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Atmospheric chemistry of carboxylic acids: microbial implication versus photochemistry

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Abstract. The objective of this work was to compare experimentally the contribution of photochemistry vs. microbial activity to the degradation of carboxylic acids present in cloud water. For this, we selected 17 strains representative of the microflora existing in real clouds and worked on two distinct artificial cloud media that reproduce marine and continental cloud chemical composition. Photodegradation experiments with hydrogen peroxide (H_2O_2) as a source of hydroxyl radicals were performed under the same microcosm conditions using two irradiation systems. Biodegradation and photodegradation rates of acetate, formate, oxalate and succinate were measured on both media at 5 °C and 17 °C and were shown to be on the same order of magnitude (around 10^{-10} – 10^{-11} M s^{-1}). The chemical composition (marine or continental origin) had little influence on photodegradation and biodegradation rates while the temperature shift from 17 °C to 5 °C decreased biodegradation rates of a factor 2 to 5.

In order to test other photochemical scenarios, theoretical photodegradation rates were calculated considering hydroxyl (OH) radical concentration values in cloud water estimated by cloud chemistry modelling studies and available reaction rate constants of carboxylic compounds with both hydroxyl and nitrate radicals. Considering high OH concentration ($[\text{OH}] = 1 \times 10^{-12}$ M) led to no significant contri-

bution of microbial activity in the destruction of carboxylic acids. On the contrary, for lower OH concentration (at noon, $[\text{OH}] = 1 \times 10^{-14}$ M), microorganisms could efficiently compete with photochemistry and in similar contributions than the ones estimated by our experimental approach.

Combining these two approaches (experimental and theoretical), our results led to the following conclusions: oxalate was only photodegraded; the photodegradation of formate was usually more efficient than its biodegradation; the biodegradation of acetate and succinate seemed to exceed their photodegradation.

1 Introduction

Clouds play a key role in the transformation of chemical species through exchanges between the gaseous and the aqueous phase and reactivity in each compartment (Jacob et al., 1986; Lelieveld and Crutzen, 1991; Ravishankara, 1997; Monod and Carlier, 1999). In cloud droplets the organic matter represents a significant fraction of the soluble matter (Hadi et al., 1995; Saxena and Hildemann, 1996; Fuzzi et al., 2002). Among the dissolved organic compounds, carboxylic acids are ubiquitous (Chebbi and Carlier, 1996; Löflund et al., 2002) and come from various sources. They are produced in the gas-phase and dissolved into the aqueous phase (main source of monocarboxylic acids such as acetic and formic acids) (Winiwarter et al., 1994; Laj et al., 1997); they can



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also result from the dissolution of the soluble part of organic particles (source of dicarboxylic acids like oxalic, succinic, malonic and maleic acids ...) (Sempéré and Kawamura, 1994; Sellegri et al., 2003; Legrand et al., 2005); and they also mainly derive from the aqueous phase reactivity (Ervens et al., 2004; Carlton et al., 2007; Tan et al., 2010). The oxidation of organic matter due to the presence of free radicals such as OH can be considered as an important source of carboxylic acids in the aqueous phase, but also as one of their main sinks (Karpel Vel Leitner and Doré, 1997; Ervens et al., 2003b; Herrmann, 2003; Warneck, 2003; Herrmann et al., 2010; Sun et al., 2010).

The aqueous phase of clouds also contains insoluble biological material such as bacteria, fungal spores, pollens, plant debris, etc. (Matthias-Maser et al., 2000; Bauer et al., 2002; Jaenicke, 2005; Burrows et al., 2009). The study from Matthias-Maser and Jaenicke (1995) indicates that bioaerosols can represent up to 30 % of the total number of insoluble aerosols in clouds. Viable microorganisms have been recovered from rain (Casareto et al., 1996), cloud and fog samples (Fuzzi et al., 1997; Amato et al., 2005, 2007b; Ahern et al., 2007). In clouds, concentration of bacteria range from 0.8×10^3 to 2.4×10^5 cells ml⁻¹ and for fungi and yeasts from 8.9×10^2 to 2.5×10^4 cells ml⁻¹ (Sattler et al., 2001; Amato et al., 2007c). The microbial strains isolated from the atmosphere were likely originating from vegetation, soil, aquatic environments (Amato et al., 2007b; Burrows et al., 2009), and most of these microorganisms were able to survive and develop under the specific conditions existing in cloud water (chemical composition, low temperature, UV exposure) (Sattler et al., 2001; Amato et al., 2007b). Possible C-sources for microorganisms in atmospheric waters are carboxylic acids, aldehydes and alcohols (Herlihy et al., 1987; Fuzzi et al., 1997; Ariya et al., 2002; Amato et al., 2005, 2007a; Côté et al., 2008; Deguillaume et al., 2008; Delort et al., 2010). Biological activity could therefore influence the budget of the organic matter in cloud droplets.

To test this hypothesis, in previous works, our strategy was first to screen the ability of a large number of microbial strains (60) isolated from clouds to degrade organic compounds (formate, acetate, lactate (D & L), succinate, formaldehyde and methanol) under optimal conditions (27 °C) (Amato et al., 2005, 2007a). We have shown that enzymes responsible for these transformations were present in these microorganisms. To go further, we studied the response of one single strain (*Pseudomonas graminis*) under more realistic atmospheric conditions (Vaitilingom et al., 2010). We used a microcosm mimicking cloud chemical composition typical from continental influence at two different temperatures representative of warm clouds (5 °C and 17 °C). Biodegradation rates of acetate, formate, succinate and oxalate were measured and compared to radical chemistry. For this, the reactions of these organic compounds with the two major free radicals OH and NO₃ were considered. We calculated theoretical photodegradation rates from radical con-

centrations simulated by cloud chemistry models (Ervens et al., 2003b; Herrmann, 2003). This comparison showed that microorganisms would dominate the degradation of certain organic compounds in clouds during the night.

The objective of this work was to generalize our conclusions previously obtained on a single strain. For this, we selected 17 strains representative of the microflora existing in clouds and worked on two distinct artificial cloud media that reproduce marine and continental cloud chemical composition. Biodegradation rates of acetate, formate, oxalate and succinate were measured for all the strains on both media at 5 °C and 17 °C. The goal was to investigate the effect of environmental parameters on the biodegradation efficiency of microorganisms. Biodegradation rates in natural cloud water sampled at the puy de Dôme station (France) with its own endogenous microflora and chemical composition was also investigated. The objective was to compare artificial microcosm and natural clouds. In addition, photodegradation experiments with hydrogen peroxide (H₂O₂) as a source of hydroxyl radicals were performed under the same microcosm conditions to compare directly photochemistry to biodegradation processes. This is the first report of such comparison using experimental data. In the discussion, theoretical calculations based on various scenarios of the OH reactivity are presented.

2 Material and methods

2.1 Bacterial strains

Seventeen bacterial strains isolated from cloud water were selected; they are among the microbial genera that are most frequently recovered by culture from cloud water sampled at the puy de Dôme station (1465 m a.s.l.) between December 2003 and March 2009 (see Amato et al., 2007c). The strains used are: 2 *Pseudomonas* sp. strains PDD-6b-4 (DQ512766) and PDD-14b-10 (DQ512794), 3 *Pseudomonas syringae* strains PDD-13b-1 (DQ512737), PDD-12b-8 (DQ512783) and PDD-13b-2 (DQ512785), 1 *Pseudomonas viridiflava* strain PDD-14b-14 (DQ512797), 1 *Pseudomonas graminis* strain PDD-13b-3 (DQ512786), 3 *Sphingomonas* sp. strains PDD-3b-10 (DQ512744), PDD-14b-5 (DQ512789) and PDD-14b-6 (DQ512790), 2 *Frigoribacterium* sp. strains PDD-14b-13 (DQ512796) and PDD-30b-1 (HQ256816), 1 *Rhodococcus* sp. strain PDD-23b-5 (HQ256785), 1 *Arthrobacter rhombi* strain PDD-3b-7 (DQ512742), 1 *Bacillus pumilus* strain PDD-5b-1 (DQ512749) and 1 *Bacillus* sp. PDD-16b-1 (not available in GenBank), and 1 *Clavibacter michiganensis* strain PDD-25b-3 (HQ256805).

2.2 Biodegradation tests

Biodegradation experiments consisted of “marine” or “continental” artificial cloud water solutions inoculated with bacterial cells and incubated at 5 °C and 17 °C for 120 h under

aerobic conditions. Incubation media consisted of “marine” and “continental” artificial cloud water solutions (see Table 1 for composition) made by diluting stock solutions in ultrapure water (resistivity $\geq 18.2 \text{ M}\Omega \text{ cm}^{-1}$). Stock solutions were prepared from the following products: acetic acid (Aldrich, $\geq 99.99\%$), formic acid (Fluka, $\sim 98\%$), oxalic acid (Fluka, $\geq 99\%$), succinic acid (Fluka, $\geq 99\%$), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (Fluka, $\geq 99\%$), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Aldrich, 99%), K_2SO_4 (Fluka, $\geq 99\%$), NaCl (Aldrich, 99.5%), NH_4NO_3 (Fluka, $\geq 99.5\%$), NaOH (Merck, 99%) and H_2SO_4 (Acros Organics, $\geq 95\%$). The artificial cloud water solutions were sterilized by autoclave (20 min at 121°C) and stored at 5°C for less than 24 h. In the case of biodegradation experiments, in order to have a better stabilization of the chemical composition and pH after the sterilization process, the artificial cloud media were prepared 10 times more concentrated than indicated in Table 1. In previous work (Vaitilingom et al., 2010), we showed that, at constant ratio “cell concentration/degraded chemical compound concentration” and in the range of concentrations investigated, biodegradation rates are independent of the absolute cell and chemical concentrations.

Artificial cloud water solutions were inoculated with bacterial cells from pure cultures grown aerobically at 17°C (200 rotations per minute (rpm)) in R2A broth (Reasoner and Geldreich, 1985). Cells were harvested by centrifugation ($4000 \times g$, 15 min., 4°C) after 18 h of growth (exponential phase), rinsed once in NaCl solution (8 g per liter) and twice in the incubation medium and finally resuspended in the incubation medium. The concentrations of cells in the test media were adjusted to $10^6 \text{ cells ml}^{-1}$ (~ 10 times higher than the average bacterial concentration found in clouds sampled at the puy de Dôme station (Amato et al., 2007c) based on the turbidity of the culture (optical depth at 575 nm). Serial dilutions of the suspension were plated (0.1 ml) on R2A (solid medium in a petri dish) and incubated at 17°C for Colony Forming Unit (CFU) counts, that allowed to determine the bacterial concentrations of the incubated media. These microbial concentrations were used to normalize the biodegradation rates (biodegradation rates per cell expressed in $\text{mol cell}^{-1} \text{ s}^{-1}$).

At defined time steps (every hour from 0 to 8 h and at times zero plus 24, 48, 72 and 120 h), samples of 1 ml were taken for analysis by ion chromatography and were pelleted ($12\,000 \times g$, 3 min) to eliminate cells; the supernatants were kept frozen until analysis. No chemical modification was observed during the time of incubation in the reference flask (cloud water solution without microorganisms).

2.3 Photodegradation tests

For photodegradation experiments, artificial cloud water solutions were prepared as indicated in Table 1, supplemented with $20 \mu\text{M}$ of hydrogen peroxide H_2O_2 (Fluka, 30%) (less than 30 min before irradiation), and exposed to controlled

lights under agitation. The maximal concentration of H_2O_2 measured in natural cloud water sampled at the puy de Dôme were in the same range ($\sim 19 \mu\text{M}$) (Marinoni et al., 2011). H_2O_2 was added for generating hydroxyl radicals in the solutions by photolysis reaction ($\lambda < 380 \text{ nm}$).

Two irradiation setups, characterized by different polychromatic light spectra were used:

The **Photochemical setup 1 (P1)**: consisted in a light beam generated by a 1000 W Xenon lamp used on the top of a cylindrical reactor equipped by a Pyrex filter (thickness 3.3 mm, wavelength cut-off: 290 nm) containing 500 ml of magnetically stirred solution at 17°C (by circulating temperature-regulated water through an outer jacket).

The **Photochemical setup 2 (P2)**: consisted in a box equipped by 12 Philips Actinic BL 15W fluorescent tubes emitting within the wavelength range 340–420 nm with a maximum emission at 365 nm and placed above the incubation flasks. The incubation flasks were cylindrical Pyrex crystallizer (100 ml) dishes covered with a Pyrex filter and incubated at 17°C under agitation (110 rpm).

The wavelength-dependent light fluxes reaching the solutions were measured by placing an optical fiber coupled to a spectrograph with a CCD detector (Ocean Optics SD 2000 CCD spectrophotometer) previously calibrated by using a DH-2000-CAL Deuterium Tungsten Halogen reference lamp inside a chemical reactor. Emission spectra of both photochemical setups and the UV-Vis spectra of nitrate and hydrogen peroxide in water solution are displayed in Fig. 1.

Effective degradation of hydrogen peroxide was verified in both setups by a fluorimetry assay involving a Horse Radish Peroxidase in 4-Hydroxyphenylacetic acid solution (Lazrus et al., 1985). At defined time points, two aliquots of 1 ml of the solution incubated at 17°C (no experiment of photochemistry was made at 5°C due to technical limitations) were taken and kept frozen until ion chromatography analysis.

A control experiment was performed under irradiation without H_2O_2 . No degradation was observed for all the organic compounds except for oxalate. This degradation was less than 5% over the whole incubation time and thus negligible as compared to this degradation in presence of H_2O_2 .

2.4 Natural cloud water sample collection and conditions of incubation

Cloud water was collected at the puy de Dôme station using a sterilized cloud water sampler (Kruisz et al., 1993). The sampling method was identical to those described in Amato et al. (2005). The collection was performed on 17 June 2008, during a North-Western flow air mass and the average temperature over the time of sampling was 11°C .

Table 1. Chemical composition of the artificial cloud water media and of the natural cloud water sample used for incubation experiments, and range of values observed in natural cloud water collected at the puy de Dome station.

Compound	Artificial cloud water medium		Natural cloud water sample	Cloud water sampled at the puy de Dome station*	
	Marine	Continental		min	max
	Concentration (μM)				
Acetate	20	20	24.3	0.6	48.7
Formate	15	15	48.1	0.8	71.4
Succinate	1.5	1.5	4.7	0.1	4.1
Oxalate	3	3	9.8	0.1	17.4
Cl^-	320	250	60.4	0.5	1948.9
NO_3^-	70	200	192.1	3.4	766.8
SO_4^{2-}	35	50	78.2	1.9	369.5
Na^+	310	250	46.4	0.4	681.0
NH_4^+	70	200	287.6	6.3	1801.7
K^+	10	10	11.7	0.1	124.1
Mg^{2+}	10	10	31.3	0.2	45.6
Ca^{2+}	40	40	45.9	0.3	74.8
pH	6–6.5	4.7–5.2	5.1	3.9	7.6

* from the OPGC database on cloud chemical composition at the puy de Dome station: <http://www.obs.univ-bpclermont.fr/SO/beam/data.php>.

Briefly, 50 ml of cloud water were collected and kept cold until laboratory investigations that were started within a few hours after sampling. Ten minutes before incubation, half of the volume of the cloud water sample was sterilized by filtration (0.22 μm porosity, nylon filter) to be considered as the “reference sample” free of microorganisms. The other half was kept intact with its endogenous microflora, and both subsamples were incubated under agitation (200 rpm) during 120 h. The incubation temperature was 12 $^\circ\text{C}$ to fit the temperature of the natural cloud. Every 24 h, 1 ml was taken out and frozen until ion chromatography analyses.

2.5 Analyses by ion chromatography

Artificial solutions and the natural cloud water samples were analyzed using ion chromatography Dionex DX320 for anions (column AS11, eluent KOH) and Dionex ICS1500 for cations (column CS16, eluent hydroxymethanesulfonate acid) (Jaffrezo et al., 1998; Marinoni et al., 2004). Samples were thawed 15 min before their dilution in ultrapure water, and transferred in vials (5 ml) previously rinsed with ultrapure water. No chemical transformations were observed in our sample after one freeze/thaw cycle. The accuracy of ion chromatographic analyses is 10 % for samples 10 times the detection limit, and around 50 % for samples twice the detection limit (Marinoni et al., 2004).

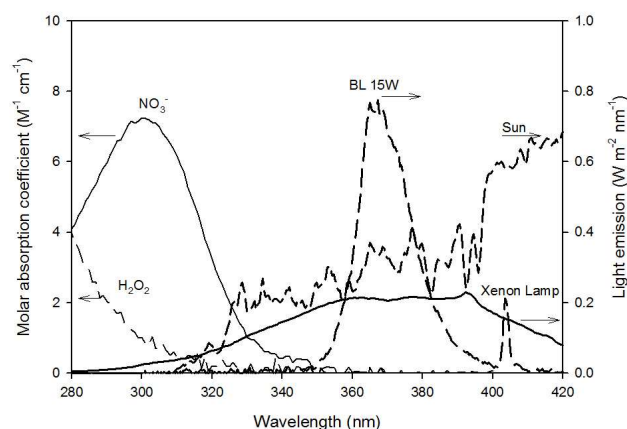


Fig. 1. Emission spectra of the 1000 W xenon lamp (P1), of the Philips Actinic BL 15 W lamps (P2), of the Sun at the Earth’s surface and molar absorption coefficients of nitrate and H_2O_2 aqueous solutions.

2.6 Calculations of bio- and photo-degradation rates

To calculate the initial bio- and photo-degradation rates, time evolution of each carboxylic acid concentration was plotted (Fig. S1 for illustration). Then the pseudo-first order decay “ k ” (s^{-1}) is determined by the linear regression of:

$$\ln([C]/[C]_0) = f(t) = -k \times t \quad (\text{see Fig. S2}).$$

With $[C]_0$ (mol l^{-1}) the initial concentrations of selected carboxylic acid C .

In biodegradation experiments, the pseudo-first order decays was determined over the 6 and the 8 first hours of incubation at 17 °C and 5 °C, respectively. For all strains tested in these experiments, no cellular growth was observed in the 24 first hours of incubation at 5 and 17 °C. After the 48 first hours of incubation, the cell growth factor was between 1 and 1.8.

In photo-degradation experiments, the pseudo-first order decays were determined over the 4 and the 8 first hours of irradiation in the photochemical setups P1 and P2, respectively; at this incubation time, H_2O_2 was available since it was totally consumed after 6 h in P1 and 48 h in P2.

The measured degradation rates of the compound C (v_c) have been determined as follows:

- for biodegradation rates per cell:

$$v_c = (k \times [C]_0) / N_{\text{cells}} \quad [\text{mol cell}^{-1} \text{s}^{-1}] \quad (1)$$

- for photodegradation rates:

$$v_c = k \times [C]_0 \quad [\text{Ms}^{-1}] \quad (2)$$

With N_{cells} (cells l^{-1}) the concentration of cells participating to the biodegradation.

The estimated photodegradation rates resulting from the reactivity with free radicals from data of the literature:

$$v_c = K \times [\bullet\text{OH}] \times [C]_0 \quad [\text{Ms}^{-1}] \quad (3)$$

with $[C]_0$ the initial concentrations of selected carboxylic acid C (mol l^{-1}), $[\text{OH}]$ is the concentration of free radical OH from literature and K ($\text{M}^{-1} \text{s}^{-1}$) is the degradation rate constant of the carboxylic acid C by OH.

3 Results

3.1 Biodegradation

3.1.1 Artificial cloud media

The artificial cloud water media used in these experiments contained the 4 major carboxylic compounds dominating the soluble organic composition of clouds (acetate, formate, succinate, and oxalate) and 8 major inorganic ions (see composition in Table 1). The inorganic composition differed by higher sodium and chloride concentrations in “marine” than in “continental” medium and inversely for nitrate, ammonium, and sulfate. The continental medium was more acidic ($\text{pH} \sim 5$) than the marine one ($\text{pH} \sim 6.3$). The organic compositions in “marine” and in “continental” media were similar to allow comparisons of the measured biotransformation

rates and to detect possible effects of the concentration of inorganic ions and of the pH on the metabolic activity of microorganisms.

These media were incubated with a pure microbial culture at 5 °C and 17 °C. The colder temperature (5 °C) corresponds to the mean annual value measured at the puy de Dôme summit, while 17 °C is approximately the maximal temperature observed there when a cloud forms (see <http://www.obs.univ-bpclermont.fr/SO/mesures/pdd.php>).

During the incubation period, only the degradation of organic species was observed and no extracellular accumulation of intermediary carboxylic acids was detected. The measured rates of biotransformation per cell of each compound by each microbial strain are reported in Table 2. The value “zero” was considered when no degradation was detected and was taken into account in the calculation of the average biodegradation rate presented further. Nine and eight strains out of seventeen were able to degrade carboxylic acids at 17 °C and 5 °C, respectively. Although some of the microorganisms used could have the enzymatic material necessary for transforming oxalate (Sahin, 2003), none was degrading it under our incubation conditions. Biodegradation rates per cell of acetate ranged from 0 to $7 \times 10^{-18} \text{ mol cell}^{-1} \text{ s}^{-1}$ (strain 14b10, “continental” medium) at 17 °C, and from 0 to $2 \times 10^{-18} \text{ mol cell}^{-1} \text{ s}^{-1}$ (strains 14b10 and 23b5, continental) at 5 °C. For formate, they reached $1 \times 10^{-17} \text{ mol cell}^{-1} \text{ s}^{-1}$ (strain 6b4, continental) at 17 °C, and $5 \times 10^{-18} \text{ mol cell}^{-1} \text{ s}^{-1}$ (strain 12b8, marine) at 5 °C. For succinate, biodegradation rates per cell ranged 0 to $1 \times 10^{-18} \text{ mol cell}^{-1} \text{ s}^{-1}$ (strains 3b1 and 13b2, marine) at 17 °C, and from 0 to $3 \times 10^{-19} \text{ mol cell}^{-1} \text{ s}^{-1}$ (strain 13b2, marine) at 5 °C.

Except in a few cases (*Pseudomonas* toward acetate at 5 °C), *Pseudomonas* strains and *Rhodococcus* were active towards all the tested compounds. On the contrary, *Bacillus*, *Arthrobacter*, *Clavibacter*, and *Frigoribacterium* strains were not able to degrade any of those under any of our experimental conditions. One of the three *Sphingomonas* strains tested was able to degrade formate and succinate at 17 °C, but none at 5 °C.

Regarding the influence of the chemical composition on the degradation rates, 55 % of the measured biodegradation rates per cell were higher in marine medium than in continental cloud water solution. However, no significant difference of the average biodegradation rate per cell of a given compound between these two artificial media was observed. Some strains degraded carboxylic acids more efficiently in the marine than in the continental medium, and *vice versa*.

As expected, when detected, activities of biodegradation at 5 °C were lower than at 17 °C by factors ranging from 2.1 to 5.5 (considering the average biodegradation rates). No relationship between the chemical composition of the incubation medium and the temperature dependence of biodegradation activities was detected.

Table 2. Measured rates of biodegradation of carboxylic acids by pure strains at 5 and 17 °C in the “marine” and “continental” artificial cloud water media.

		Marine			Continental		
17 °C		Rates of biodegradation per cell (mol cell ⁻¹ s ⁻¹)*					
		Acetate	Formate	Succinate	Acetate	Formate	Succinate
3b-10	<i>Sphingomonas</i> sp.	0	0	0	0	0	0
14b-5	<i>Sphingomonas</i> sp.	0	2×10 ⁻¹⁹	2×10 ⁻¹⁹	0	2×10 ⁻¹⁹	3×10 ⁻¹⁹
14b-6	<i>Sphingomonas</i> sp.	0	0	0	0	0	0
6b-4	<i>Pseudomonas</i> sp.	7×10 ⁻¹⁹	1×10 ⁻¹⁸	1×10 ⁻¹⁹	5×10 ⁻¹⁹	1×10 ⁻¹⁷	6×10 ⁻¹⁹
14b-10	<i>Pseudomonas</i> sp.	2×10 ⁻¹⁸	2×10 ⁻¹⁸	2×10 ⁻¹⁹	7×10 ⁻¹⁸	7×10 ⁻¹⁸	3×10 ⁻¹⁹
3b-1	<i>Pseudomonas syringae</i>	1×10 ⁻¹⁸	2×10 ⁻¹⁸	1×10 ⁻¹⁸	5×10 ⁻¹⁹	1×10 ⁻¹⁸	4×10 ⁻¹⁹
12b-8	<i>Pseudomonas syringae</i>	6×10 ⁻¹⁹	8×10 ⁻¹⁸	4×10 ⁻²⁰	2×10 ⁻¹⁸	2×10 ⁻¹⁸	5×10 ⁻¹⁹
13b-2	<i>Pseudomonas syringae</i>	1×10 ⁻¹⁸	3×10 ⁻¹⁸	1×10 ⁻¹⁸	4×10 ⁻¹⁹	2×10 ⁻¹⁸	6×10 ⁻¹⁹
13b-3	<i>Pseudomonas graminis</i>	4×10 ⁻¹⁹	5×10 ⁻¹⁸	5×10 ⁻¹⁹	5×10 ⁻¹⁹	4×10 ⁻¹⁸	4×10 ⁻¹⁹
14b-14	<i>Pseudomonas viridiflava</i>	9×10 ⁻¹⁹	2×10 ⁻¹⁸	6×10 ⁻¹⁹	9×10 ⁻²⁰	7×10 ⁻¹⁹	1×10 ⁻¹⁹
16b-1	<i>Bacillus</i> sp.	0	0	0	0	0	0
5b-1	<i>Bacillus pumilus</i>	0	0	0	0	0	0
3b-7	<i>Arthrobacter rhombi</i>	0	0	0	0	0	0
14b-13	<i>Clavibacter michiganensis</i>	0	0	0	0	0	0
30b-1	<i>Frigoribacterium</i> sp.	0	0	0	0	0	0
14b-13	<i>Frigoribacterium</i> sp.	0	0	0	0	0	0
23b-5	<i>Rhodococcus</i> sp.	5×10 ⁻¹⁸	8×10 ⁻¹⁹	5×10 ⁻²⁰	4×10 ⁻¹⁸	1×10 ⁻¹⁸	7×10 ⁻²⁰
Microbial average rate		7×10 ⁻¹⁹	1×10 ⁻¹⁸	2×10 ⁻¹⁹	8×10 ⁻¹⁹	2×10 ⁻¹⁸	2×10 ⁻¹⁹
5 °C							
3b-10	<i>Sphingomonas</i> sp.	0	0	0	0	0	0
14b-5	<i>Sphingomonas</i> sp.	0	0	0	0	0	0
14b-6	<i>Sphingomonas</i> sp.	0	0	0	0	0	0
6b-4	<i>Pseudomonas</i> sp.	5×10 ⁻¹⁹	6×10 ⁻¹⁹	3×10 ⁻²⁰	2×10 ⁻¹⁹	2×10 ⁻¹⁸	1×10 ⁻¹⁹
14b-10	<i>Pseudomonas</i> sp.	7×10 ⁻¹⁹	1×10 ⁻¹⁸	9×10 ⁻²¹	2×10 ⁻¹⁸	3×10 ⁻¹⁸	8×10 ⁻²¹
3b-1	<i>Pseudomonas syringae</i>	0	4×10 ⁻¹⁹	2×10 ⁻¹⁹	0	2×10 ⁻¹⁹	4×10 ⁻²⁰
12b-8	<i>Pseudomonas syringae</i>	2×10 ⁻¹⁹	5×10 ⁻¹⁸	3×10 ⁻²⁰	2×10 ⁻¹⁹	9×10 ⁻¹⁹	1×10 ⁻¹⁹
13b-2	<i>Pseudomonas syringae</i>	0	1×10 ⁻¹⁸	3×10 ⁻¹⁹	0	6×10 ⁻¹⁹	2×10 ⁻¹⁹
13b-3	<i>Pseudomonas graminis</i>	1×10 ⁻¹⁹	1×10 ⁻¹⁸	1×10 ⁻¹⁹	5×10 ⁻²⁰	7×10 ⁻¹⁹	7×10 ⁻²⁰
14b-14	<i>Pseudomonas viridiflava</i>	0	6×10 ⁻¹⁹	7×10 ⁻²⁰	0	9×10 ⁻²⁰	1×10 ⁻²⁰
16b-1	<i>Bacillus</i> sp.	0	0	0	0	0	0
5b-1	<i>Bacillus pumilus</i>	0	0	0	0	0	0
3b-7	<i>Arthrobacter rhombi</i>	0	0	0	0	0	0
25b-3	<i>Clavibacter michiganensis</i>	0	0	0	0	0	0
14b-13	<i>Frigoribacterium</i> sp.	0	0	0	0	0	0
30b-1	<i>Frigoribacterium</i> sp.	0	0	0	0	0	0
23b-5	<i>Rhodococcus</i> sp.	1×10 ⁻¹⁸	4×10 ⁻¹⁹	4×10 ⁻²⁰	2×10 ⁻¹⁸	8×10 ⁻¹⁹	4×10 ⁻²⁰
Microbial average rate		2×10 ⁻¹⁹	6×10 ⁻¹⁹	5×10 ⁻²⁰	2×10 ⁻¹⁹	4×10 ⁻¹⁹	4×10 ⁻²⁰

* Global experimental uncertainties result from two additive errors: (1) the one from the analysis with the ionic chromatography (less than 10%); (2) the one from the biological variability that was calculated from five replicates on 2 strains (between 20 and 25 %).

3.1.2 Natural cloud

Natural cloud water was used without any chemical modification and in the presence of its own microflora. The chemical composition of this cloud water sample is given in Table 1

and present concentrations typical from continental influence and similar to the composition from the artificial continental cloud medium.

The cloud water was incubated at 12 °C and the biodegradation of organic acids was monitored. Under this condition,

Table 3. Estimated rates of photo- and bio-degradation in artificial cloud water at 17 °C.

17 °C	Marine				Continental			
	Rate of degradation (M s ⁻¹)							
	Acetate	Formate	Succinate	Oxalate	Acetate	Formate	Succinate	Oxalate
P1*	0	$7.1 (\pm 2.2) \times 10^{-10}$	0	$2.7 (\pm 1.2) \times 10^{-10}$	0	$7.4 (\pm 2.1) \times 10^{-10}$	0	$3.4 (\pm 0.9) \times 10^{-10}$
P2*	0	$0.2 (\pm 0.1) \times 10^{-10}$	0	$0.2 (\pm 0.1) \times 10^{-10}$	0	$1.5 (\pm 0.4) \times 10^{-10}$	0	$1.9 (\pm 0.7) \times 10^{-10}$
Microbial**	$6.6 (\pm 9.1) \times 10^{-11}$	$1.2 (\pm 1.8) \times 10^{-10}$	$2.4 (\pm 3.2) \times 10^{-11}$	0	$8.3 (\pm 1.4) \times 10^{-11}$	$1.7 (\pm 2.3) \times 10^{-10}$	$1.9 (\pm 1.8) \times 10^{-11}$	0

* Photodegradation rates (\pm absolute uncertainties for 3 replicates) determined experimentally in M s⁻¹. ** Biodegradation rates expressed in M s⁻¹ is calculated by considering the average biodegradation rates per cell (in mol cell⁻¹ s⁻¹) (see Table 2) and the cellular concentration equal to 8×10^7 cells l⁻¹ (mean value observed in natural cloud water sampled at the puy de Dôme station, Amato et al., 2007c).

acetate, formate and succinate were degraded while oxalate was not. The measured biodegradation rates were 1.2×10^{-10} , 0.9×10^{-10} and 3.3×10^{-11} M s⁻¹, for acetate, formate and succinate, respectively. In the reference sample free of microbes, carboxylic acids were not transformed during the incubation period.

To compare these rates with the one measured in artificial microcosms, we converted the average biodegradation rates per cell (mol cell⁻¹ s⁻¹) obtained for the 17 bacterial strains at 17 °C into biodegradation rates expressed in M s⁻¹. For that, we have considered the mean bacterial concentration in cloud water sampled at the puy de Dôme station to 8×10^7 cells l⁻¹. We obtained the following rates: 0.7×10^{-10} , 1.4×10^{-10} , 1.5×10^{-11} M s⁻¹, for acetate, formate and succinate, respectively (see Table 3).

These values are on the same order of magnitude than values obtained at 12 °C in the natural cloud. This shows that our artificial microcosms are a rather good experimental model to mimic real cloud water. To constitute our microcosms of cloud water, a panel of strains representative of the bacteria usually encountered in cloud water was chosen along with typical chemical compositions and cell numbers in natural clouds. Using this very simplified setup, we were able to reproduce the results obtained with a natural cloud sample whose composition is usually much more complex (more diverse organic and inorganic compounds, presence of HULIS, wider biodiversity, etc.).

3.2 Photodegradation in artificial cloud water media

To compare microbial and photochemical activities on carboxylic acid transformations, irradiation experiments were conducted in the artificial cloud solutions. Preliminary irradiation tests with no supplementation of H₂O₂ showed, as expected, that no direct photochemical degradation of carboxylic acids happened in both photochemical setups.

In the presence of H₂O₂, no organic oxidation products were detected by ion chromatography analyses. The photodegradation rates of carboxylic acids in “marine” and “continental” media measured at 17 °C in experimental setups P1 and P2 are given in Table 3.

Acetate and succinate were not degraded by photolysis in all experiments. In the experimental setup P1, there was no influence of the incubation medium (“marine” or “continental”) on the photodegradation rates of formate and oxalate ($\sim 7 \times 10^{-10}$ M s⁻¹ and $\sim 3 \times 10^{-10}$ M s⁻¹ for formate and oxalate, respectively). In P2, the degradation rates of formate and oxalate were higher in continental than in marine medium by about one order of magnitude ($\sim 2 \times 10^{-10}$ M s⁻¹ and $\sim 2 \times 10^{-11}$ M s⁻¹ in continental and in marine medium, respectively, for both species). The difference of pH is thought not to influence the production of hydroxyl radical from photolysis of H₂O₂ and nitrates (Mack and Bolton, 1999). We can expect the difference of behaviour observed between continental and marine solutions to be due the relative contribution of nitrate and H₂O₂ to the total concentration of free radicals in P1 and P2 which is directly related to the respective light emission spectra. Nitrate concentration in continental medium was about 3 times higher than in marine medium (70 and 200 μM in marine and in continental medium, respectively). In the setup P1, the smallest emission wavelength was ~ 290 nm (Fig. 1) and the formation of hydroxyl radicals was mainly attributed to the photolysis of H₂O₂. Hence, there was no or very little influence of the concentration of nitrates on the concentration of total free radicals. In the setup P2, the smallest emission wavelength was ~ 340 nm (Fig. 1) and the photolysis rate of H₂O₂ was lower than in P1. Consequently, the relative contribution of nitrates to the concentration of total free radicals was higher in P2 than in P1 and could explain the differences observed between irradiations of “marine” and “continental” cloud solutions.

3.3 Comparison of microbial and photochemical experiments

Biodegradation rates measured in artificial cloud media were compared with the photodegradation rates resulting from irradiation in P1 and P2. The aim was to evaluate the relative contribution of microbial activity and of photochemistry on the fate of carboxylic acids. The estimated degradation rates of carboxylic compounds in artificial cloud water by microorganisms and photochemical processes at 17 °C are

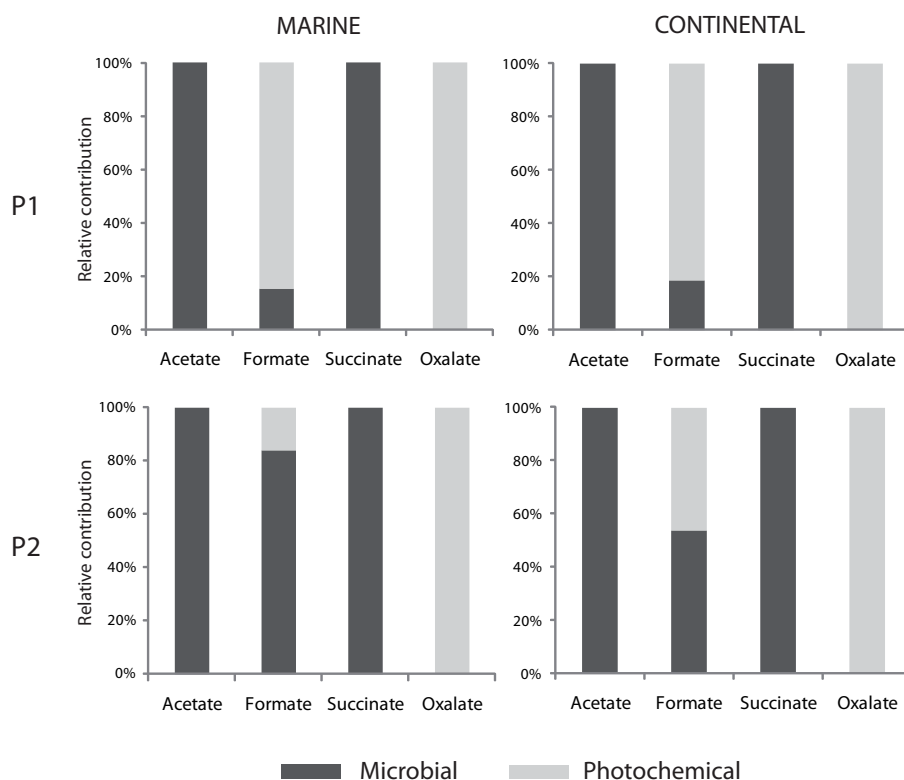


Fig. 2. Microbial and photochemical relative contributions to the degradation of carboxylic compounds in “marine” (left side) and “continental” (right side) artificial cloud media at 17 °C and 5 °C. P1 and P2 correspond to the 2 irradiation setups. This figure is based on the experimental photodegradation and biodegradation rates shown in Table 3.

given in Table 3. The values provided for microbial degradation correspond to the average of the biodegradation rates given in Table 2. Figure 2 shows, for marine and continental cloud media, the relative contribution of biodegradation and of photochemistry to the degradation of acetate, formate, succinate and oxalate.

Considering the values of photodegradation rates obtained in P1 and P2, only microbial activity participated to the degradation of acetate and succinate, while oxalate was exclusively degraded by photochemical processes. For formate, microbial activity in the marine and continental medium contributes to 14 % and 83 % of its degradation in P1 and P2, respectively; in the continental medium, microbial contribution to the degradation of formate are equal to 16 % and 48 % respectively.

4 Atmospheric implications

The aim of this laboratory study was to measure biodegradation and photodegradation rates of organic acids under the same experimental conditions to quantify the relative contribution of microorganisms in cloud chemistry vs. photochemistry. Our results have shown that these degradation rates

were on the same range of order, and suggested thus that microbes could be competitive with solar light.

However, we unexpectedly found the photodegradation rates of acetate and succinate to be undetectable in the two photo-reactors used for these experiments. In order to test other photochemical scenarios, we made a theoretical study taking into account hydroxyl radical concentrations in cloud water simulated by cloud chemistry models and the available reaction rate constants of the studied carboxylic compounds with this radical (Chin and Wine, 1994; Ervens et al., 2003b). The simulated aqueous OH concentrations from several models are displayed in Table 4. Since the 80’s, these numerical tools were developed (Graedel et al., 1986; Jacob et al., 1989) and were based on up-to-date chemical mechanisms that contained explicit chemical processes in the aqueous phase (Herrmann et al., 2005; Tilgner and Herrmann, 2010). They also considered complex microphysical processes that redistribute chemical species among the various phases of the cloud (Leriche et al., 2001; Wolke et al., 2005). Simulated OH concentrations are dependent on the chemical scheme that is considered in the model and also on the cloud microphysical properties that control the mass transfer between the liquid and gas phases. As shown in Table 4, the concentrations reported in the literature can differ by two orders of magnitude depending mainly on the chemical

Table 4. Maximum aqueous OH concentrations simulated by various multiphase cloud chemistry models. These models consider different explicit chemical mechanisms and simulate clouds with different microphysical properties (monodisperse to polydisperse).

Chemical mechanisms	Cloud simulation	Chemical scenario	Simulated OH concentration (max) (M)	References
<i>Aqueous:</i> Chemistry of some inorganic and organic compounds	– Permanent – Monodisperse	– Polluted cloud	2×10^{-13}	Graedel et al. (1986)
<i>Gas phase:</i> Lurmann et al. (1986) <i>Aqueous phase:</i> Chemistry of some inorganic and organic compounds	– Permanent – Polydisperse	– Polluted cloud	1.6×10^{-14}	Jacob et al. (1989)
<i>Gas phase:</i> RACM <i>Aqueous phase:</i>	– Permanent – Monodisperse	– Remote – Urban – Marine	2×10^{-13} 1×10^{-13} 4.5×10^{-13}	Ervens et al. (2003a)
CAPRAM 3.0 Chemistry of inorganic and organic compounds (up to 5 carbon atoms)	– Permanent – Polydisperse	– Remote	1.4×10^{-13}	Herrmann et al. (2005)
	– Non-permanent – Polydisperse	– Remote – Urban	5×10^{-14} 1×10^{-14}	Tilgner and Herrmann (2010)
	– Non-permanent – Polydisperse	– Remote – Urban – Marine	1.6×10^{-14} 6.9×10^{-14} 5.3×10^{-12}	Herrmann et al. (2010)
<i>Gas:</i> Madronich and Calvert (1990)	– Monodisperse – Permanent	– Polluted cloud	2×10^{-14}	Leriche et al. (2001)
<i>Aqueous:</i> chemistry of inorganic and organic compounds (up to 2 carbon atoms)	– Permanent – Monodisperse	– Remote – Urban	1.7×10^{-12} 1×10^{-12}	Deguillaume et al. (2004)
	– Polydisperse – Non-permanent	– Polluted cloud	7×10^{-14}	Leriche et al. (2007)
	– Monodisperse – Permanent	– Remote	3.5×10^{-13}	Deguillaume et al. (2010)

scenario (marine, remote, or urban) and on the microphysical scheme. For our calculation, we decided to consider maximum OH concentrations (simulated at noon and during summertime) that range from 1×10^{-12} to 1×10^{-14} M. We selected the most extreme OH concentrations to evaluate the role of biodegradation in the most “unfavourable” conditions.

To compare these theoretical photodegradation rates to microbial activity, we used the average of biodegradation rates measured in our “continental” and “marine” artificial media (Table 5). The activity of microorganisms is clearly temperature dependant; however the activity of microbes only decreased by a factor 2 to 5 when the temperature shifted from 17 °C to 5 °C. The relative contributions of microbial activity vs. radical chemistry for the various scenarios and the two temperatures are shown in Fig. 3.

During the day and for the highest OH concentrations (i.e. 1×10^{-12} M), microbial activity has clearly a minor contribution both at 17 °C and 5 °C. It would contribute to 4.8 % (17 °C) or 2.0 % (5 °C) of the degradation of acetate, 2.6 % (17 °C) or 0.6 % (5 °C) for succinate, and would be negligible for formate (0.4 % at 17 °C and 0.2 % at 5 °C). However, at lower OH concentrations (i.e. 1×10^{-14} M), microbial contribution could become very important compared to radical chemistry: 27.3 % (17 °C) and 13.6 % (5 °C) for formate, 83.5 % (17 °C) and 67.6 % (5 °C) for acetate, 72.5 % (17 °C) and 37.5 % (5 °C) for succinate.

The contribution of microbes on the degradation of carboxylic acids does not change dramatically when decreasing the temperature from 17 to 5 °C. The main sensitive factor is clearly the concentrations of OH radicals that are generated

Table 5. Experimental biodegradation rates in artificial cloud water at 17 °C and 5 °C and calculated photodegradation rates by OH of acetate, formate and succinate.

		Acetate	Formate	Succinate
17 °C		Estimated degradation rate (M s ⁻¹)		
OH*	10 ⁻¹² M	1.2 × 10 ⁻⁹	3.2 × 10 ⁻⁸	6.6 × 10 ⁻¹⁰
	10 ⁻¹⁴ M	1.2 × 10 ⁻¹¹	3.2 × 10 ⁻¹⁰	6.6 × 10 ⁻¹²
Microbial**	8 × 10 ⁷ cells l ⁻¹	6.0 × 10 ⁻¹¹	1.2 × 10 ⁻¹⁰	1.7 × 10 ⁻¹¹
5 °C				
OH*	10 ⁻¹² M	9.1 × 10 ⁻¹⁰	2.7 × 10 ⁻⁸	5.4 × 10 ⁻¹⁰
	10 ⁻¹⁴ M	9.1 × 10 ⁻¹²	2.7 × 10 ⁻¹⁰	5.4 × 10 ⁻¹²
Microbial**	8 × 10 ⁷ cells l ⁻¹	1.9 × 10 ⁻¹¹	4.3 × 10 ⁻¹¹	3.2 × 10 ⁻¹²

* The kinetic data for OH reactions with acetate is k_1 (25 °C) = $7.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$; $E_{A,1} = 15 \text{ kJ mol}^{-1}$ (Chin and Wine, 1994); with formate: k_2 (25 °C) = $(2.4 \pm 0.4) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$; $E_{A,2} = (9 \pm 5) \text{ kJ mol}^{-1}$ and with succinate (dianion): k_3 (25 °C) = $(5.0 \pm 0.5) \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$; $E_{A,3} = (11 \pm 5) \text{ kJ mol}^{-1}$ (Ervens et al., 2003b).

** Biodegradation rates expressed in M s^{-1} are calculated by considering the average biodegradation rates per cell (in $\text{mol cell}^{-1} \text{ s}^{-1}$) determined in the “marine” and “continental” media (see Table 2) and the cellular concentration equal to $8 \times 10^7 \text{ cells l}^{-1}$ (see Amato et al., 2007c).

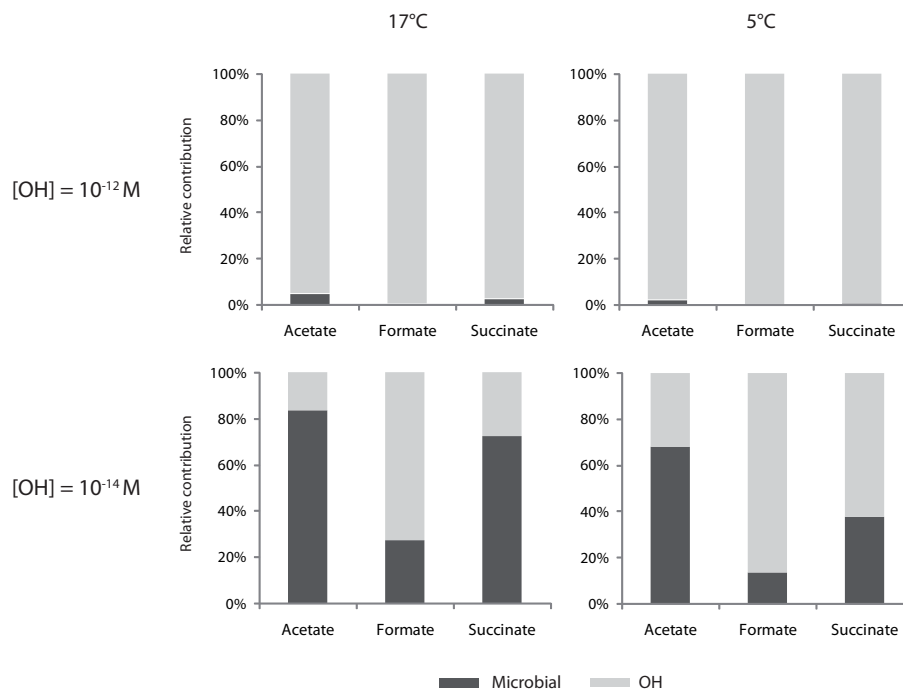


Fig. 3. Theoretical comparisons of microbial activity vs. radical chemistry to the degradation of carboxylic compounds in artificial cloud media. Relative contributions of each process were determined by comparison of biodegradation and photodegradation rates. Biodegradation rates per cell were measured experimentally (Table 2). Photodegradation rates were calculated using simulated standard OH concentrations in cloud water and reaction rates of OH towards the respective acids for two radical concentrations ($[\text{OH}] = 10^{-12} \text{ M}$ and $[\text{OH}] = 10^{-14} \text{ M}$) (see Table 5).

in our photochemical setups using H_2O_2 as a precursor of OH or calculated from modelling studies on cloud chemistry. Our experimental results with both systems (P1 and P2) and calculations using OH concentration equal to 1×10^{-14} M, led to similar contributions of the efficiency of microbes to degrade the studied carboxylic acids. In these conditions, microorganisms were always more efficient to degrade acetate and succinate than photodegradation; the degradation of formate was usually in favour of photodegradation vs. biodegradation; and oxalate was only photodegraded.

5 Conclusions and perspectives

In conclusion, this paper brings new answers to the emerging scientific question related to the relative contribution of microbial activity to atmospheric chemistry. Our results derived from laboratory experiments and theoretical calculations contribute to reinforce the validity of the hypothesis of potential microbial activity in warm clouds:

- the influence of aqueous chemical composition (continental vs. marine) does not seem to influence strongly microbial activity;
- the biodegradation rates not drastically decrease when the temperature of incubation varies from 17 to 5 °C;
- the measured biodegradation rates even at 5 °C are competitive with measured and calculated photodegradation rates especially for acetate and formate.

However more work is required to see if the conclusions obtained in this work can be applied to the more complex environments of ambient cloud droplets. Our detailed experiments have been performed in bulk media with simplified situations. These biodegradation rates might represent the upper limit of the possible biological activity taking place in real clouds where several stressful conditions for microbes can occur (evaporation/condensation cycles, strong oxidants, high UV radiation etc.). But, this first step is crucial to understand the mechanisms taking place and to highlight the potential role of microbes. Chemistry in real clouds is even more complex as it involves multiphase mechanisms and transfers. Only models will be able to reproduce these complex scenarios. In this frame, an urgent task is to experimentally measure radical concentrations in real cloud water. Future work should also combine both photo- and biodegradation experiments both in natural cloud water and in microcosms of various chemical compositions. It should include more parameters as descriptors of the processes going on in these environments. In particular the evolution of microbial population and concentrations of oxidants such as H_2O_2 during the incubation period seem to be relevant parameters to consider. To go further, in cloud processes can also contribute to the modification of pre-existing particles

through new mass formation of organics and/or to new particle formation (Blando and Turpin, 2000; Lim et al., 2010). Microorganisms through the production of metabolites could potentially participate to this in-cloud process. Laboratory experiments have to be performed on individual biological processes under controlled conditions to create parameterizations that could be incorporated in cloud chemistry models. In this study, we showed that our artificial microcosms could realistically mimic real cloud water since biodegradation rates measured in these two media were in the same range. By this way, effect of biological activity on cloud chemistry could be quantified.

Supplementary material related to this

article is available online at:

<http://www.atmos-chem-phys.net/11/8721/2011/acp-11-8721-2011-supplement.pdf>.

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