

Short communication

# Ion chromatography–electrospray mass spectrometry for the identification of low-molecular-weight organic acids during the 2,4-dichlorophenol degradation

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## Abstract

End-product identification of organic pollutants during oxidation water treatments is of environmental concern due to their potential toxicity. In this work the coupling of ion chromatography (IC), equipped with a membrane ion suppressor, and electrospray ionization mass spectrometry (ESI-MS) has been successfully exploited for the identification of novel low molecular weight organic acids formed as final by-products of 2,4-dichloro-phenol (DCP) degradation by Fenton's reagent, an oxidation method widely employed to degrade recalcitrant organic pollutants in industrial wastewater. The IC–ESI-MS analytical set-up not only allowed the detection of known organic acids but also the identification of 13 new low molecular weight organic acids as a result of extensive oxidation of the parent organic pollutant. Some of the novel organic acids were shown to be chlorine containing by-products and, consequently, of environmental concern. The analytical set-up could be used for characterizing the unknown organic carbon fraction arising from oxidation water treatments.

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**Keywords:** Water analysis; Ion chromatography; Mass spectrometry; Organic acids; Oxidation by-products

## 1. Introduction

Identification of by-products arising from organic pollutant degradation in water treatments, such as biological oxidation [1], disinfection [2] and chemical oxidation [3] is a key step in assessing the environmental fate of such pollutants. In fact, identifying by-products, whose toxicity may be even higher than that of the parent compounds [4], is an important issue as little is known about their nature. The identification of such by-products, usually carried out by GC/MS and LC/MS, is not exhaustive, as a large fraction of the total organic carbon present in the solutions analyzed still remains unknown [2]. This is because the by-products are further degraded to organic acids not LC detectable in standard reverse phase operation mode. Accordingly, in order to identify these latter end by-products, ion chromatography (IC) or ion exclusion chromatography (IEC) with conductivity detection, i.e. without MS, have been employed making the detection

of some organic acids possible on the basis of the authentic standards available [5]. However, IC–MS and IEC–MS have not been employed up to now for the identification of novel ionic by-products arising from organic pollutant degradation even though these techniques have been successfully used for the detection of organic as well as inorganic species [6–12] in a number of applications where low detection limits were also required [13–16]. In particular, in IC–ESI-MS the use of a membrane ion suppressor has been proved to remove effectively from the mobile phase the sodium ions that cause the formation in the MS interface of uncharged species (i.e. organic/inorganic anion and sodium ion as counter-ion). The latter decreases the transfer efficiency from the atmospheric part of the interface to the vacuum region causing, in turn, a decrease of MS detection sensitivity. Therefore, the use of an ion suppressor makes it possible to combine the advantages of the unique selectivity offered by IC with the specificity and structural elucidation capability of MS [6,14,16,17].

In the present work the coupling of IC, equipped with a membrane ion suppressor, and ESI-MS has been successfully

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used to identify low molecular weight organic acids during the degradation of 2,4-dichloro-phenol (DCP) by Fenton's reagent ( $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ ), a typical chemical oxidation method widely used to degrade recalcitrant organic pollutants in industrial wastewater [19]. 2,4-Dichloro-phenol has been selected as a model organochloro pollutant as it has been frequently found in industrial landfill leachates.

## 2. Experimental

### 2.1. Chemicals and Fenton's reactions

2,4-Dichloro-phenol of purity higher than 98% (Aldrich) was used as-received. Hydrogen peroxide solution (ca. 300 g/L) was from Baker and its concentration was assayed iodometrically before each experiment. Ferrous sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 99.5%), sulfuric acid and potassium hydroxide were obtained from Carlo Erba (Italy) and were used as-received. Water used for IC as well as for preparing all aqueous solutions (18.2 M $\Omega$ cm, organic carbon content  $\leq 4 \mu\text{g/L}$ ) was obtained by a Milli-Q Gradient A-10 system (Millipore). NaOH for IC was from Baker. Reagent stock solutions were prepared daily.

Details about Fenton's reactions are reported elsewhere [20]. Initial reagent concentrations (total volume 500 mL) were as follows: [DCP] = 2.3 mM,  $[\text{Fe}^{2+}] = 0.1 \text{ mM}$  and  $[\text{Cu}^{2+}] = 0.1 \text{ mM}$ . The progress of the reactions was monitored at scheduled reaction times by withdrawing 2 mL samples that were immediately frozen.

### 2.2. Ion chromatography–mass spectrometry

Organic acids as well as inorganic anions determination were carried out by a GS50 system (Dionex) equipped with an AS50 autosampler, an ED50 conductivity detector and a ASRS-ultra suppressor, operated at 100 mA in external water mode. Samples, injected via a 25  $\mu\text{L}$  loop, were eluted at a flow rate of 0.5 mL/min through an analytical IonPac AS-11 column (250 mm  $\times$  2 mm) equipped with a IonPac AG-11 guard-column (50 mm  $\times$  2 mm) with the following gradient: from 10/0/90 (NaOH 5 mM/NaOH 100 mM/water), held for 2.5 min, to 100/0/0 in 3.5 min, then to 50/50/0 in 12 min, held for 5 min.

The flow from the conductivity detector of the IC system was split 1:1, by means of a zero dead volume T-piece, to allow one half to enter a Ultimate series UV detector (LC-Packing Dionex), set at 220 nm, and the other half to enter the mass spectrometer interface. The optimization of the ESI-MS interface was performed by using just the ionPac AG-11 guard column in isocratic mode (0/20/80 of NaOH 5 mM/NaOH 100 mM/water) at a flow rate of 0.5 mL/min. These analytical conditions were carefully chosen in order to elute all the organic acids within 2 min and, consequently, to run several injections during a MS acquisition for optimizing each ESI interface parameter. Ten microliters of a standard

solution of organic acids (1 mg/L) was injected through a 7125-Ti Rheodyne valve.

An API 165 mass spectrometer (Applied Biosystems/MSD Sciex) equipped with a turboionspray interface was used throughout the present work. The resolution of the mass spectrometer was set so that peaks full width at half-height was 0.7 Da. Full scan acquisition were acquired in negative ion mode using 0.1 amu step sizes with a dwell time of 0.8 ms, resulting in total scan time of 2.16 s. Other mass spectrometric conditions were as follows: nebulizer gas flow (air) = 1.5 L/min, curtain gas flow (nitrogen) = 1 L/min, mass range = 30–300, needle voltage =  $-4000 \text{ V}$ , orifice voltage =  $-25 \text{ V}$ , ring voltage =  $-110 \text{ V}$ , auxiliary gas flow (air) delivered by a turbo heated probe = 6 L/min at  $450^\circ\text{C}$ .

## 3. Results and discussion

An optimization of the ESI-MS interface was initially performed in order to verify whether standard HPLC–MS parameters apply to organic acids detection. It was found that a careful optimization of the critical ESI-MS parameters (needle voltage, orifice voltage, ring voltage and auxiliary gas temperature), carried out with citric, muconic and chloroacetic acids as representative compounds, allowed an increase of  $[M - \text{H}]^-$  intensities of more than one order of magnitude with respect to standard HPLC–MS parameters. In particular, the parameter that most affected the sensitivity was the ring voltage showing a gain between two- and four-fold (Fig. 1) with respect to the standard value ( $-200 \text{ V}$ ). In addition, the influence of methanol was also investigated as it has been reported that adding it can result in an increase in MS sensitivity [14,21]. However, it was found that methanol addition did not cause any improvement of the sensitivity of the  $[M - \text{H}]^-$  ions probably because in the interface used the

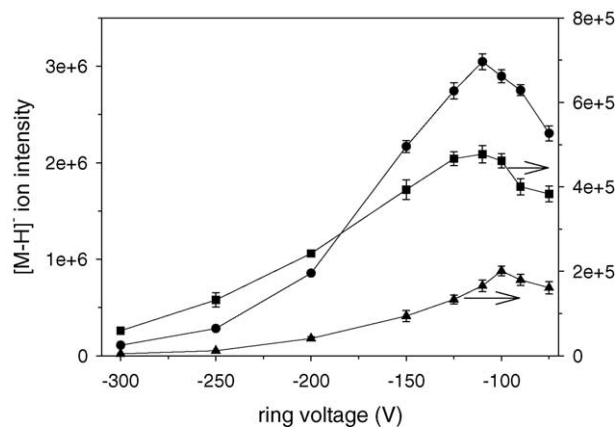


Fig. 1. Dependence of the intensity of  $[M - \text{H}]^-$  ions (citric acid (●), muconic acid (■) and chloroacetic acid (▲)) on ring potential. Plotted data points are averages of duplicate measurements. Other MS conditions: nebulizer gas flow (air) = 1.5 L/min, curtain gas flow (nitrogen) = 1 L/min, mass range = 30–300, needle voltage =  $-4000 \text{ V}$ , orifice voltage =  $-25 \text{ V}$ , auxiliary gas flow (air) delivered by a turbo heated probe ( $450^\circ\text{C}$ ) = 6 L/min.

Table 1

Chemical names and  $[M - H]^-$  ions of the organic acids detected by IC–ESI-MS and confirmed by the use of authentic standards during 2,4-dichlorophenol degradation by Fenton's reagent

Retention time (min)	Name	$[M - H]^-$
2.6	Acetate	59
2.65	Glycolate	75
3.5	Formiate	45
5.9	Chloroacetate	93
8.9	Dichloroacetate	127
11.4	Malonate	103
11.4	Tartrate	149
11.7	Fumarate	115
12.3	Oxalate	89

Instrumental conditions as in Fig. 2.

formation of ions is already enhanced by the auxiliary gas flow kept at a high temperature (450 °C). Overall, the results reported above suggest that a careful optimization of interface voltages is necessary when analyzing ionic compounds and this could be of some utility even when using other MS instruments.

The conductivity and UV chromatograms obtained for 2,4-dichloro-phenol degradation are reported in Fig. 2. Several peaks, on the basis of authentic standards, were identified as low molecular weight organic acids (Table 1) and as chloride, sulfate, nitrite and nitrate. An inspection of Fig. 2 reveals that some of the organic acids coelute partially or completely in the IC chromatogram with conductivity detection. This coelution results in complications during species identification but with MS detection such ambiguities are removed. This is demonstrated by the bottom chromatogram of Fig. 2 showing the single ion traces of oxalate, fumarate and malonate ( $m/z$  89, 115 and 103, respectively). From this chromatogram it is evident that the fumarate, whose detection in conductivity is quite critical due to coelution with sulfate, can be detected without interferences and the malonate can be selectively detected although it coelutes with tartrate. The same chromatogram also shows that, compared to conductivity and UV detection, the employment of IC–ESI-MS allowed most of the organic acids to be detected with higher sensitivity. Furthermore, it can be surmised that if the IC–ESI-MS runs were carried out in SIM mode, an even higher sensitivity would be obtained.

Further inspection of the conductivity and/or UV chromatograms of Fig. 2 reveals that other by-products are formed as small peaks during 2,4-dichloro-phenol degradation. The identification of these by-products was performed by interpreting the background subtracted IC–ESI-MS spectra. Table 2 reports the proposed chemical structures,  $[M - H]^-$  ions and molecular formulas of the detected by-products. It can be seen from this table that by-products with a very different chemical structure were identified, i.e. chlorine containing organic acids (4–7, 9, 13), non-hydroxylated ones (1, 5, 7–9, 13), hydroxylated ones (2–4, 6, 10–12). From Table 2 it is also possible to note that for some by-products more than one structure was proposed. This comes from the fact that the

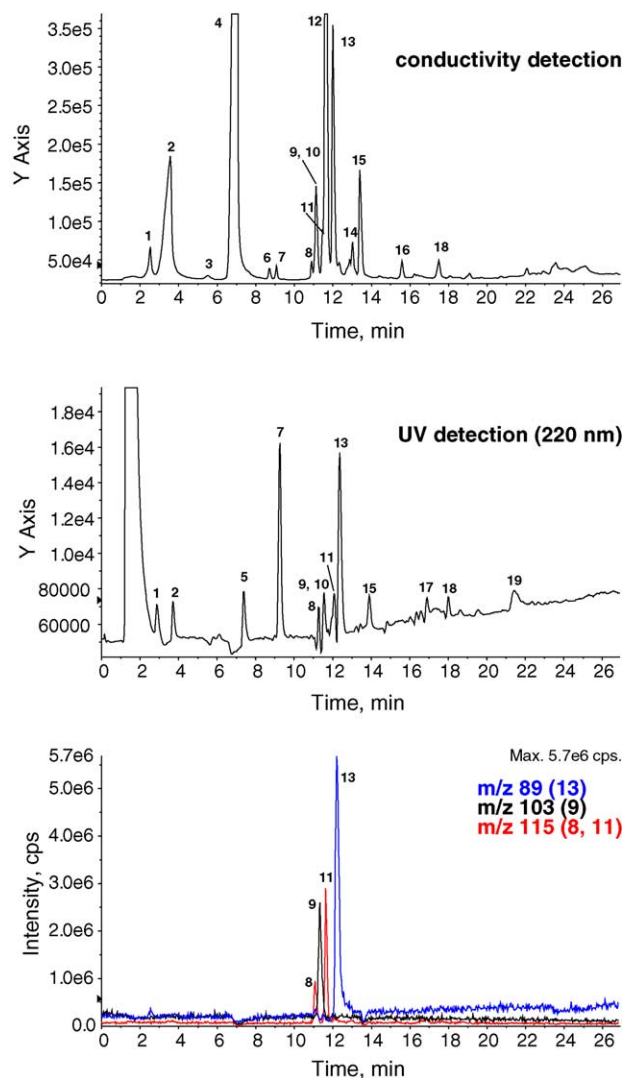


Fig. 2. IC chromatograms in conductivity, UV and ESI-MS detection obtained after 120 min of 2,4-dichloro-phenol degradation by Fenton's reagent. Conditions: guard column, IonPac AG-11 (2 mm i.d.); analytical column, IonPac AS-11 (2 mm i.d.); gradient: from 10/0/90 (NaOH 5 mM/NaOH 100 mM/water), held for 2.5 min, to 100/0/0 in 3.5 min, then to 50/50/0 in 12 min, held for 5 min; injection volume 25  $\mu$ L; ASRS-ultra operated at 100 mA in external water mode; detection, negative ESI-MS nebulizer gas flow (air) = 1.5 L/min, curtain gas flow (nitrogen) = 1 L/min, mass range = 30–300, needle voltage = –4000 V, orifice voltage = –25 V, ring voltage = –110 V, auxiliary gas flow (air) delivered by a turbo heated probe = 6 L/min at 450 °C. Peaks, (1) acetate/glycolate; (2) formiate; (3) chloroacetate; (4) chloride; (5) nitrite; (6) dichloroacetate; (7) nitrate; (8) by-product 2; (9) malonate; (10) tartrate; (11) fumarate; (12) sulfate; (13) oxalate; (14) by-product 6; (15) by-product 7; (16) by-product 9; (17) by-product 11; (18) by-product 12; (19) by-product 13. By-product structures are reported in Table 2.

organic acids were identified on the basis of  $[M - H]^-$  ion, isotopic ion cluster and, when present, of the few fragments obtained and this information for some by-products was consistent with two or three structures. An unequivocal assignment of the chemical structures would have been possible by running accurate mass determinations of  $[M - H]^-$  ions by IC–ESI-TOF-MS analyses. As an example of the by-products

Table 2

Proposed chemical structures,  $[M - H]^-$  ions and molecular formula of the organic acids identified by IC-ESI-MS as by-products of Fenton's degradation of 2,4-dichloro-phenol (for convenience just one possible isomeric structure is reported for each by-product)

By-product number	Retention time (min)	Chemical structure	$[M - H]^-$	Molecular formula
1	8.3		113	C <sub>6</sub> H <sub>10</sub> O <sub>2</sub>
2	11.1		163	C <sub>5</sub> H <sub>8</sub> O <sub>6</sub> /C <sub>6</sub> H <sub>12</sub> O <sub>5</sub> /C <sub>4</sub> H <sub>4</sub> O <sub>7</sub>
3	11.3		133	C <sub>4</sub> H <sub>6</sub> O <sub>5</sub> /C <sub>5</sub> H <sub>10</sub> O <sub>4</sub>
4	12.6		209	C <sub>6</sub> H <sub>7</sub> O <sub>6</sub> Cl/C <sub>7</sub> H <sub>11</sub> O <sub>5</sub> Cl
5	12.7		181	C <sub>6</sub> H <sub>8</sub> O <sub>2</sub> Cl <sub>2</sub>
6	13.3		137	C <sub>4</sub> H <sub>7</sub> O <sub>3</sub> Cl/C <sub>3</sub> H <sub>3</sub> O <sub>4</sub> Cl
7	13.7		149	C <sub>6</sub> H <sub>11</sub> O <sub>2</sub> Cl/C <sub>4</sub> H <sub>3</sub> O <sub>4</sub> Cl
8	15.5		155	C <sub>7</sub> H <sub>8</sub> O <sub>4</sub>
9	15.8		171	C <sub>3</sub> H <sub>2</sub> O <sub>4</sub> Cl <sub>2</sub>
10	16.5		207	C <sub>6</sub> H <sub>8</sub> O <sub>8</sub>
11	16.8		161	C <sub>6</sub> H <sub>10</sub> O <sub>5</sub> /C <sub>5</sub> H <sub>6</sub> O <sub>6</sub>
12	17.7		175	C <sub>7</sub> H <sub>12</sub> O <sub>5</sub> /C <sub>6</sub> H <sub>8</sub> O <sub>6</sub>
13	21.1		173	C <sub>8</sub> H <sub>11</sub> O <sub>2</sub> Cl

Instrumental conditions as in Fig. 2.

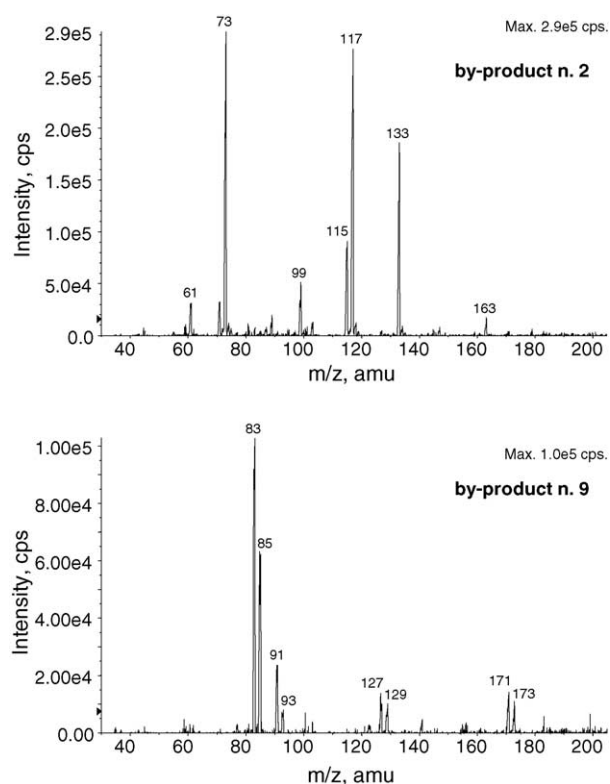


Fig. 3. IC-ESI-MS spectra of by-products 2 and 9 identified during the degradation of 2,4-dichloro-phenol degradation by Fenton's reagent. Instrumental conditions as in Fig. 2.

identification performed, the mass spectra of by-products 2 and 9 whose identification was also supported by fragment ion are depicted in Fig. 3. In these spectra it is possible to note the  $\text{CO}_2$  neutral loss of 44 Da, characteristic of many dicarboxylic acids, the HCl loss of 36 Da (by-product 9) and the oxygen and  $\text{H}_2\text{O}$  loss of 16 and 18 Da (by-product 2). It should be noted that the present analytical set-up for organic acids identification as a result of organic pollutant degradation is of environmental interest, as most of the substances in question are chlorinated by-products and could well be toxic. Moreover, the set-up can be used for characterizing the unknown organic carbon fraction arising from oxidation water treatments.

The extracted ion chromatograms of the organic acids identified, reported in Table 2, are depicted in Fig. 4. It can be noted, comparing chromatograms in Figs. 2 and 4, that most of the organic acids identified were minor compounds with respect to those identified on the basis of authentic standards (Table 1). However, even though some of them partially coeluted with known organic acids, by IC-ESI-MS it was possible to detect them with high specificity and sensitivity. It was not possible to analyze these organic acids quantitatively nor, consequently, determine the detection and

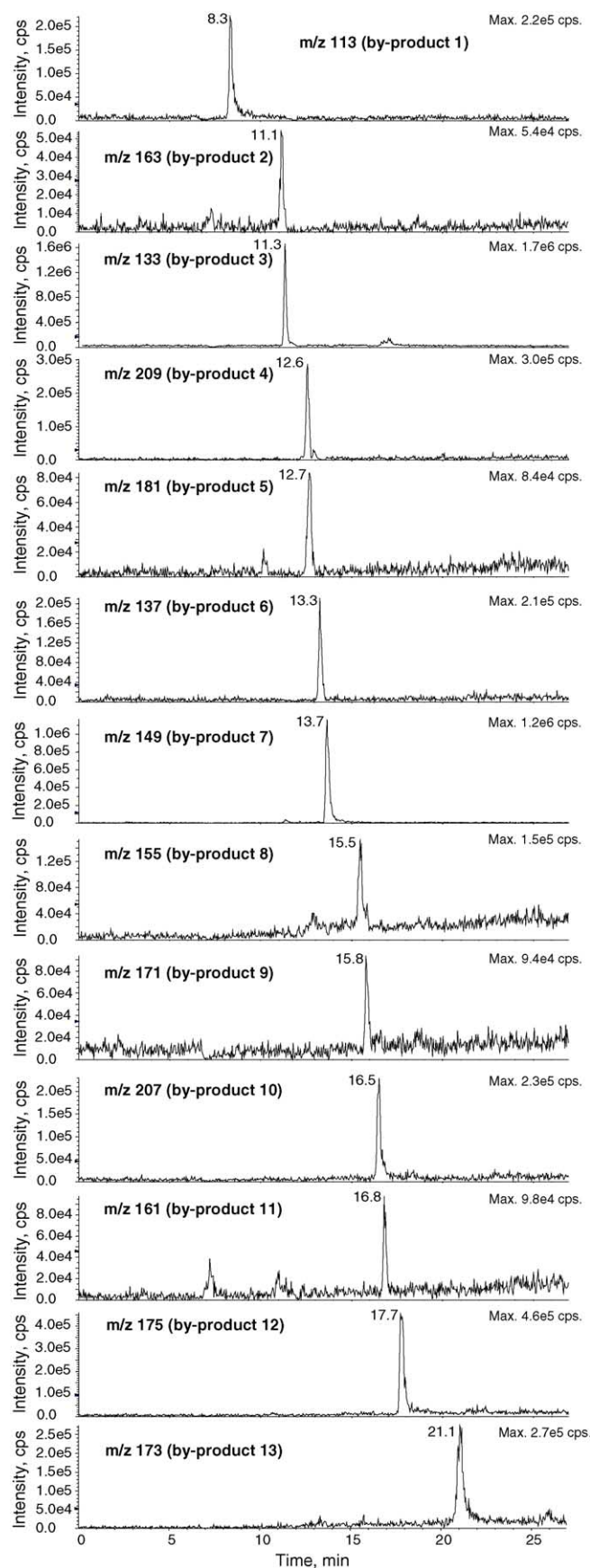


Fig. 4. IC-ESI-MS single ion chromatograms of by-products identified during 2,4-dichloro-phenol degradation by Fenton's reagent. Instrumental conditions as in Fig. 2.

quantitative limits of the method, due to the lack of authentic standards. However, assuming that the MS response of the identified organic acids would be comparable to those for which the standards were available, it can be inferred that the detection of even a few  $\mu\text{g/L}$  of such organic acids could be achieved in selected ion monitoring IC–MS analyses. The employment of a NaOH gradient was necessary for the separation of these organic acids and the high background resulting from the mobile-phase eluent was efficiently reduced by the micromembrane suppressor that allows the specific movement of ions between the suppressor regenerant and the eluent stream leading to the exchange of sodium ions for hydronium ions [14,18]. Hence, the background spectral interferences were minimized and the detection of  $[M - H]^-$  ions of organic acids was enhanced.

#### 4. Conclusion

The coupling of IC and ESI-MS proved to be a powerful technique for the identification of low molecular weight organic acids that are the end by-products of the degradation of 2,4-dichloro-phenol by Fenton's reagent ( $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ ), a typical chemical oxidation method widely employed to degrade recalcitrant organic pollutants in industrial wastewater. As a result of the extensive oxidation of 2,4-dichloro-phenol, nine organic acids were detected on the basis of authentic standards and 13 low molecular weight organic acids were identified by interpreting the background subtracted IC–ESI-MS spectra. The analytical procedure employed for the identification of novel organic acids is of environmental interest, as most of the latter are chlorinated by-products and could well be toxic. It could be used for characterizing the unknown organic carbon fraction arising from oxidation water treatments. Organic acids identification was possible due to the employment of a NaOH gradient and a membrane ion suppressor that minimized the background spectral interferences and enhanced the  $[M - H]^-$  ions signals. However, for some by-products the identification was not unequivocal because it was mainly based on  $[M - H]^-$  ion information. The unequivocal assignment of the chemical structures

would be possible by running accurate mass determinations of  $[M - H]^-$  ions by IC–ESI-TOF-MS analysis.

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