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Biotransformation of methanol and formaldehyde by bacteria isolated from clouds. Comparison with radical chemistry

Slavomíra Husárová [a,e], Mickaël Vaïtilingom [a,c], Laurent Deguillaume [c,d], Mounir Traikia [a,b], Virginie Vinatier [a,b], Martine Sancelme [a,b], Pierre Amato [a,b], Mária Matulová [e], Anne-Marie Delort [a,b,*]

[a] Clermont Université, Université Blaise Pascal, Laboratoire SEESIB, BP 10448, F-63000 Clermont-Ferrand, France
[b] CNRS, UMR 6504, Laboratoire SEESIB, F-63177 Aubière, France
[c] Clermont Université, Université Blaise Pascal, OPGC/Laboratoire de Météorologie Physique LaMP, BP 10448, F-63000 Clermont-Ferrand, France
[d] CNRS, UMR 6016, LaMP, F-63177 Clermont-Ferrand, France
[e] Institute of Chemistry, Centre for Glycomics, Slovak Academy of Sciences, Dúbravská cesta 9, SK-845 38 Bratislava, Slovakia

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ABSTRACT

The kinetics of biodegradation of methanol and formaldehyde in phosphate buffer at pH 7 by 4 bacterial strains (*Pseudomonas* spp., *Bacillus* sp. and *Frigoribacterium* sp.) isolated from cloud water at the puy de Dôme mountain have been investigated using ^{1}H and ^{13}C NMR spectroscopy. We showed that biodegradation occurred at 5 °C and 17 °C, respectively average and summertime temperature considered within the cloud system at this site. They ranged from 10^{-19} to 10^{-21} mol cell^{-1} s^{-1} both at 5 and 17 °C for formaldehyde, and from 10^{-21} to 10^{-23} mol cell^{-1} s^{-1} at 5 and 17 °C for methanol. Metabolic intermediates were identified, with notably production of C3 compounds (glycerol, 1,2- and 1,3-propanediol) from formaldehyde by the strain *Bacillus* sp. In order to evaluate to which extent microbiological oxidation of organic compounds has to be considered as an alternative route to radical chemistry in cloud water, the biodegradation rates measured were compared with rates related to the reactivity of organic species with free radicals $^{\bullet}OH$ (daytime chemistry) and $NO_3^{\bullet}$ (nighttime chemistry) under two cloud situations (urban and remote cases). Clearly, measured biological and chemical reaction rates were in the same range of magnitude and their relative contribution varies according to the scenarios we tested, including the temperature of the clouds (5 or 17 °C), the category of the clouds (urban and remote) and the diurnal cycle (day and nighttime). Except for the degradation of methanol at 5 °C in remote clouds, our results show that biotransformation processes could be the main sink for C1 compounds in liquid clouds ($T \geq 5$ °C ≡ "warm cloud") during the night and both in polluted and non polluted clouds.

1. Introduction

Large quantities of Volatile Organic Compounds (VOCs) are emitted into the atmosphere by natural and/or anthropogenic sources. Oxidation of VOCs leads to the formation of gaseous and particulate secondary products which, in turn, have significant impact on the atmospheric composition (Atkinson et al., 2006; Kawamura et al., 2005). In this context, secondary organic aerosols (SOA) are formed by gas-to-particle conversion of these low volatile organic products. These particles account for a significant fraction of ambient tropospheric aerosols and impact on atmospheric processes, climate and human health (Finlayson-Pitts and Pitts, 2000; Hallquist et al., 2009).

Oxidation of VOCs occurs both in the atmospheric gas and liquid phases (cloud, rain). In the gas phase, VOCs are primarily transformed by photolysis and/or radical chemistry and reactions with other oxidants such as O_3. In the presence of clouds and depending on their solubility, several VOCs (Van Pinxteren et al., 2005) are significantly transferred into the atmospheric liquid phase where they can be oxidized. Methanol (CH_3OH) is a significant component of the volatile organic carbon in the atmosphere (Heikes et al., 2002) and is the predominant oxygenated compound in the mid to the upper troposphere (Singh et al., 2000, 2001); its life time in the atmosphere has been evaluated to 1–2 weeks (Dufour et al., 2007). The anthropogenic primary source and oxidation taking place in the atmosphere represent 11–20% of the methanol while the biogenic contribution mainly issued from plant metabolism is the most significant part (80–90%) (Galbally and Kirstine, 2002;

* Corresponding author. Clermont Université, Université Blaise Pascal, Laboratoire SEESIB, BP 10448, F-63000 Clermont-Ferrand, France. Tel.: +33 473 40 77 14; fax: +33 473 40 77 17.
E-mail address: A-Marie.DELORT@univ-bpclermont.fr (A.-M. Delort).

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Tie et al., 2003; Jacob et al., 2005; Brunner et al., 2007). Methanol plays an important role in atmospheric chemistry as it influences the oxidizing capacity of the atmosphere by reacting with hydroxyl radicals ($^{\bullet}OH$) or by producing peroxy radical (RO_2) and formaldehyde (HCHO). Formaldehyde is a key atmospheric VOC since it is an important tracer of Non-Methane Volatile Organic Compound (NMVOC) emissions and photochemical activity. HCHO is a primary emission product from biomass burning (Carlier et al., 1986) and fossil fuel combustion (Anderson et al., 1996; Holzinger et al., 1999). However, its major source in the atmosphere is the photochemical oxidation of methane and non-methane hydrocarbons (Altshuller, 1993).

Methanol and formaldehyde are soluble and efficiently transferred into atmospheric waters (Henry's law constants respectively equal to 2.2×10^2 M atm^{-1}and 10^3 M atm^{-1}) (Sander, 1999). Very few papers report methanol concentrations in atmospheric waters; it was found at concentrations of 0.7 μM in rain (Snider and Dawson, 1985). Formaldehyde concentration varies in a very wide range of values (see Table 1); from 0.001 μM in rainwater in rural areas (Kieber et al., 1999) to 710 μM in highly polluted fog (Jacob et al., 1984). The average concentration measured in cloud water at the puy de Dôme station is 2.9 μM (Parazols, 2007) and depends on the air mass origins (6.5 μM for "polluted" category and 2.0 μM for "remote" category).

It is generally admitted that the reactivity of organic acids in clouds is driven by the presence of free radicals ($^{\bullet}OH$, $NO_3^{\bullet}$) or oxidants such as O_3 or H_2O_2 (Herrmann et al., 2005; Tilgner and Herrmann, 2010). However, recent studies raised the idea that microbial activity can play a significant role in the liquid phase transformation of some organic compounds (Ariya and Amyot, 2004; Deguillaume et al., 2008; Delort et al., 2010). It is now well established that living microorganisms are present in clouds (Ahern et al., 2007; Amato et al., 2005, 2007a,c; Bauer et al., 2002; Fuzzi et al., 1997; Sattler et al., 2001). They are active in clouds as shown by ATP (Adenine Tri Phosphate) measurements (Amato et al., 2007c) and CTC (5-Cyano-2,3-ditotyl Tetrazolium Chloride) staining (Hill et al., 2007), these two techniques describe the metabolic activity and the energetic state of the cells. This implies the uptake of nutrients from the liquid phase by cells and suggests that they can develop within cloud water. One key question for cloud chemistry now is to quantify biological oxidation as respect to chemical and photochemical processes. In the recent paper, from Vaïtilingom et al. (2010), it was shown that cloudborne microorganisms could indeed contribute to the transformation of atmospheric organic compounds (carboxylic acids: acetate, succinate and formate), particularly during the night.

The aim of this study is to extend this work to the transformation processes of two important atmospheric organic compounds in clouds: methanol and formaldehyde, considering both biological activity and photochemical reactivity. It is known that the metabolic pathway involving C1 compounds results in very similar intermediates as radical chemistry (Fig. 1). However, contrarily to radical chemistry, only microorganisms can either reduce or oxidize compounds such as formaldehyde.

14 cloud samples have been collected between March 2003 and October 2004 at the puy de Dôme station (1465 m.a.s.l, 45°46′ North, 2°57′ West, France, see for more information: http://wwwobs.univ-bpclermont.fr/SO/beam) (Marinoni et al., 2004; Amato et al., 2005, 2007a). From these samples, we isolated by cultivation more than one hundred strains of bacteria, fungi and yeasts. These included a large proportion of non sporing bacteria able to grow at low temperature. Amato et al. (2005, 2007b) demonstrated their abilities to degrade methanol and formaldehyde at 27 °C. Here, we examined the effect of temperature on biological activity by determining the rates of biodegradation at 5 °C and 17 °C; these temperatures correspond to the mean annual temperature and to the maximal temperature observed at the puy de Dôme summit when clouds form, respectively (see the database at: http://wwwobs.univ-bpclermont.fr/opgc/index.php).

The results obtained with 4 bacterial strains belonging to the genera *Pseudomonas*, *Bacillus* and *Frigoribacterium*, the most current microorganisms isolated from clouds, are presented. The metabolism of these strains was investigated using ^{1}H and/or ^{13}C NMR spectroscopy. This provided indication about the pathways

Table 1
Methanol and formaldehyde concentrations measured in atmospheric waters (rain, fog and cloud) at different sampling sites under various influence (continental, marine, urban, polluted).

Compound	Sites	Samples	Concentrations (μM) Min–Max or (average)	References
HCHO	Wilmington, Rural with maritime influence (NC, US)	Rain	<0.001–13	Kieber et al., 1999
	San Rita, Rural (Arizona, US)	Rain	(7.33)	Snider and Dawson, 1985
	Florence, Urban (Italy)	Rain	0.16–14.8	Largiuni et al., 2002
	Heraklion, Coastal Urban (Crete)	Rain	0.4–11.1	Economou and Mihalopoulos, 2002
	Gdansk Wrzeszcz, Urban (Poland)	Rain	1.7–44	Polkowska et al., 2006
	Los Angeles, Urban (California, US)	Rain	0.85–45	Kawamura et al., 2001
	Los Angeles, Urban (California, US)	Rain	0–20	Grosjean and Wright, 1983
	Galicia, rural with anthropogenic influence from a thermal power plant (Northwest Spain)	Rain	0.2–2.1	Peña et al., 2002
	Sierra Nevada, Rural (US)	Cloud	8–14	Collett, Jr. et al., 1990
	5 sites, Virginia, Rural (US)	Cloud	0.8–228	Munger et al., 1995
	Los Angeles, Urban (US)	Cloud	6–43	Richards, 1995
	Henninger flat, Rural (California, US)	Cloud	0.3–36	Grosjean and Wright, 1983
	San Pedro Hill, Coastal (California, US)	Cloud	13.6–37.3	Igawa et al., 1989
	Henninger flat, Inland Mountainous site (California, US)	Cloud	45.9–61.5	Igawa et al., 1989
	puy de Dôme mountain (air masses from various origins) (Fr)	Cloud	0.1–14.3 (2.0–remote) (6.5–polluted)	Parazols, 2007
	San Joaquin, Polluted (California, US)	Fog	53–710	Jacob et al., 1984
	San Joaquin, Polluted (California, US)	Fog	2.3–410.2	Collett, Jr. et al., 1999
	5 sites, Urban/remote/marine (California, US)	Fog	0.3–76.7	Grosjean and Wright, 1983
	Fairbanks, Urban (Alaska, US)	Ice fog	16.7–38.7	Grosjean and Wright, 1983
	Riverside campus, Polluted (California, US)	Fog	4.1–228	Igawa et al., 1989
CH_3OH	San Rita, Rural (US)	Rain	0.7	Snider and Dawson, 1985