 100kV, with a precooled specimen chamber.

Results and Discussion

Surfactant Properties of Humic Acids. Humic acids (ash content below 1% w/w) were extracted from parent materials before and after 15 days of composting at a yield of 6.98 ± 0.46% of dry matter (dm) (mean ± SD, n = 3), 8.14 ± 0.1% of dm, 8.75 ± 0.21% dm and 8.07 ± 0.17% of dm from L (HA$_L$), and composted L (HA$_{LC}$), and LF (HA$_{LF}$) and composted LF (HA$_{LFC}$), respectively.

HA aqueous solutions at concentrations from 0.005 to 15 g L^{-1}, and adjusted to pH 7 by adding NaOH 0.1 mol L^{-1} (16), were used to measure the ability of HAs to reduce surface tension. Results (Figure 1) showed that the solution surface tension decreased as HA concentration increased until at some point the diminishing became less marked (Figure 1) (23, 17). This point represents the HA CMC (otherwise identified as the aggregation concentration) (24). At this point, the surface tension of water was reduced from 74 mN m^{-1} to 45.4 ± 4.4 mN m^{-1} (mean ± SD, n = 4) (Table 1). This value is comparable with that of synthetic surfactants such as Tween 80 (41 mN m^{-1}) (25) and sodium dodecyl sulfate (39.5 mN m^{-1}) (17) but higher than that of other synthetic surfactants such as Triton X 100 (30 mN m^{-1}) (25). The surface area occupied by HA molecules (Table 1) is inversely related to surface tension reduction, as lower surface area corresponded to an increased amount of HA at the water–air interface (17) and greater reduction in surface tension (Figure 1).

The CMC is an important parameter to describe a surfactant as it defines the surfactant concentration at which solubility ability increase as consequence of the micelle formation. For each HA, CMC was calculated from the intersection of the two lines describing premicellar and postmicellar regimes (17) (Figure 1). CMC$_S$ were quite diverse
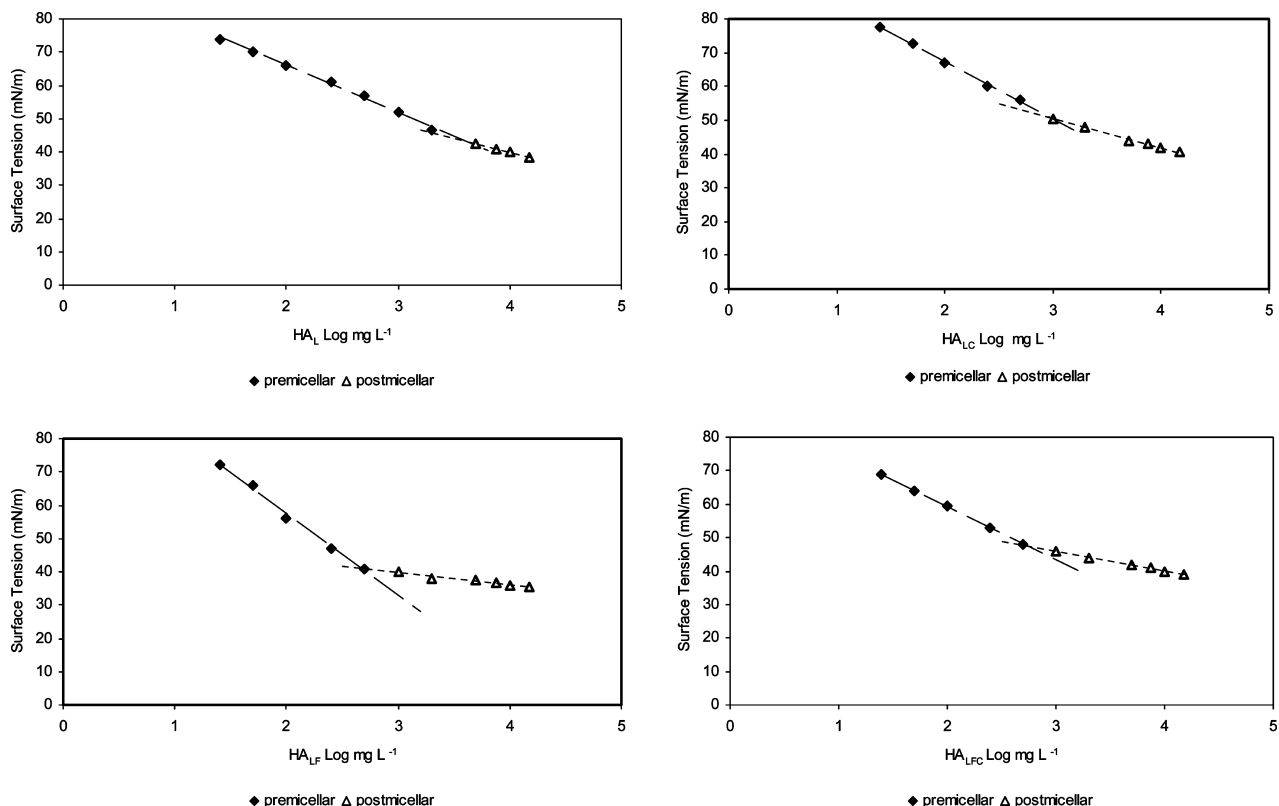
FIGURE 1. Surface tension versus humic acid (HA) concentration (mg L^{-1}). CMC was calculated from the intersection of the premicellar and postmicellar straight lines (see ref (17)).

surfactant	critical micelle concentration (g L^{-1})	γ_{CMC} (mN m^{-1})	surface area occupied by HA molecule (Å^2)	HA yield (% dry matter) (mean ± SD, $n = 3$)
	data from this work			
HA$_L$[a]	4.04	43.5	65.91	6.99 ± 0.47
HA$_{LC}$[b]	0.999	50.4	55.37	8.15 ± 0.10
HA$_{LF}$[c]	0.471	40.8	37.98	8.75 ± 0.21
HA$_{LFC}$[d]	0.665	47.6	57.98	8.08 ± 1.79
	data from literature			
HA$_{LFC}$[e]	0.403	36.1	32.73–589	n.a.[f]
HA$_{commercial}$[g]	7.4	50	n.a.	n.a.

[a] Humic acids obtained from lignocelluloses wastes. [b] Humic acids obtained from lignocelluloses wastes, after 15 days of composting process. [c] Humic acids obtained from lignocelluloses plus food wastes in the ratio of 1/1 (v/v). [d] Humic acids obtained from lignocelluloses plus food wastes in the ratio of 1/1 (v/v), after 15 days of composting process. [e] Humic acids obtained from food residues and public parks green wastes in the ratio of 1/1 (v/v), after 15 days of composting process (17). [f] Data not available. [g] Humic acids from Aldrich (31).

for the different HAs (Table 1), with an average value of 1.54 ± 1.68 g L^{-1} (mean ± SD, $n = 4$). This result is very interesting because it suggests that one could modify the HA surfactant properties by changing the waste mixture and using composting biotechnology.

Simply adding food waste to the lignocellulose reduced CMC by a factor of 8.6 (Table 1). On the other hand, composting reduced CMC only for lignocellulose wastes (Table 1). CMCs obtained for HAs from organic wastes were lower than those reported for both commercial HAs and HAs from natural sources (Table 1) and comparable to the CMC of 0.403 g L^{-1} detected in our previous work for HAs derived from composted organic waste (17).

^{1}H NMR and ^{13}C CPMAS NMR suggested HAs from lignocelluloses contained less aliphatic hydrocarbon and more O and N aliphatic and aromatic carbons than HAs from lignocelluloses plus food wastes (Supporting Information Tables S2 and S3). These different characteristics of HAs were the consequence of the different composition of the parent materials (Table S1) from which the HAs were extracted.

Nevertheless, composting did not significantly affect the average chemical composition of HAs (Tables S2 and S3). CMC has been reported to be affected by molecule hydrophobicity and hydrophilicity (23). Moreover, a higher content of carboxylic groups allowing an extensive H bonding with water hampers the development of amphiphilic characteristics of HAs increasing the CMC (26). Hydrophobic/ hydrophilic carbon ratio (Hydrophobic degree = Hb/Hi) (Hb/ Hi = 1.49 ± 0.11; mean ± SD, $n = 4$) (see Supporting Information Table S4) calculated from ^{13}C CP MAS NMR data (27) and the content of −COOH groups (−COOH = 3.51 ± 0.26 meq g^{-1} HAs; mean ± SD, $n = 4$) calculated from ^{13}C CP MAS NMR and micro analytical data (17) (see Supporting Information Tables S3, and S5) were very similar for all HAs studied. Thus, at pH 7, we concluded that average HA-characteristic did not affect CMC. Nevertheless, the nature of HAs is complex and sometimes there is no agreement among researchers about their structure (28). Only recently have researchers agreed that HAs are composed of low molecular weight (MW) hydrophobic and amphiphilic compounds derived from the degradation and decomposition of dead biological material (lignin, polysaccharides, lipids, and peptides), arranged in supramolecular structures (28). These structures at pH 7 are stabilized by dispersive hydrophobic forces (van der Waals, $\pi-\pi$, and CH$-\pi$ bonding) and hydrogen bonds (29). Therefore if synthetic surfactants are
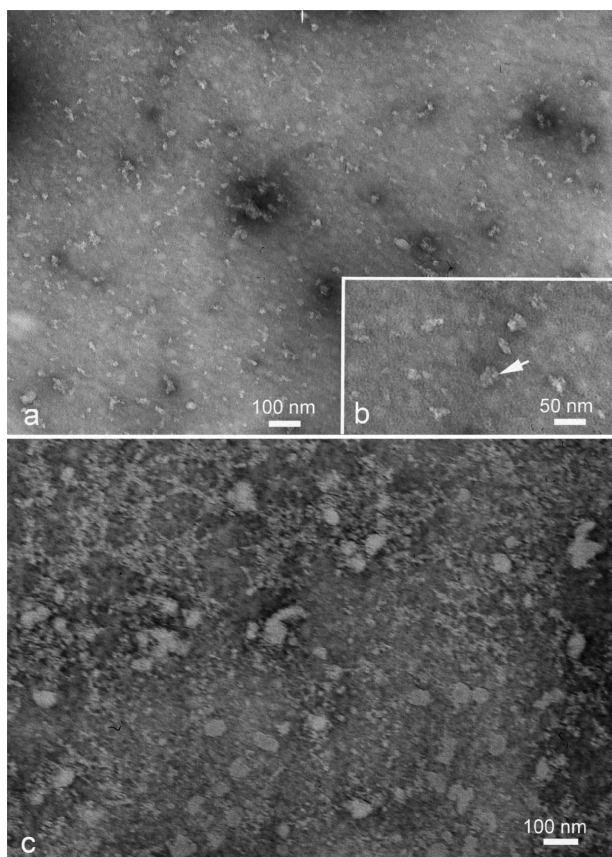
FIGURE 2. TEM micrographs of HA$_{LFC}$ at concentration below and above critical micelle concentration. (a) Small globular units of about 10 nm and aggregates of 20–30 nm visible at the 0.4 × CMC HA$_{LFC}$ concentration. (b) Enlargement of the association in different patterns of three to five 10 nm units, indicated by an arrow. (c) Larger aggregates of about 70–100 nm visible at 4 × CMC HA$_{LFC}$ concentration. Similar results were obtained with an aqueous solution of 2% uranyl acetate (see Supporting Information Figure S1).

composed of amphiphilic molecules which, at concentrations lower than CMC, exist as monomers (23), HAs consist of a mixture of organic molecules forming amphiphilic aggregates at concentrations below CMC. Consistent with this understanding, TEM images of HA$_{LFC}$ performed at concentration less than CMC (0.4 × CMC) showed relatively uniform small globular particles of about 10 nm, usually aggregated in 3–5 units in different aggregation patterns (Figures 2a and b). Above CMC, HAs cluster forming larger aggregates. TEM images of HA$_{LFC}$ solution at a concentration higher than CMC (4 × CMC) showed homogeneous particles of about 70–100 nm (Figure 2b), intermingled with the 10 nm subunits visible in Figure 2a. In some instances it was apparent that the larger aggregates were formed by the 10 nm subunits.

At pH 7, the HA MWs, characterized by high performance size exclusion chromatography (HPSEC) were 13 986 ± 435 Da (mean ± SD, $n = 3$), 16 632 ± 444 Da, 16 331 ± 554 Da and 17 376 ± 951 Da for HA$_L$, HA$_{LC}$, HA$_{LF}$ and HA$_{LFC}$, respectively (Supporting Information Table S6). Polydispersity indexes ranged from 7.7 to 9.5. These MWs represent the average MW of HA-aggregates and can thus be considered as a measure of the tendency of simple molecules to associate into HA-aggregates (29).

The lowest-MW HA$_L$ had the highest CMC (see Table S6 and Table 1). On the other hand, a commercial HA characterized by a lower MW (4100 Da) (30) than the HAs studied here had a very high CMC (7.4 g L^{-1}) (31). Using data for HAs from this and previous work (17), and commercial-HAs

(Aldrich) (30) (see Table S6), MW and CMC were found to be strongly linearly correlated ($r = 0.92$; $P < 0.05$, $n = 6$). Therefore, we conclude that, although average chemical composition of HAs cannot be used to infer CMC, average MW detected by HPSEC at pH 7 could be a measure of the tendency of HAs to aggregate. The size of the HA aggregates has been reported to be related to the nature of the molecules in the aggregates (29). These considerations suggest that any prior recommendations to use average analytical data (e.g., NMR, elemental analysis, acidity etc.) to predict surfactant properties (CMC) of HAs should be reconsidered (17) and that surfactant properties should be correlated to the kind of molecules composing the HA aggregates. Fractionation of HAs in low MW aggregates (29) indicated that larger molecular size fractions were mainly composed of alkyl chains, whereas lower MW fractions were increasingly richer in aromatic and more polar groups including carbohydrates. Therefore, we can asses that adding food waste to the lignocelluloses increased the alkyl chain content, as revealed by ^{1}H NMR, ^{13}C CP MAS NMR (see Tables S2 and S3) and C/H ratio calculated from micro-analytical data (see Table S5), and contributed to the greater tendency of HAs to cluster, as revealed by both higher average MW and lower CMC detected for HAs derived from lignocelluloses plus food wastes. A higher content of alkyl chains increases the hydrophobicity of a HAs, and increases the free energy of the water/surfactant-like system that can be reduced anticipating the process of micellization (23).

Moreover, composting also contributed to compositional changes in HAs. Elemental analysis (see Table S5) showed, after composting, an increase of the contents of both N and S, indicating higher presence of amide molecules of probably microbial origin (18). Amides have been reported to be incorporated in the aggregate/micelle thus lowering CMC. Amide molecules are probably adsorbed at the outer portion of the micelle, close to the water−micelle interface reducing the work required for micellization as a consequence of the reduction of the mutual repulsion of the polar groups (−COO$^-$) of HA (23).

These findings are supported by a multiple linear regression for CMC versus alkyl-C and N + S contents [CMC = 24.6 − 0.189 alkyl C − 2.64 (N + S); $R^2 = 0.77$, $P < 0.10$, $n = 6$] that was determined from data from this and previous works (17) and from commercial-HAs (Aldrich) (32) (Tables S3 and S5).

Solubilization of PCE by Humic Acids. When 1 mL of PCE (99% + pure, density of 1670 kg m^{-3}) was equilibrated (∼72 h) with 4 mL of HA solution, the apparent PCE solubility increased linearly (R^2 of 0.995, 0.982, 0.988, and 0.998 for HA$_L$, HA$_{LC}$, HA$_{FL}$, and HA$_{LFC}$, respectively; $P < 0.05$, $n = 7$) with increasing concentration of HAs (HAs concentration ranged from 1 to 10 g L^{-1}; pH 7 adjusted with NaOH 0.1 mol L^{-1}) (Figure 3). The ability of HAs to solubilize PCE is represented by the partitioning coefficient (K_{dom}) or its logarithmic form (Log K_{dom}) (16) (Table 2), which represents the ratio of the equilibrium concentrations of a hydrophobic organic compound within a HA complex and in the free aqueous phase (16, 33–35).

The slope of the line in Figure 3 indicates the apparent solubility of the PCE per gram of HA-surfactant (Table 2). From the slope we calculate that 0.18, 0.22, 0.14, and 0.48 g of PCE were solubilized for each gram of aqueous biosurfactant for HA$_L$, HA$_{LC}$, HA$_{LF}$, and HA$_{LFC}$, respectively. These values were 5.4−18.2 times higher than those reported for commercial HAs (0.026 g g^{-1} HA) (16).

HAs isolated in this work are more hydrophilic than commercial-HAs, as suggested by the hydrophilic/hydrophobic average ratio of 1.49 ± 0.11 (mean ± SD, $n = 4$), which is lower than the value calculated for commercial-HAs of 2.70 (32) (see Table S4). Greater hydrophilicity was the consequence of a higher content of nonionic hydrophilic
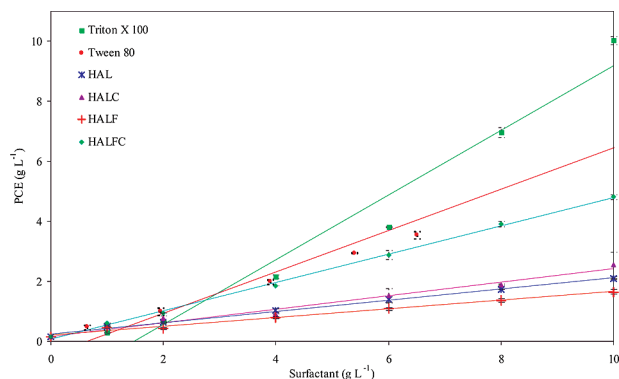
FIGURE 3. Equilibrium solubilization data for PCE by humic acids (HA) and synthetic surfactants (Triton X 100 and Tween 80). The regression of the total PCE concentration (free aqueous plus surfactant-bound), S_W^* (g L^{-1}), against the surfactant concentration is described by the equation $S_W^* = C_W^{sat} + C_W^{sat} K_{dom} C_{SU}$, in which C_W^{sat} (g L^{-1}) is the normal water solubility of PCE, K_{dom} represents the concentration of hydrophobic organic compound within humic acids that is in equilibrium with a particular concentration of hydrophobic organic compound in the free aqueous phase, and C_{SU} is the concentration of surfactant in water solution (g L^{-1}). The slope of the regression calculated as $K_{dom} C_W^{sat}$, represents grams of PCE solubilized per grams of surfactant. Data points were averaged from triplicate; error bars $\pm$ SD are not visible if they are hidden by the symbol. HA$_L$ are humic acids obtained from lignocelluloses wastes; HA$_{LC}$ are humic acids obtained from lignocellulose wastes after 15 days of composting process; HA$_{LF}$ are humic acids obtained from lignocellulose plus food waste mixture in the ratio of 1/1 (v/v); HA$_{LFC}$ are humic acids obtained from lignocellulose plus food waste mixture in the ratio of 1/1 (v/v), after 15 days of composting process.

TABLE 2. Solubilization Data of PCE for Humic Acids (HAs) Studied in This Work in Comparison with Commercial-HA and Synthetic Surfactants

	Log K_{dom} (mL g^{-1})	slope ($K_{dom} C_w^{sat}$) (g PCE g^{-1} aqueous surfactants)
HA$_L$[a]	3.09[b]	0.1842
HA$_{LC}$[c]	3.18[b]	0.2253
HA$_{LF}$[d]	2.99[b]	0.1454
HA$_{LFC}$[e]	3.50[b]	0.4720
Tween 80	3.62[b]	0.6895
Triton X 100	3.86[b]	1.0766
HA$_{commercial}$[f]	2.1	0.0263

[a] Humic acids obtained from lignocelluloses waste. [b] Data from this work, calculated considering the aqueous solubility of PCE of 0.15 g L^{-1} (37). [c] Humic acids obtained from lignocelluloses waste, after 15 days of composting process. [d] Humic acids obtained from lignocelluloses plus food wastes mixture in the ratio of 1/1 (v/v). [e] Humic acids obtained from lignocelluloses plus food wastes mixture in the ratio of 1/1 (v/v), after 15 days of composting process. [f] Humic acids from Aldrich (16).

molecules as polysaccharide and proteins as detected by ^{13}C CP MASNMR (see ^{13}C CP MAS NMR area of 47–108 ppm in Table S3).

An increase of hydrophilicity with respect to lipophilicity (hydrophile–lipophile balance designated HLB) in polyethoxylate nonionic surfactants has been reported to increase the micelle–water partition coefficient (Log K_m) of chlorinated alkenes (including PCE) based on the two-site model of solubilization: solute partitioning into the micellar hydrophobic core and dissolution and/or binding into the hydrophilic polyethoxylene shell (5). A possible explanation of the dissolution/binding process can be found in the

formation of an electron donor–acceptor complex between PCE and the nonionic portion of HAs (5). Electron donors are represented by R_2O and R_3N, contained in carbohydrate and amide fractions of HA. Electron donors are represented by the unsaturated hydrocarbons of PCE. Thus, we explain the greater solubilization of PCE by HAs from organic waste than by commercial HAs as the consequence of the presence of a more defined and consistent hydrophilic shell, able to interact with PCE.

The ability of nonionic synthetic surfactants to solubilize PCE has been reported in the literature (12, 13). When synthetic nonionic surfactants Triton X-100 and Tween 80 were dosed at the same concentration as HAs their capability to solubilize PCE were described by power equations ($y = a + bx^c$) (R^2 of 0.998 and 0.997 for Triton X-100 and Tween 80, respectively; $P < 0.05$, $n = 7$) (Figure S2). As at surfactant concentration lower than 1 g L^{-1}, no significant enhancement for PCE solubility was observed with respect to water solubility, which is consistent with previous research (12). PCE solubility for both Triton X-100 and Tween 80, was modeled starting from the concentration of 1 g L^{-1} using a linear regression (36) (Figure 3). The data were well described by linear models (R^2 of 0.962 and 0.972 for Triton X-100 and Tween 80, respectively; $P < 0.05$, $n = 6$). The slope of the line in Figure 3 indicates the apparent solubility was 1.08 and 0.69 g of PCE for each gram of aqueous surfactant for Triton X-100 and Tween 80, respectively (Table 2). These solubilities were only 2.3 and 1.5 times greater than the best-performing tested HAs (HAL$_{FC}$), but 41 and 25 times greater than commercial-HAs (16).

The development of environmentally friendly biobased surfactants that have solubilization capacities for hydrophobic organic compounds (such as PCE) that are comparable to synthetic surfactants could be of great importance for technologies that require massive utilization of surfactants. Our result showed a high capability of HAs isolated from organic wastes to solubilize PCE. Moreover, the combination of organic wastes with different compositions and simple biotechnology, such as composting to modify waste characteristics, offers the opportunity to obtain, theoretically, infinite HAs with different chemical characteristics and surfactant properties. This fact appears more important in the context of the modern issue of renewable versus nonrenewable sources of chemical compounds for the above uses (17).

Further research is needed to better understand the correlation between waste composition, composting dynamics, waste-derived HA characteristics, and their surfactant properties, including solubilizing ability for organic compounds. Such work could allow producing tailor-made HA-surfactants for different applications.

Supporting Information Available

Tables S1−S6, Figures S1 and S2, and refs S1−S5 are shown. This material is available free of charge via the internet at http://pubs.acs.org.

Literature Cited

(1) West, C. C.; Harwell, J. H. Surfactants and subsurface remediation. *Environ. Sci. Technol.* **1992**, *26* (12), 2324–2330.
(2) Chandler, D. Intefaces and the driving force of hydrophobic assembly. *Nature* **2005**, *437* (7059), 640–647.
(3) Ying, G. G. Fate, behaviour and effect of surfactants and their degradation products in the environment. *Environ. Int.* **2006**, *32* (3), 417–431.
(4) Holmberg, K. Natural surfactants. *Curr. Opin. Colloid Interface Sci.* **2001**, *6* (2), 148–159.
(5) Diallo, M. S.; Abriola, L. M.; Weber, W. J. Solubilization of nonaqueous phase liquid hydrocarbon in micellar solution of dodecyl alcohol ethoxylates. *Environ. Sci. Technol.* **1994**, *28* (11), 1829–1837.

(6) Johansson, I.; Svensson, M. Surfactants based on fatty acids and other natural hydrophobes. *Curr. Opin. Interface Sci.* **2001**, *6* (2), 178–188.

(7) Clifford, J.; Ioannidis, M. A.; Legge, R. L. Enhanced aqueous solubilization of tetrachloroethylene by rhamnolipid biosurfactant. *Curr. Opin. Interface Sci.* **2007**, *305* (2), 361–365.

(8) Berselli, S.; Milone, G.; Canepa, P.; Di Gioia, D.; Fava, F. Effects of cyclodextrins, humic substances, and rhamnolipids on the washing of a historically contaminated soil and on the aerobic bioremediation of the resulting effluents. *Biotechnol. Bioeng.* **2004**, *88* (1), 111–120.

(9) Moran, J. M.; Zogorsky, J. S.; Squillace, P. J. Chlorinated solvents in groundwater of the United States. *Environ. Sci. Technol.* **2007**, *41* (1), 74–81.

(10) Diodovich, C.; Ferrari, D.; Casati, B.; Malerba, I.; Marafante, E.; Parent-Massin, D; Gribaldo, L. Sensitivity of human cord blood cells to tetrachloroethylene. Cellular and molecular endpoints. *Arch. Toxicol.* **2005**, *79* (9), 508–514.

(11) He, J.; Ritalahti, K. M.; Yang, K. L.; Koenigsberg, S. S.; Löffler, F. E. Detoxification of vinyl chloride to ethane coupled to growth of an anaerobic bacterium. *Nature* **2003**, *424* (1), 62–65.

(12) Zhao, B.; Chen, X.; Zhu, K.; Zhu, L. Micellar solubilization of TCE and PCE by mixed surfactant: effects of surfactant partitioning and DNAPL mixing. *Colloid Surf. A* **2007**, *296* (1–3), 167–173.

(13) Kim, E. S.; Lee, D. H.; Yum, B. W.; Chang, H. W. The effect of ionic strength and hardness of water on the non-ionic surfactants-enhanced remediation of perchloroethylene contamination. *J. Hazard. Mater.* **2005**, *119* (1–3), 195–203.

(14) Chin, Y. P.; Aiken, G. R.; Danielsen, K. M. Binding pyrene to acquatic and commercial humic substances: the role of molecular weight and aromaticity. *Environ. Sci. Technol.* **1997**, *31* (6), 1630–1635.

(15) Cho, H. H.; Choi, J.; Goltz, M. N.; Parl, J. W. Combined effect of natural organic matter and surfactant on the apparent solubility of polycyclic aromatic hydrocarbons. *J. Environ. Qual.* **2002**, *31* (1)), 275–280.

(16) Johnson, W. P.; John, W. W. PCE solubilization and mobilization by commercial humic acid. *J. Contam. Hydrol.* **1999**, *35* (4), 343–342.

(17) Quagliotto, P. L.; Montoneri, E.; Tambone, F.; Adani, F.; Gobetto, R.; Viscaridi, G. Chemicals from wastes: Compost-derived humic acid-like matter as surfactant. *Environ. Sci. Technol.* **2006**, *40* (5), 1686–1692.

(18) González-Vila, F. J.; Almendros, G.; Madrid, F. Molecular alteration of organic fraction from urban waste in the course of composting and their further transformation in amended soil. *Sci. Total Environ.* **1999**, *236* (1–3), 215–229.

(19) Adani, F.; Lozzi, P.; Genevini, P. L. Determination of biological stability by oxygen uptake on municipal solid waste as derived product. *Compost Sci. Util.* **2001**, *9* (2), 163–178.

(20) Szymczyk, K.; Jańczuk, B. The properties of a binary mixture of nonionic surfactants in water at the water/air interface. *Langmuir* **2007**, *23* (9), 4972–4981.

(21) Terashima, M.; Fukushima, M.; Tanaka, S. Influence of pH on the surface activity of humic acid: micelle-like aggregate formation and interfacial adsorption. *Colloid Sur. A.* **2004**, *247* (1–3), 77–83.

(22) Hayat, M. A. *Basic Technique for Trasmission Electron Microscopy*; Academic Press: San Diego, CA, 1985.

(23) Rosen, M. J. *Surfactant and Interfacial Phenomena*; Wiley-Interscience: New York, 2004.

(24) Hayase, K.; Tsubota, H. Sedimentary humic acid and fulvic acid as fluorescent organic materials. *Geochim. Cosmochim. Acta* **1985**, *49* (1), 159–163.

(25) Wong, J. W. C.; Fang, M.; Zhao, Z.; Xing, B. Effect of surfactants on solubilization and degradation of phenanthrene under thermophilic conditions. *J. Environ. Qual.* **2004**, *33* (6), 2015–2025.

(26) Yates, L. M.; Von Wandruska, R. Effects of pH and metals on the surface tension of aqueous humic materials. *Soil Sci. Am. J.* **1999**, *63* (6), 1645–1649.

(27) Spaccini, R.; Mbagwu, J. S. C.; Conte, P.; Piccolo, A. Changes of humic acid characteristics from forested to cultivated soils in Ethiopia. *Geoderma* **2006**, *132* (1–2), 9–19.

(28) Sutton, R.; Sposito, G. Molecular structure in soil humic substances: The new view. *Environ. Sci. Technol.* **2005**, *39* (23), 9009–9015.

(29) Piccolo, A.; Conte, P.; Trivellone, E.; van Lagen, B.; Buurman, P. Reduced heterogeneity of a lignite humic acid by preparative HPSEC following interaction with an organic acid. Characterization of size-separates by Pyr-GC-MS and 1H-NMR spectroscopy. *Environ. Sci. Technol.* **2002**, *36* (1), 76–84.

(30) Chin, Y. P.; Aiken, G. R.; O'Loughlin, E. Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. *Environ. Sci. Technol.* **1994**, *28* (11), 1853–1858.

(31) Gueztloff, T. F.; Rice, J. A. Does humic acid form a micelle. *Sci. Total Environ.* **1994**, *152* (1), 31–35.

(32) Malcolm, L. R.; Mac Carhty, P. Limitations in the use of commercial humic acids in water and soil research. *Environ. Sci. Technol.* **1986**, *20* (9), 904–911.

(33) Chiou, C. T.; Malcolm, R. L.; Briton, T. I.; Kile, D. E. Water solubility enhancement of some organic pollutants and pesticides by dissolved humic and fulvic acids. *Environ. Sci. Technol.* **1986**, *20* (5), 502–508.

(34) Chiou, C. T.; Kile, D. E.; Briton, T. I.; Malcolm, R. L.; Leenheer, J. A. A Comparison of water solubility enhancements of organic solutes by aquatic humic material and commercial humic acids. *Environ. Sci. Technol.* **1987**, *21* (12), 1231–1234.

(35) Abdul, A. S.; Gibson, T. L.; Rai, D. N. Use of humic acid solution to remove organic contaminants from hydrogeologic system. *Environ. Sci. Technol.* **1990**, *24* (3), 328–333.

(36) Zhang, C.; Zheng, G.; Nichols, C. M. Micellar partitioning and its effects on Henry's law constant of Chlorinated solvents in anionic and nonionic surfactant solution. *Environ. Sci. Technol.* **2006**, *40* (1), 208–214.

(37) Yaws, L. *Chemical Properties Handbook*; McGraw-Hill Book Co.: New York, 1999.

ES702144T