Soap Determination in Sewage Sludge by High-Performance Liquid Chromatography

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A specific analytical method for soaps in the environment has been developed. Three main steps are involved in the process: (i) removal of fatty materials (lipids) other than soaps; (ii) derivatization of soaps with either bromomethyl-methoxy-coumarin or *p*-bromo phenacyl bromide to get a suitable response by ultraviolet; and (iii) separation of different carbon-chain fatty soaps by high-performance liquid chromatography, followed by either ultraviolet or fluorometry detection. The method has been applied successfully to anaerobically digested sludges produced in several waste water treatment plants. Substantial amounts of soaps have been found in sludges entering and leaving the anaerobic digestor. After conducting a mass balance, 70% soap biodegradation was found in the above digestor.

KEY WORDS: Analysis, biodegradation, environment, HPLC, sewage sludge, soap.

Worldwide soap production in 1991 was nearly nine million tons, and soap was the most important surfactant on a tonnage basis. Soap has been used continuously since ancient times and is based on natural raw materials (tallow, etc.). It has always been taken for granted that its environmental behavior was benign. In fact, this belief seems to be correct because no accumulation has been observed in environmental compartments and no aquatic toxicity problems have been detected, despite the paucity of information available from actual monitoring. There is, however, one aspect that is partially ignored and little studied, namely the potential interactions between Ca/Mg soaps and microorganisms living in aerobic and in anaerobic environmental compartments.

The (Ca + Mg)/Na ratio in anaerobically digested sludges is substantially higher than in either raw or treated waters (1). This could be an indication that organic matter, including soaps, may be precipitated as Ca/Mg salts in waste water treatment plants (WWTP). This would enable them to escape the biological treatment as they follow the anaerobic process together with all solids and sludge products.

Our work has focussed on monitoring soaps during the anaerobic treatment step (30 d average residence time) to better understand their behavior during this process. As a first step, a new specific analytical method for soap was developed and subsequently used during the monitoring work. During the last few years, several authors have been working on soap biodegradability as well as on soap analysis with modern techniques, such as high-performance liquid chromatography (HPLC).

Loehr and Roth (2) have found lower biodegradation for Ca-soaps than for Na-soaps. Novak and Carlson (3) have established criteria for anaerobic digestion operations based on the evolution of fatty acids. Novak and Kraus (4) also have studied the aerobic biodegradation of fatty acids and found an influence of unsaturation on the biodegradation, as well as different kinetics, for Ca-soaps. Wei Min *et al.* (5) used the anaerobic degradation of fatty acids to establish the criteria for microorganism inhibition.

The derivatization technique of soaps and fatty acids with some chromophore groups sensitive to ultraviolet (UV) or fluorescence has been developed to monitor either environmental or biological matrices at low levels (ppm). Examples are given in Table 1. In all cases, HPLC was used for chromatographic separation, with detection based on UV, fluorometry or electrochemical principles.

BrMmC also has been used by Voelter *et al.* (12) and Lam and Grushka (13) to derivatize fatty acids. Hayashi *et al.* (14) uses the same derivatization agent with Na or Ca salts of fatty acids and has reported on its use to detect soaps in river waters.

Our work has been based on the model developed by Hayashi *et al.* (14), although adapted to sludge samples. In some cases, we have also used derivatization techniques with *p*-bromo phenacyl bromide (DAP) (15). Regardless of the derivatization agent used, the first step when working with sludges is to extract all lipids present to avoid any interference in the subsequent analytical steps.

EXPERIMENTAL PROCEDURES

Materials. Pure (>99%) fatty acids (C_{12} to C_{18}), used in the soap form for calibration, were obtained from Chem-Service (Westchester, PA). 4-Bromomethyl-7-methoxy coumarin (BrMmC) was obtained from Sigma Chemical Co. (St. Louis, MO), as were the C_{17} fatty acid internal standard, margaric acid (purity: 97% by HPLC). *p*-Bromo phenacyl bromide (purity >97%), ethylenediaminetetraacetic acid-3K (EDTA-3K), potassium carbonate (purity 97%) and 18-crown ether (1,4,7,10,13,16-hexa-oxacyclooctadecane) (purity >99%) were all obtained from Merck (Darmstadt, Germany). Lichrocart columns used in HPLC were also obtained from Merck.

Samples. We have used anaerobically digested sludges from the WWTP of Alicante, Estepona (Málaga) and Madrid in Spain and also from Austin, TX. We have also used the raw sludge (influent to the anaerobic digestor) of the Estepona treatment plant. In all cases the sludges were dried in air and stabilized with 0.1% formaldehyde. Once dried they were homogenized and ground in an agatha mortar.

Methods. The Na-salts of extra-pure fatty acids were prepared by precipitation with caustic soda in acetonic media. The precipitates were washed with petroleum ether and dried at 60°C in an electric oven. The corresponding calcium salts were obtained from the Na-salts by precipitation with calcium chloride in hot water, then washing the precipitates with distilled water and finally drying at 80°C in nitrogen atmosphere.

The following analytical methods have been used: (i) Soxhlet extraction of all lipids present in the sludges

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TABLE 1

Examples of Chromophoric Derivatization for Soaps and Fatty Acids

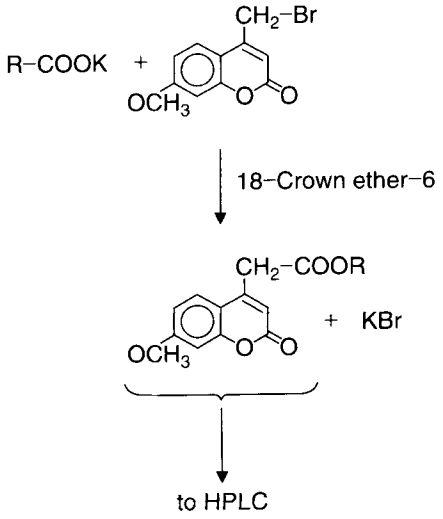
Authors	Derivatization	References
Politzer *et al.*	1-Benzyl-3 *p*-tolyl triazine	6
Durst *et al.*	*p*-Bromo phenacyl bromide (DAP)	7
Hoffmann and Liao	*p*-Methoxy aniline	8
Dünges	4-Bromomethyl-7 methoxy coumarin (BrMmC)	9
Shimada *et al.*	3-Bromoacetyl-1,1'dimethyl ferrocene	10
Hublet and Eisenreich	Phenacyl ester	11

with petroleum ether (40–60°C) for 70 h, according to Almendros *et al.* (16). (ii) Derivatization of extracted sludge with either BrMmC or DAP. In the first case (BrMmC), 5 mg of the Ca-salt of margaric acid, as internal standard to quantitate the soap by HPLC, was added to 0.5 g of extracted dried sludge. Then, 0.3 g K_2CO_3 and 0.1 g EDTA-3K are also added, and the mixture is diluted in 2.5 mL of water, heated to boiling for a few minutes and subsequently dried at 60°C in an oven. The reaction produced is:

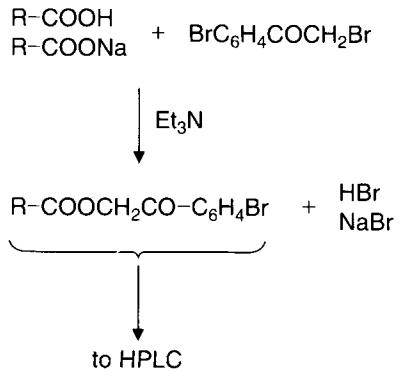
$$\text{Ca-soap} + K_2CO_3 + \text{EDTA-3K} \rightarrow \text{K-soap} + \text{EDTA-Ca} + K_2CO_3 \text{ (excess)} \quad [1]$$

The potassium soaps are then extracted in a Soxhlet with 200 mL methanol for 24 h. At the end of the extractions, the methanol is eliminated by evaporation to obtain a total of 100 mL solution in a volumetric flask.

This solution (0.5 mL) is placed in a microreactor, and the methanol is eliminated to dryness with a cold nitrogen stream, and then 1 mL acetone plus 1 mL of the crown ether (0.07% in acetone) and 0.2 mL BrMmC (0.1% wt in acetone) are added. The mixture is digested for 30 min at 70°C, and the final product is immediately injected into the HPLC equipment. Rapid injection is recommended because the color of the chemical complex may degenerate with time. The reaction produced is as follows:



For the derivatization with DAP, the procedure is as follows: 0.1 g of extracted sludge is placed in a glass minireactor with 5-mL vol before adding 1 mL acetone, 0.5 mL DAP (12 mg/mL in acetone) and 0.5 mL triethylamine (10 mg/mL in acetone). The reactor is closed and kept at 60°C overnight. The reaction product is then settled, and the liquid layer is directly injected into the HPLC equipment. The reaction produced with DAP is as follows:



HPLC operating conditions. Hewlett-Packard (Palo Alto, CA) HP-1090 L equipment was used with pump gradient system, automatic injection and variable UV wavelength detector. For fluorimetric detection, an HP-1046 A equipment with programmable fluorescence detector was used. Chromatographic separation was carried out in a Lichrocart column (10 cm/4.6 mm) RP-8, 5 μm (Merck).

Solvent A was a methanol/water (20:80, vol/vol) mixture, and solvent B was acetonitrile. The gradient used was A = 40% at time 0, and A = 25% at 10 min. The flow rate was kept constant at 1 mL/min during the run (40 min total approx.). The UV wavelength used for detection was 254 nm with the DAP derivative. With BrMmC we used fluorescence with excitation at 328 nm and emission at 380 nm. The sample injection volume was 10 μL.

RESULTS AND DISCUSSION

Recoveries. The anaerobically digested sludge from a WWTP of Madrid (La China) containing 25 mg/g of total soap was spiked with different quantities of Ca-salt of margaric acid (1, 5, 10 and 20 mg/g dry sludge) and re-equilibrated by agitation for 3 h.

Figure 1 reflects the analytical results obtained on spiked sludge samples by taking into account the results of the blank sample. The minimum recovery obtained was

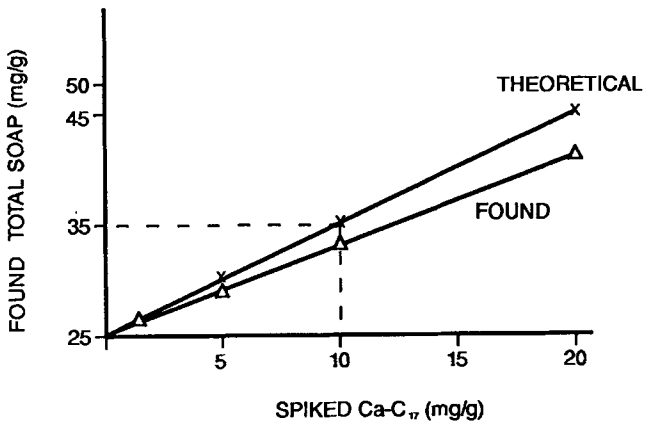


FIG. 1. Soap analysis in sludge—recovery.

nearly 90% and, therefore, the method can be considered as adequate to be used without significant errors.

Table 2 summarizes the data of percent recovery, repeatability and reproducibility. The limit of detection, based on three times the background noise (IUPAC), is 10 pmol for calcium margarate.

Soap solubility. The corresponding Na- and Ca-salts of margaric acid have been extracted separately in a Soxhlet system for 70 h with petroleum ether and methanol. After elimination of the solvents by evaporation, the amount of salt extracted was determined gravimetrically. The results obtained are summarized in Table 3.

The test was carried out to be sure that neither Na- nor Ca-soaps are present in the extract obtained from sludges with petroleum ether. This also can be observed in Figures 2–4, which show the following chromatograms: an anaerobically digested sludge spiked with internal standard (C_{17}) in Figure 2; standards of Ca-soaps in Figure 3; and the ether extract obtained after a 70-h Soxhlet extraction in Figure 4.

In the above three cases, BrMmC was used as the derivatization agent. The ether extract is free of C_{12}–C_{18}, and thus all potential soaps (Ca or Na) have remained in the residue after the ether extraction.

Fatty acid, Na-soap and Ca-soap differentiation. We have used different pure standards of fatty acids or their

TABLE 2

Soap Analysis in Sludges

Recovery of Ca-C_{17} soap				Repeatability and reproducibility		
Spiking level (mg/g)	Determined level (mg/g)	Recovery (%)	SD	Spiking	SD repeatability	SD reproducibility
1	0.95	95.0	0.014	1	0.010	0.016
5	4.40	88.0	0.018	5	0.009	0.020
10	9.27	92.7	0.068	10	0.010	0.070
20	19.01	95.0	0.291	20	0.020	0.290

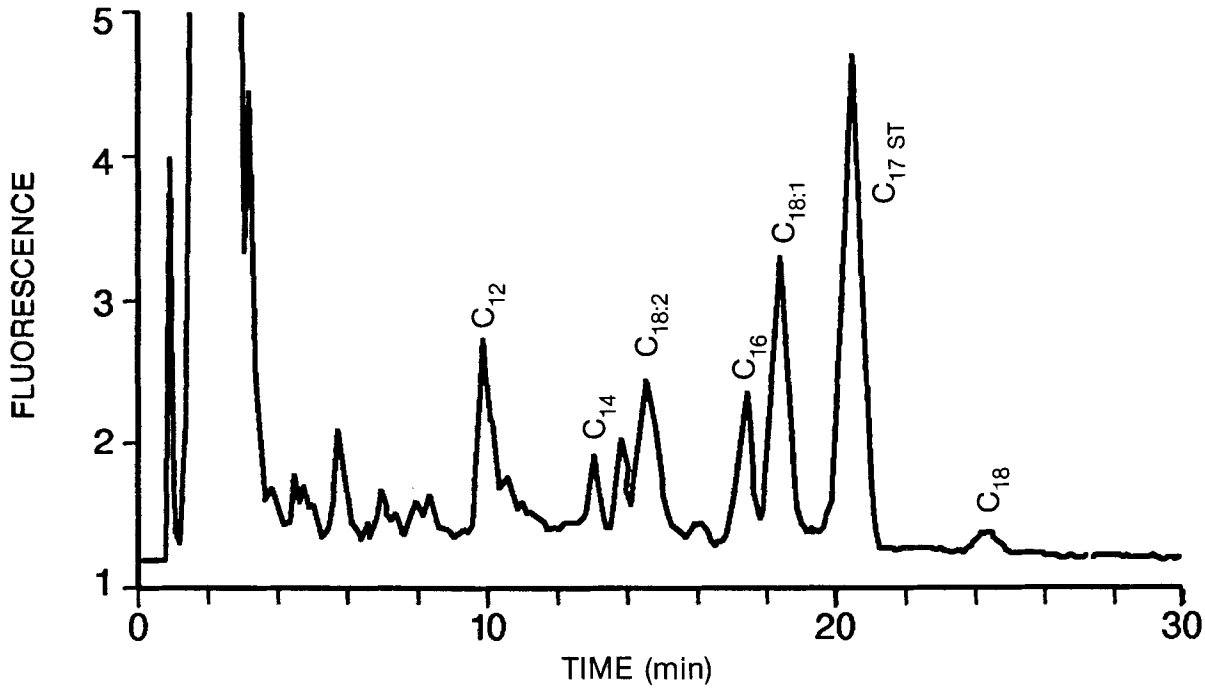


FIG. 2. Soap analysis in sludge—typical chromatogram. Anaerobically digested sludge from Alicante (Spain) plant. Derivatization, 4-bromomethyl-7-methoxy coumarin.

TABLE 3

Solubility of C_{17} Soaps

	Solvent	
	Petroleum ether	Methanol
C_{17}-Na	Insoluble 100%	Soluble 100%
C_{17}-Ca	Insoluble 100%	Partially soluble (20% approx.)

Na- or Ca- salts and, after derivatization with DAP or BrMmC, their reactivities can be summarized as shown in Table 4. Based on these results, it was possible to first check that the ether extract does not contain either Na- or Ca- soaps, and then that basically all soap found in the extracted sludge is in the form of Ca-soap, which justifies the idea mentioned in the introduction of this paper.

Quantitation. All Ca-soaps in this study (C_{12}–C_{18} even, saturated and unsaturated) have been quantitated by using C_{17} (margaric acid) as internal standard. The calibration curves have been obtained by plotting peak areas *vs.* soap quantities used in the range of concentrations indicated in the method.

Results for WWTP sludges. The analytical method for soap has been applied to anaerobically digested sludge produced in several WWTPs in Spain and the United States. All plants monitored operate mainly with domestic waste water. The results obtained are summarized in Table 5. The carbon-chain distribution of the fatty acids in the analyzed soaps are shown in Table 6.

Neither the total soap concentration nor the fatty acid distribution is substantially different for the different WWTPs monitored. The presence of soap in anaerobically digested sludges indicates that the biodegradation was

TABLE 4

Reactivity of Fatty Acid and Na- and Ca-Soaps[a]

Reagent	R-COOH	R-COONa	$(R\text{-}COO)_2Ca$
DAP	+	+	− (app)
BrMmC	−	+	− (app)
BrMmC + K_2CO_3 EDTA-3K	+	+	+

[a]+, Derivation occurs; −, derivation does not occur; − (app), derivation occurs insignificantly. EDTA, ethylenediaminetetraacetic acid. See Table 1 for other abbreviations.

TABLE 5

Soap Analysis in Sludge

Treatment plant	Soap in dry sludge (mg/g)
Alicante (Spain)	23.6
Madrid (Spain)	25.8
Estepona (Spain)	51.9
Austin (U.S.)	18.8

TABLE 6

Fatty Acid Distribution in Sludge (wt%)

WWTP[a]	C_{12}	C_{14}	C_{16}	$C_{18:2}$[b]	$C_{18:1}$[c]	C_{18}
Alicante	15.8	6.3	17.5	21.8	34.1	4.5
Madrid	11.6	6.0	34.3	16.4	23.5	8.2
Estepona	8.9	5.3	44.1	14.3	17.7	9.7
Austin	11.3	9.4	27.2	25.6	19.8	6.7

[a]Waste water treatment plants. Locations of plants given in Table 5.
[b]$C_{18:2}$, linoleic.
[c]$C_{18:1}$, oleic.

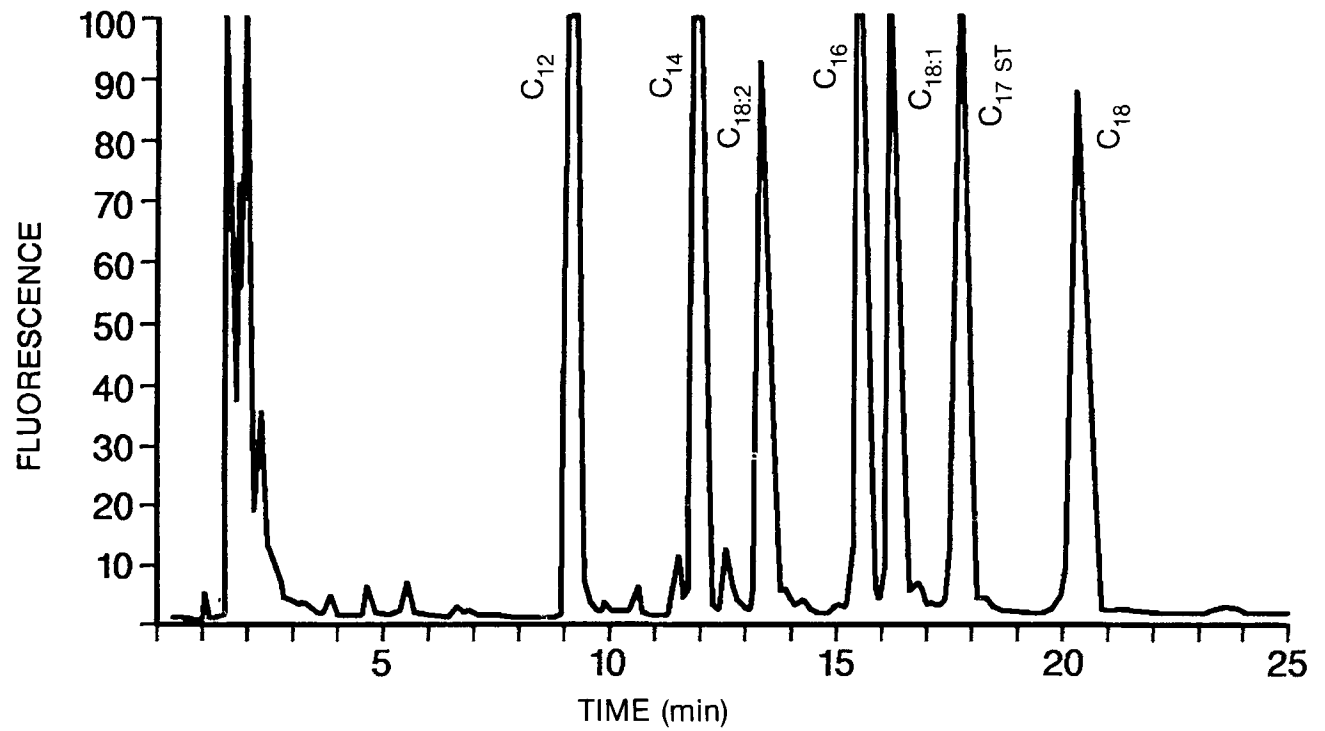


FIG. 3. Soap analysis in sludge—typical chromatogram. Standard mixture, 4-bromomethyl-7-methoxy coumarin.

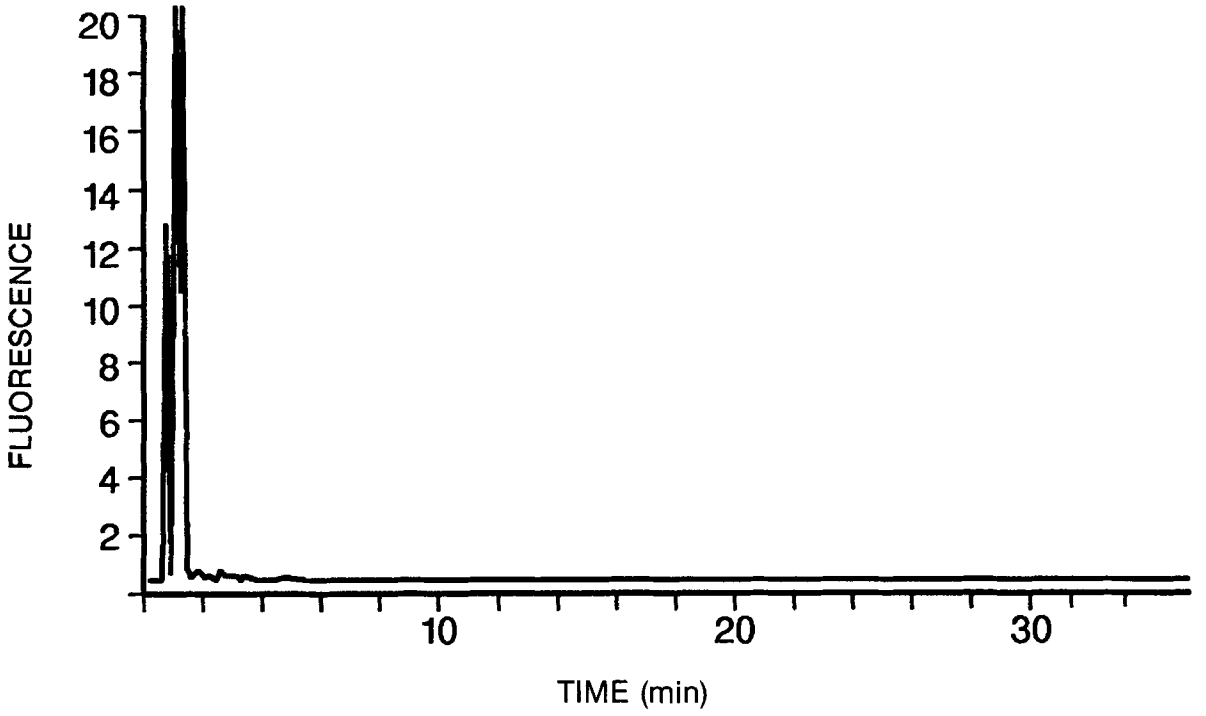


FIG. 4. Soap analysis in sludge—typical chromatogram. Ether extract of Alicante sludge. Derivatization, 4-bromomethyl-7-methoxy coumarin.

not completed in the digestor. To better evaluate this, we have conducted a mass balance in the Estepona treatment plant to know the biodegradation level reached in the digestor. The results were: soap in influx to anaerobic digestion, 231 kg/d; soap in digested sludge, 69.1 kg/d; and biodegradation, 70.1%. There is 30% soap left in the sludge that is normally used in soil amendment operations.

It is our understanding that the reason for not reaching 100% biodegradation is due to the agglomeration degree of Ca-soaps in the anaerobic digestor, which may inhibit the bioavailability of such soaps to the microorganisms. It is highly probable that soap present in sludge-amended soils will further biodegrade as a consequence of being finely dispersed and in contact with air.

The numbers in Table 4 also indicate that Ca-soap precipitation is not a direct function of water hardness because even at low water hardness (Madrid, <100 ppm as $CaCO_3$) the amount of Ca-soap is similar to instances where high water hardness (Alicante, 600 ppm as $CaCO_3$) is prevalent. Differences in soap content may also be due to other factors, such as pH, temperature and ionic strength, in addition to water hardness.

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