Determination of Structure and Origin of Refractory Organic Matter in Bio-epurated Wastewater via Spectroscopic Methods. Comparison of Conventional and Ozonation Treatments

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With the widely used activated sludge treatment most of the organic matter (OM) of wastewater can be removed, but the composition and origin of the refractory OM in treated water are poorly known. The structures occurring in treated water are more difficult to elucidate than those of the OM originally present in wastewater, since the classical hydrolysis—chromatography methods are not suitable for the analysis of such samples. ^{13}C and ^{15}N solid-state Nuclear Magnetic Resonance (NMR), liquid-state ^{1}H NMR and infrared (IR) were used in the present study. A treatment procedure including a stage of ozonation of the sludge, used to reduce the sludge production, was studied along with a conventional treatment line. The OM in the water treated through the ozonated line was mainly composed of proteins and sugars moieties, while the aliphatic compounds dominant in the wastewater were efficiently removed. The conventional treatment was more efficient in terms of OM removal but led to the formation and/or enrichment of unknown products with high oxidation and branching levels. These products contained C=O groups, while the nitrogen was found in amide groups, refractory to the treatment.

Introduction

The organic matter (OM) of wastewater is highly heterogeneous in size and composition (*1*). The most usual technique to remove OM from wastewater is the biological treatment with activated sludge. In the aeration basin, a complex population of microorganisms degrades and metabolizes the OM. The treated water is then separated from the microbial biomass by sedimentation in a clarifier. The concentrated biomass is partly recycled to the aeration basin, to keep a high concentration of microorganisms. The concentration of OM remaining in the treated water must be as low as possible, since the effluent is generally discharged in the aquatic environment. As an alternative, the use of the treated water for irrigation is becoming more and more frequent, especially in arid regions (*2*). In both cases, it is important to know the composition of the residual OM, to evaluate its influence on the quality and quantity of natural OM.

Apart from its concentration and its global composition, obtained by colorimetric measurements, very few data are available on the composition and structure of residual OM (*3*). The origin of residual OM remains also unclear. It is highly probable that this OM is not merely a residue of the pollution originally present in the wastewater but is mainly composed of products generated during the treatment, generally called Soluble Microbial Products (SMP) (*4*). These products resist further bacterial degradation, or at least, they cannot be degraded at the time scale of treatment operation (*5*). The recent review of Barker and Stuckey (*6*) underlines the difficulties still encountered in finding a commonly accepted definition of the SMP, in analyzing them or in determining their biodegradability.

The chemical characteristics of the products so formed are still to elucidate (*3*, *6*, *7*). The major difficulty for the chemical analysis of complex OM in wastewater and treated water resides in the preliminary hydrolysis used to break down complex structures (*8*). If molecular composition can be determined in a bacterial media like activated sludge (63% of the Total Organic Carbon (TOC) characterized), only 50% of the OM of wastewater and 20% of the OM of treated water can be recognized as simple biological molecules after hydrolysis (*8*). The same analytical difficulties are encountered in studies of complex OM from other environments, like ocean particulate OM (*9*) and dissolved OM of surface waters (*10*).

Solid-state Nuclear Magnetic Resonance (NMR) with cross-polarization and magic angle spinning (CPMAS) (*11*) is a powerful technique for the examination of the chemical composition of complex OM, since it can be used on the bulk sample, without prior chemical treatment or dissolving. The resolution of CPMAS ^{13}C NMR, although less than in liquid-state experiments, has been greatly improved, thanks to careful optimization of the instrument parameters. This allows the use of this technique on various kinds of samples to describe the functionalities of organic carbon. Nitrogen is also an important element in biological molecules, but nitrogen study via NMR has long been impeded because of sensitivity problems. The most abundant isotope, ^{14}N, cannot be studied via NMR, because of its large quadrupolar moment, that broadens dramatically the resonance signals. High-resolution NMR is only possible with ^{15}N isotope. However, the sensitivity of this technique is very low, because of both the low natural abundance of this isotope (0.37%) and the low value of its gyromagnetic ratio. Until recently, ^{15}N NMR spectra were only acquired for ^{15}N-enriched material. Thanks to important improvements of the instruments and method (*12*), it is now possible to obtain ^{15}N spectra at natural abundance in samples with rather low nitrogen content (around 1%).

Solid-state ^{13}C and ^{15}N NMR have been widely used during the past years to study the complex chemical structures of OM in soils, but it was rarely applied to the characterization of the OM of wastewater and treated water. Only the humic and fulvic fractions of the OM extracted from wastewater

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treatment effluents have been studied so far via ^{13}C NMR spectroscopy (2, 13), but this method was not used for the bulk OM.

In the present study, solid-state ^{13}C and ^{15}N NMR were used to characterize the transformations of the OM upon biological treatment of wastewater. These analytical methods were used along with liquid-state ^{1}H NMR, which gives complementary information on proton environment in OM and Fourier Transform Infrared (FTIR). The combination of these methods provides an in situ insight into the functional groups present in the OM and was used to compare the chemical properties of waters treated with a conventional activated sludge treatment and with a process involving the ozonation of a part of the recycled sludge.

Experimental Section

Samples Collection. Samples originated from a pilot plant located in Le Pecq (France), where two lines were operated under different conditions. The concentration of suspended solids in the activated sludge was 4 g/L. The treatment line 1 was operated according to the conditions of Sakai et al. (14): 20 L of sludge were ozonated per day in an ozonation reactor (8 L), with an ozone dosage to the ozone reactor of 0.04 g O_3/g of suspended solids. In the conventional treatment line 2, the carbon and nitrogen elimination was achieved with a sludge retention time of 10 days and the hydraulic retention time in the aeration tank was 20 h. Wastewater and treated water were collected as 24 h composite samples, to get rid of possible daily variations in their composition. Wastewater was collected at the entrance of the aeration basin and treated water at the outlet of the clarifier. Samples were collected during a dry spring period.

Sample Preparation. The samples collected were pumped, successively through prefilters of 100, 50 and 1 μm porosity and through a cation exchanger that replaced other cations with Na^+, to a 50 L reservoir, kept at 4 °C. After filtration, the water was circulated through an electrodialysis (ED) unit. The conductivity in the sample (between 900 and 1200 μS initially) decreased slowly, and the experiment was stopped when it reached 50–100 μS. After electrodialysis, the 50 L of water was concentrated by rotary evaporator, at a temperature of 30 °C, at a rate of about 1 L/h, to a volume of 100 to 200 mL, and then freeze-dried.

Elemental Analysis. C (total and inorganic), H and N contents of the samples were determined by standard elemental analysis at the "Service Central d'Analyse" of CNRS, Vernaison.

Total Organic Carbon (TOC). TOC in liquid fractions was measured with a Dohrman DC80 analyzer after acidification of samples and elimination of inorganic carbon. Organic carbon was oxidized by potassium persulfate oxidation catalyzed by ultra-violet light.

Chemical Oxygen Demand (COD) (mg O_2/L). COD was measured by the potassium bichromate method (AFNOR T90–101).

Solid-State ^{13}C NMR. Using the standard cross-polarization magic angle spinning technique (CPMAS), ^{13}C NMR spectra were recorded at 100 MHz for ^{13}C on a Bruker MSL 400. Freeze-dried sample were placed into a rotor and spun at a frequency of 4 kHz at the magic angle. Contact time was 1 ms, with a recycle time of 5 s. The spectrum was the result of 12×10^3 scans for wastewater, 6×10^3 scans for treated water from line 1, and 16×10^3 scans for treated water from conventional line 2. Chemical shifts are referred to an external reference: tetramethylsilane (TMS) (0 ppm).

Solid-State ^{15}N NMR. Freeze-dried samples were spun at 5.5 kHz in a zirconium dioxide rotor. ^{15}N NMR spectra were recorded at 40 MHz for ^{15}N on a Bruker DMX 400, acquiring 3×10^5 scans for wastewater, 2×10^6 for the treated water from line 1 (ozonated sludge), and 1×10^6 scans for treated

TABLE 1. Bulk Features of the Water Samples Before and After Electrodialysis (ED) and Elemental Composition (After ED) of the Freeze-Dried Material Isolated from the Wastewater and Treated Waters from Line 1 (Ozonated Sludge) and Line 2 (Conventional)

		treated waters	
	wastewater	line 1	line 2
TOC before ED (mg/L)	105	14.7	8.0
TOC after ED (mg/L)	66	12.9	7.3
TOC recovery (%)	63	88	92
conductivity[a] before ED (μS)	1222	1075	965
conductivity[a] after ED (μS)	112	65	95
conductivity decrease (%)	91	94	90
elemental analysis after ED			
total C (%)	33.7	17.6	10.1
inorganic C (%)	<0.1	0.9	2.8
organic C (%)	33.7	16.7	7.4
N (%)	2.4	1.95	1.05
atomic ratio (organic C)/N	14.0	8.6	7.0
ash (%)	33.0	39.2	50.4

a Conductivity is measured at 20 °C.

water from conventional line 2. Chemical shifts are expressed relative to nitromethane (0 ppm). The pulse delay was 300 ms for the wastewater and 150 ms for the two treated waters.

Liquid-State ^{1}H NMR. Freeze-dried samples were solubilized in D_2O (SDS, purity > 99.97%). Liquid NMR spectra were recorded on a Bruker 400 MHz, acquiring 250 scans for the wastewater and 1000 scans for the two treated waters. Chemical shifts are expressed relative to water at 4.8 ppm.

Fourier Transform Infrared Spectroscopy. FTIR spectra were recorded on freeze-dried samples as KBr pellets, using a Bruker IFS 48 spectrometer.

Results and Discussion

Bulk Characterization of the Samples. The bulk characteristics of the samples before and after electrodialysis are presented in Table 1. The high content of inorganic matter in the crude samples (high conductivity before electrodialysis) may affect the spectroscopic analyses, because it decreases the OM concentration and hence the sensitivity of the analyses. In addition, the response of minerals can disturb the response of OM, especially for infrared experiments. The electrodialysis step was thus essential to isolate the OM. However, if the electrodialysis procedure appeared to be very efficient in salt elimination (the conductivity was decreased by 91%, 94% and 90% for wastewater, treated water from line 1 and line 2, respectively, Table 1), it also resulted in some OM loss. The organic carbon recoveries were of 88 and 92% for the treated water from line 1 and line 2, respectively, but only of 63% for the wastewater. Such losses in the case of the wastewater may be due to adsorption on membranes, but they may also include volatile compounds, known to account for about 10% of the original TOC (8). After ED, the ash content of treated water from line 2 (50%) was higher than that of treated water from line 1 (39%) and of wastewater (33%) (Table 1). This is in agreement with the larger value of the conductivity in the treated water from line 2, combined with a lower TOC value (Table 1).

The TOC decreases from 105 mg/L in the wastewater to 14.7 mg/L with treatment 1 (86% decrease) and 8.0 mg/L with treatment 2 (92% decrease). The biological treatment is efficient for the removal of organic carbon. The ozonation of the sludge is known to result in an increase in the amount of TOC rejected (15), The wastewater had the highest C/N ratio (14.0). The decrease of this ratio was more pronounced with treatment 2 (7.0) than with treatment 1 (8.6). This trend indicates that the aliphatic character of the wastewater is
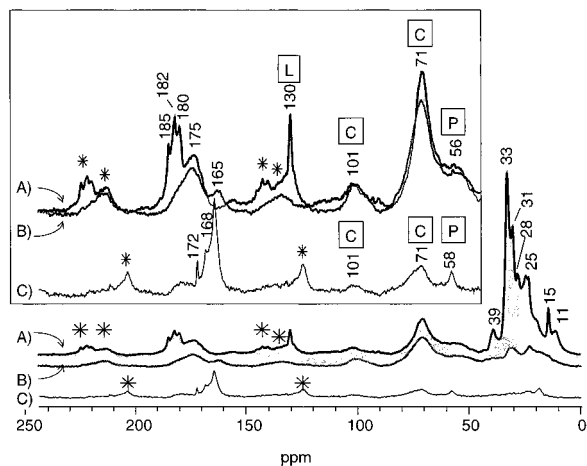
FIGURE 1. ^{13}C NMR spectra of wastewater (A), effluent after treatment 1 with ozonated sludge (B), effluent after conventional treatment 2 (C). Chemical shifts in the 50−250 ppm range are expanded in the inset. *: spinning sidebands. Origin of the peaks: C (carbohydrates); L (lignin); P (proteins).
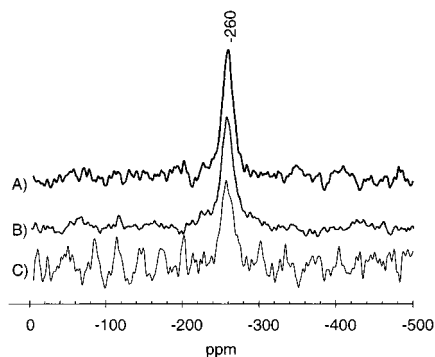

FIGURE 2. ^{15}N NMR spectra of wastewater (A), effluent after treatment 1 with ozonated sludge (B), effluent after conventional treatment 2 (C). The unique peak observed on this figure has a chemical shift of about −260 ppm.

FIGURE 3. ^{1}H NMR spectra of wastewater (A), effluent after treatment 1 with ozonated sludge (B), effluent after conventional treatment 2 (C). All the samples were dissolved in D$_2$O. Note scale-break for the peak at 1.8 ppm. Intensity of peak at 1.8 ppm is approximately 4 times the intensity of the peak at 1.0 ppm.

FIGURE 4. IR spectra of wastewater (A), effluent after treatment 1 with ozonated sludge (B), effluent after conventional treatment 2 (C).

highly reduced in the treated waters, where N-containing structures are enriched.

Spectroscopic Analyses. After ED, wastewater and treated waters are analyzed by solid-state CPMAS ^{13}C and ^{15}N NMR (Figures 1 and 2), liquid-state ^{1}H NMR (Figure 3) and FTIR (Figure 4).

The CPMAS ^{13}C NMR spectra (Figure 1) can be divided into 5 main regions, corresponding to different carbon environments.

(1) In the aliphatic region (0−45 ppm), resonances can originate from fatty acids and other lipids (16) or from proteins (17). In the wastewater spectrum (Figure 1A), three main peaks are observed between 11 and 33 ppm, corresponding to terminal methyl groups (maximum at 15 ppm, shoulder at 11 ppm), aliphatic carbons β to heteroatoms or branching (ca. 25 ppm) and methylene groups (30−35 ppm). A small peak at 39 ppm is also noted. It may be assigned to carbon atoms β to an hydroxy group. The predominance of the signal at 33 ppm indicates that aliphatic carbons essentially occur as long polymethylenic chains. This peak has completely disappeared in the spectrum of the treated water from conventional line 2. The resonance at 25 ppm, likely reflecting the presence of some amino acids, is important in the spectrum of wastewater, sharply decreased in the spectrum of treated water from line 1, and is no longer observed after treatment 2. The terminal methyl groups observed in the spectrum of the wastewater occur at the characteristic chemical shift of 15 ppm, and a shoulder at 11 ppm indicates
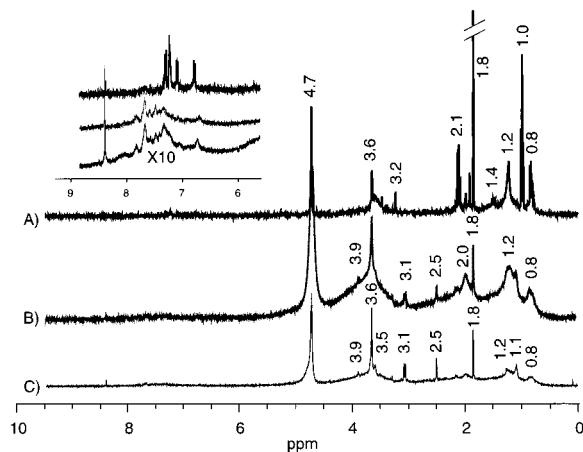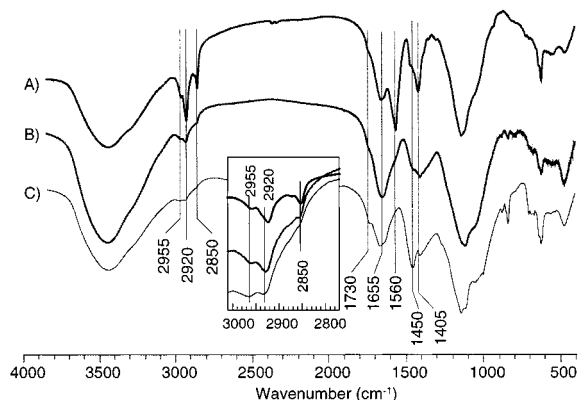
that some methyl groups are located in a position β to methyl branching. The methyl peaks are markedly decreased in the spectrum of the treated waters.

(2) Between 45 and 110 ppm are found the resonances of aliphatic carbon atoms linked to heteroatoms (oxygen and nitrogen). In the spectra of wastewater and treated waters, a peak at 71 ppm, with a shoulder at 56 ppm and a peak centered around 100 ppm are characteristic of three different types of functional groups. The peak at 56 ppm corresponds to carbon atoms substituted by amino groups, i.e., in peptides and amino acids (17), and is also sometimes attributed to methyl esters and methyl ethers in lignin derivatives (18). The peak at 71 ppm is due to O-alkyl C such as C-2, C-3 and C-5 atoms of polysaccharides (18), and chemical shifts of the anomeric carbon atoms (C-1) of carbohydrates occur around 100 ppm.

(3) The resonances of unsaturated carbons are found between 110 and 160 ppm, but this region is affected by the presence of the spinning sidebands of the peaks of the carboxyl/amide region (Figure 1). The spinning sidebands occur due to incomplete removal of chemical shift anisotropy. The only peak detected in this region in the spectrum of the wastewater (130 ppm) corresponds to aromatic-C such as those found in lignins or to olefinic-C. The presence of this resonance at 130 ppm indicates that the peak at 55 ppm of the wastewater spectrum may also originate, at least partly, from lignin derivatives. This unsaturated component of the OM is completely eliminated by the biological treatment; in

the spectra of the treated waters, only spinning sidebands can be seen in the aromatic region.

(4) The chemical shifts of C=O of carboxylic acids, esters and peptides are found between 160 and 190 ppm, producing several overlapping peaks. In the wastewater, a complex peak occurs between 180 and 185 ppm. It should correspond to carboxyl groups of low molecular weight aliphatic acids. The latter are completely removed by the treatments. The resonance at 175 ppm is generally assigned to amide groups of peptides (17) and amino sugars (19) and carboxyl groups of lipids (16). The groups responsible for this peak are not eliminated after treatment 1, but they disappear in the treated water from the conventional line 2. In the treated water from line 1, some of these groups may derive from proteins, and may be related to the peak at 58 ppm, still present after treatment 1 (Figure 1B). In contrast, the very low intensity of the peak at 30 ppm shows that only a very small part of this carboxyl peak can be attributed to lipids (Figure 1B). A peak at 165 ppm appears in the treated waters and especially in that from line 2, where a shoulder at 168 ppm is also observed. The nature of the chemical groups thus formed or preserved upon treatment is still to elucidate, although the resonances of esters groups in unsaturated compounds could occur around 165 ppm.

(5) In the last region (190–210 ppm), corresponding to carbonyl groups, no significant peaks are found, and only the spinning sidebands of the carboxyl/amide region can be seen.

^{13}C NMR observations indicate that the dominant moieties are aliphatic in the wastewater, polysaccharides in the treated water from line 1, and carboxyl, ester or amide-containing groups in the treated water from the conventional line 2. The relative intensity of the aliphatic signal in the ^{13}C NMR spectra is highly reduced for treated waters, compared to that of the wastewater. This is in accordance with the decrease in the C/N ratios after the biological treatments (Table 1). Some aliphatic chains are still present in the treated water from line 1 (ozonated sludge); however, the aliphatic signal is less intense in this sample than that related to polysaccharide moieties (71 and 100 ppm).

In the spectra of the two treated waters, the carboxyl signal is modified when compared to the corresponding region in wastewater spectrum. In the OM of the treated water from the conventional line 2, the products formed and/or enriched during the treatment become dominant. These products are highly oxidized, with a dominant peak in the C=O region, and could contain ester groups.

The spectra of the bulk OM of the treated waters obtained in this study are rather different from the spectra of the humic substances extracted from a bio-epurated treated water studied by Peschel et al. (13). The fraction adsorbed on a XAD-2 resin, examined in the latter study, exhibited a peak at 175 ppm, relatively intense peaks characteristic of polysaccharides, and some weak resonances in the aromatic region. The spectrum of the hydrophobic acid extracted from a treated water by Chefetz et al. (2) also showed a resonance in the aromatic region, a weak component in the polysaccharide region, and a dominant peak corresponding to CH$_2$. The peak at 165 ppm observed in the treated waters studied here seems to be rather uncommon, since it has not been mentioned in other studies of humic substances extracted from the OM of soils or waters.

Figure 2 shows the solid-state ^{15}N NMR spectra of the OM from the wastewater and the two treated waters. Despite low nitrogen abundances (ca. 1% for the most depleted sample, Table 1), solid-state ^{15}N NMR was applied successfully on the material collected before and after the biological treatments. The ^{15}N spectra do not change much with the biological treatment and are all dominated by a signal at −260 ppm, assigned to amide/peptide groups (between −240

and −285 ppm). The spectrum of the treated water from line 2, which is characterized by the lowest concentration of nitrogen (Table 1), suffers from a modest signal-to-noise ratio (Figure 2C), but it still clearly shows that amide/peptide is the dominant form of nitrogen. This amide peak should correspond to the peaks at 58 and 175 ppm observed in the ^{13}C NMR spectra of the wastewater and treated waters (Figure 1), while the peak at 165 ppm is certainly due to another complex form of C=O groups.

In the spectra of the wastewater and of the treated water from line 1 (ozonated sludge), the broad shoulder at the low-field basis of the main amine/peptide peak (−200 to −240 ppm) could be due to heterocycles, observed for example in melanoidin structures (20), or in indole and imidazole moieties of some amino acids (histidine, tryptophan), occurring at −245 ppm. In the spectrum of the treated water from line 2 (Figure 2C), the shoulder at −200 to −240 ppm may also be present, but it is not distinguishable from the noise. No signal distinguishable from the noise is found for the amino groups of free amino acids or free amino sugars (−325 to −375 ppm) in any of the three samples.

Apart from the main amide peak, no other signal can be unambiguously identified in the ^{15}N NMR spectra, indicating that most, if not all, of the organic nitrogen in the examined three water samples is bound in peptide-like structures. Amides are the most current form of nitrogen in biological samples, occurring in some structural components of bacteria (peptidoglycans), and in proteins. In a recent study (8), we have shown that 90% of the nitrogen of treated water are not characterized as amino acid nitrogen via analysis after hydrolysis. The present results reveal that this uncharacterized nitrogen corresponds to amides, with an unusual resistance to chemical and biological hydrolysis. In the OM refractory to the most efficient treatment, such amide groups are likely to be protected from biodegradation within complex, highly cross-linked organic structures. Such a steric protection would decrease the accessibility of these normally labile functions for microorganisms and their extracellular enzymes. The resistance of some amide-containing functional groups toward microbial and chemical degradation was previously demonstrated in algaenans (21), in soil samples (20), in the dissolved OM of ocean water (22) and in some immature kerogens (23).

Chemical shifts in proton NMR spectra (Figure 3) provided further information on the functional groups present in the samples. As for the ^{13}C NMR spectra, the effect of an electronegative substituent is to displace the proton resonance to higher chemical shifts. Some protons can be rapidly exchangeable, and the dissolution of the sample in D$_2$O decreases the intensity of the peaks resulting from groups exchanging their protons with deuterium, for instance amide, amine or hydroxyl groups.

The ^{1}H NMR spectrum can be split into three main regions.

(1) The chemical shifts between 0.8 and 3 ppm are attributed to aliphatic protons. Signals are especially intense in this region of the spectrum of the wastewater, with several well resolved peaks. The peak at 0.8 ppm is due to methyl protons in aliphatic structures and corresponds to the peak at 15 ppm in ^{13}C NMR. The peak at 1.2 ppm is due to protons in methyl groups α to hydroxy functions. The presence of such units is also reflected by the peaks at 25 and 39 ppm in CPMAS ^{13}C NMR. The dominant peak at 1.8 ppm is due to protons in polymethylenic chains. Its intensity is in agreement with that of the peak at 33 ppm in ^{13}C NMR. The peaks in the 2.1 and 2.5 ppm range originate from protons α or β to an acid or amine group.

(2) The chemical shifts between 3 and 4.8 ppm can originate from a wide range of protons associated with oxygen-containing functional groups. The peak at 3.6 ppm is typical for carbohydrates (>C*H*-OD resonating at 3.4–3.8

ppm) and can be related to the peak at 71 ppm of the ^{13}C NMR spectra. The chemical shifts of the proton on the anomeric carbon atoms (C-1) of carbohydrates are at 4.5–4.8 ppm and are hidden by the intense peak of protons in water (4.7 ppm).

(3) The 6–8.5 ppm region is attributed to aromatic protons. Some peaks can be found in the spectrum of the wastewater, but the unsaturated structures are almost completely eliminated by the biological treatments, in agreement with ^{13}C NMR observations.

These ^{1}H NMR spectra confirmed that biological treatment is very efficient for the removal of aliphatic and aromatic compounds, with an important decrease of the peaks in the 0.8–3 and 6–8.5 ppm regions, especially after the conventional treatment 2. The dominant peak at 1.8 ppm of the wastewater spectrum (polymethylenic long chains) is largely decreased after the two biological treatments. The peak at 2.1 ppm is decreased in the treated water from line 1 and completely eliminated with line 2. The compounds responsible for the peak at 2.5 ppm are only observed after the treatment and probably correspond to the formation of the esters groups revealed by the peak at 165 ppm in the ^{13}C NMR spectra of the treated waters. The shift of the 2.1 ppm peak to 2.5 ppm may reflect a higher branching level and a more oxidized character of the OM in the treated water compared to the OM in the wastewater. The polysaccharide peak at 3.6 ppm is retained in the treated waters, confirming that polysaccharide moieties are less decreased by the biological treatment than aliphatic ones. Since free sugars are normally among the most biodegradable compounds, the polysaccharides moieties observed after treatment may have been released by bacteria and incorporated in complex structures, that protect them from biodegradation.

The FTIR spectra of the wastewater and treated waters are shown in Figure 4. These spectra are affected by the inorganic matter present in the samples, as revealed by their high ash contents, even after the elimination of salts via electrodialysis (Table 1). The intense absorption band at 1100 cm^{-1} is mainly due to SiO_2, and the bands under 600 cm^{-1} are due to other minerals. The large band with a maximum at 3450 cm^{-1} originates from hydrogen bonds in hydroxyl groups in minerals or OM.

Some information can be derived, however, from the other peaks of the spectra. The presence of aliphatic chains is revealed by the band at 2920 cm^{-1} with a shoulder at 2955 cm^{-1} (C–H asymmetric stretching in CH_2 and CH_3, respectively), and the peak at 2850 cm^{-1} (C–H symmetric stretching in CH_2). The variation in the intensity of the aliphatic peaks confirms the differences in alkyl chain abundance between the three samples. The intensity of these bands is more strongly decreased with conventional treatment 2 than with the treatment 1 (ozonated sludge). The CH_2 band at 2920 cm^{-1} is sharply decreased in the treated water spectra, whereas the CH_3 band is still observed (2955 cm^{-1}), confirming the high branching level in the treated waters. FTIR thus indicates changes from long aliphatic chains in wastewater to less abundant and more branched structures after biological treatment, in agreement with ^{13}C and ^{1}H NMR findings.

The other noticeable regions of the spectra are a shoulder at 1730 cm^{-1} (possibly C=O of carboxylic acids or esters), two correlated amide bands at 1650 cm^{-1} (amide I: C=O absorption) and 1560 cm^{-1} (amide II), a peak at 1450 cm^{-1} (CH deformation of CH_2 or CH_3 groups), and a peak at 1405 cm^{-1} (CH_2 or COO^-). The amide II band is only present in the spectra of the wastewater. The spectrum of water treated through line 1 shows the presence of proteins (important amide I band), in agreement with the ^{13}C NMR data.

The combination of solid-state ^{13}C and ^{15}N NMR, liquid-state ^{1}H NMR and FTIR performed on wastewater and treated waters provided complementary and correlated information about the chemical transformations of OM upon biological treatments. These methods were particularly useful to underline the differences in chemical structures present in effluents treated with different processes. The treatments resulted in a sharp decrease in total OM content along with conspicuous changes in the composition of the OM of treated waters relative to initial OM. ^{13}C NMR, ^{1}H NMR and FTIR show that the wastewater contains a high level of aliphatic structures, mainly long alkyl chains, that are well eliminated under the two conditions used for the biological treatment.

Two different treatment conditions lead to differences in the OM released. If the conventional treatment removes most of the compounds present in the wastewater, some specific organic molecules are generated or concentrated in the bacterial media. The aliphatic part of the OM and the polysaccharide moieties are more efficiently decreased than after the treatment with ozonated sludge, but other compounds appeared, with chemical characteristics different from the products found in the wastewater. These compounds can have been formed and/or concentrated during the treatment. The spectra of this sample proved that these refractory products, with dominant C=O groups, have oxidized and branched structures. The presence of amide groups, evidenced by ^{15}N NMR, suggests that OM resistance is related to steric protection in highly cross-linked, protein-derived, macromolecular structures.

Larger amounts of OM are present in the water treated with ozonated sludge, but, contrary to the effluent from the conventional treatment, no compounds with structures different from that of the OM in wastewater are observed. The OM found in the water treated with a sludge ozonation stage is mainly composed of sugar and protein moieties, possibly corresponding to soluble microbial products released from the ozonation step. These compounds may be incorporated in complex organic structures and sterically protected from biodegradation. These products appeared refractory to biological degradation and should not cause oxygen consumption in natural waters. According to their structure containing essentially simple biological moieties, and no aromatic moieties, and their refractory character, these products should be harmless for the environment. Apart from limiting the sludge production, which is an important environmental concern, the treatment with sludge ozonation has the advantage to avoid the production and/or concentration of the unknown products observed after the conventional treatment.

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