

## Microbiology and atmospheric processes: chemical interactions of Primary Biological Aerosols

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# Microbiology and atmospheric processes: chemical interactions of Primary Biological Aerosols

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## Chemical interactions of PBA

L. Deguillaume et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

EGU

## Abstract

This paper discusses the influence of bioaerosols on atmospheric chemistry and vice versa through microbiological and chemical properties and processes. Several studies have shown that biological matter represents a significant fraction of air particulate matter and hence affects the microstructure and water uptake of aerosol particles. Moreover, airborne micro-organisms can transform chemical constituents of the atmosphere by metabolic activity. Recent studies have emphasized the viability of bacteria and metabolic degradation of organic substances in cloud water. On the other hand, the viability and metabolic activity of airborne micro-organisms depend strongly on physical and chemical atmospheric parameters such as temperature, pressure, radiation, pH value and nutrient concentrations. In spite of recent advances, however, our knowledge of the microbiological and chemical interactions of primary biological particles in the atmosphere is rather limited. Further targeted investigations combining laboratory experiments, field measurements, and modelling studies will be required to characterize the chemical feedbacks, microbiological activities at the air/snow/water interface supplied to the atmosphere.

## 1 Introduction

Airborne micro-organisms have long been known to spread human, animal and plant diseases, and most research on bioaerosols has been focused on health hazards (Jones and Cookson, 1983). Several studies have shown that primary biological aerosol (PBA) particles like bacteria, fungal spores, pollen and plant fragments are ubiquitous in the atmosphere, undergo long-range vertical and horizontal transport, and can influence atmospheric chemistry, physics and climate (Wainwright et al., 2003; Griffin et al., 2004; Prospero et al., 2005; Ariya and Amyot, 2004; Lohmann and Feichter, 2005; Keene and Galloway, 1988; Vali et al., 1996; Acker et al., 2002; Kanakidou et al., 2005; McFiggans et al., 2006; Sun and Ariya, 2006; Fuzzi et al., 2006; Després

**BGD**

5, 841–870, 2008

## Chemical interactions of PBA

L. Deguillaume et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

et al., 2007; Elbert et al., 2007; Möhler et al., 2007; and references therein).

Recent studies also emphasize the role of bacteria present in polar or mountain environments on the chemistry of such cold environments (Skidmore et al., 2000; Toom-Saunry and Barrie, 2002; Amato et al., 2007b; Ariya et al., 2008<sup>1</sup>; Kos and Ariya, 2008<sup>2</sup>). They demonstrate that chemical products resulting from this heterogeneous chemistry could be emitted in the air, modifying atmospheric chemistry (Ariya et al., 2008<sup>1</sup>).

On the other hand, primary biological aerosols can be modified by chemical and physical processes in the atmosphere. In this paper, we discuss the atmospheric effects and feedbacks of microbiological and chemical interactions of PBA primary biological aerosols. Section 2 outlines the abundance of bioaerosol particles and components in atmospheric aerosols and clouds. Section 3 illustrates how micro-organisms can influence cloud chemistry and multiphase processes. Section 4 investigates the influence of atmospheric chemistry on the viability of micro-organisms and other PBA properties; and Sect. 5 gives an outlook on research perspectives and suggestions for future field, laboratory and modeling studies.

## 2 Abundance of primary biological aerosols particles and components in atmospheric aerosols and clouds

Carbonaceous aerosols (organic compounds and black or elemental carbon) account for a large fraction of air particulate matter, exhibit a wide range of molecular structures, and have a major influence on the physico-chemical, biological, climate- and

<sup>1</sup>Ariya, P. A., Domine, F., Kos, G., Amyot, M., Cote, V., Vali, H., Lauzier, T., Legagneux, L., Kuhs, W. F., Techmer, K., Heinrichs, T., Mortazavi, R., Bottenheim, J.: Snow: A Photo-Bio-Chemical Exchange Platform with the Atmosphere, *Geophys. Res. Lett.*, submitted, 2008.

<sup>2</sup>Kos, G. and Ariya, P. A.: Identification of wide range of bioorganic and organic volatile compounds in snow at various Arctic and SubArctic sites, *J. Geophys. Res.*, submitted, 2008.

**BGD**

5, 841–870, 2008

### Chemical interactions of PBA

L. Deguillaume et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

health-related behaviours of atmospheric aerosols (Seinfeld and Pandis, 1998; Jacobson et al., 2000; Turpin and Saxena, 2000; Seinfeld and Pankow, 2003; Kanakidou et al., 2005; Pöschl, 2005; Fuzzi et al., 2006; and references therein). Traditionally the total carbon (TC) content of air particulate matter is divided into an organic carbon (OC) fraction and a black carbon (BC) or elemental carbon (EC) fraction. The origin of BC and EC is mostly pyrogenic (e.g., fossil fuel combustion, biomass burning, Szidat et al., 2004.). The organic fraction is a complex mixture of thousands of organic compounds. The characterisation, classification, and description of the organic fraction in atmospheric models is a major challenge of current atmospheric and climate research (Kanakidou et al., 2005; Fuzzi et al., 2006; Kalberer et al., 2006; Donahue et al., 2006). The sources of OC are much more diverse and include secondary organic aerosol (SOA) formation in the atmosphere as well as primary biogenic aerosols (PBA).

PBA particles and components are, by definition, directly emitted from the biosphere to the atmosphere. Pollen ( $>10\ \mu\text{m}$ ), bacteria ( $\sim 1\ \mu\text{m}$ ), fungal, algae, moss and fern spores ( $\sim 10\ \mu\text{m}$ ), viruses ( $<0.1\ \mu\text{m}$ ) and fragments of animals and plants constitute PBA particles (Matthias-Maser and Jaenicke, 1995, 2000; Artaxo and Hansson, 1995; Bauer et al., 2005; Després et al., 2007). PBA components, which are composed of fragments of biological particles (pollen, bacteria, etc.) can be found on other types of aerosol particles such as sea spray, dust etc. (Kellogg and Griffin, 2006). Large PBA particles such as pollen, fern spores and large fungal spores belong to the coarse mode of air particle matter, but PBA particles and components are also found in intermediate and fine fractions of aerosol particles (e.g., fungal spores, small fragments of animals and plants, bacteria, viruses, carbohydrates, proteins, waxes, biopolymers, ions) (Taylor et al., 2004; Zhang and Anastasio, 2003; Franze et al., 2005). Recent investigations suggest that cellular protein-containing particles are a major fraction of atmospheric aerosols with roughly  $1000\ \text{Tg yr}^{-1}$  compared to  $3300\ \text{Tg yr}^{-1}$  for sea salt and  $2000\ \text{Tg yr}^{-1}$  for mineral dust (Jaenicke, 2005). Recent investigations by Bauer

**BGD**

5, 841–870, 2008

---

## Chemical interactions of PBA

L. Deguillaume et al.

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

EGU

et al. (2008)<sup>3</sup> demonstrate that fungal spores contributed to 10 and 4% to OC and to 5 and 2% to PM10 in aerosols collected at a suburban and an urban site in Vienna (Austria) from April to July. Fungal spores are quantified by microscopic enumeration.

Several studies have shown that fungal spores account for a major fraction of primary biological air particulate matter, with characteristic number and mass concentrations on the order of  $10^4 \text{ m}^{-3}$  and  $1 \mu\text{g m}^{-3}$  in continental boundary layer air (Bauer et al., 2007; Elbert et al., 2007; and references therein). Based on the average concentrations of spores and molecular tracers, a global emission rate on the order of  $17 \text{ Tg yr}^{-1}$ , has been estimated for actively wet discharged spores from *Basidiomycota*, which is one of the major groups of fungi in the biosphere. The global average atmospheric abundance and emission rate of total fungal spores, including wet and dry discharged species, are estimated to be higher by a factor of about three, i.e. on the order of  $50 \text{ Tg yr}^{-1}$  (Elbert et al., 2007). Comparisons with estimated rates of emission and formation of other major types of organic aerosol ( $47 \text{ Tg yr}^{-1}$  of anthropogenic primary organic aerosol;  $12\text{--}70 \text{ Tg yr}^{-1}$  of secondary organic aerosol) indicate that emissions from fungi should be taken into account as a significant global source of organic aerosol. The effects of fungal spores and related chemical components might be particularly important in tropical regions, where both physicochemical processes in the atmosphere and biological activity at the Earth's surface are particularly intense, and where the abundance of fungal spores and related chemical compounds are typically higher than in extra-tropical regions.

The numerical density of micro-organisms at a specific site is highly variable, and is dependent on several factors. The most important factor is due to the season with an increase during the warm period (Di Gorgio et al., 1996; Katial et al., 1997). At a shorter time period, a diurnal cycle is also observed with a maximum around midday (Lighthart, 1999). Finally, the type of the air mass is also clearly a crucial factor; gener-

<sup>3</sup>Bauer, H., Schueller, E., Weinke, G., Berger, A., Hitenberger, R., Marr, I. L., Puxbaum, H.: Significant contributions of fungal spores to the organic carbon and to the aerosol mass balance of the urban atmospheric aerosol, Atmos. Environ., submitted, 2008.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

ally more bacteria are observed carried by urban-influenced air masses than rural and coastal ones (Mancinelli and Shulls, 1978; Shaffer and Lighthart, 1997). This point is developed in detail in Sect. 4.

Several studies have found biopolymers like cellulose and proteins (molecular mass  $\gg 1$  kDa) and other compounds with relatively high molecular mass ( $\gg 100$  Da) such as humic-like substances (HULIS) (analogous to humic substances in soil, surface water and groundwater) in air particulate matter as well as in fog, cloud, and rain water (Zapoli et al., 1999; Puxbaum and Tenze-Kunit, 2003; Pöschl, 2005; Graber and Rudich, 2006; Sanchez-Ochoa et al., 2007). Indeed, biopolymers and other PBA components are likely to account for a large proportion of OC. Proteins, amino acids, and related amino compounds were found to account for up to  $\sim 10\%$  of fine particulate matter and to have a strong influence on the microstructure and water uptake of aerosol particles (Saxena and Hildemann, 1996; Miguel et al., 1999; Zhang and Anastasio, 2001, 2003; Mace et al., 2003a, b, c; Mikhailov et al., 2004; Kuznetsova et al., 2005; Matsumoto and Uematsu, 2005; Pöschl, 2005; McFiggans et al., 2006). In the atmosphere, they undergo chemical reactions such as oxidation, nitration, photolysis and hydrolysis, which can modify their optical, chemical and biological properties and eventually result in the formation of HULIS (Pöschl, 2005; Fuzzi et al., 2006). However, the current understanding of aerosols transformations and the interaction of gases with particles is very limited since they involve multiple physico-chemical processes such as mass transport, phase transition and chemical reactions at the interface or in the bulk of the different compartments: gas, liquid and solid phases (see Sect. 4).

Bauer et al. (2002) showed that average mass concentrations of bacteria only amounted to 0.01% of OC in cloud water, snow, rain and aerosol samples. But, although the mass concentrations of bacteria seems low compared to other organic aerosols, their numbers range from  $\sim 10^3$  to  $\sim 10^5$  cells  $m^{-3}$  (Sattler et al., 2001; Bauer et al., 2002; Amato et al., 2005; Casareto et al., 2006). Bacterial concentrations in cloud water seem to depend mainly on the altitude and location of sampling. Sattler et al. (2001) estimated the average number of bacteria in cloud water

**BGD**

5, 841–870, 2008

---

## Chemical interactions of PBA

L. Deguillaume et al.

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

to be  $\sim 1.5 \times 10^3 \text{ mL}^{-1}$  at Mt Sonnblick (3106 m a.s.l., Austria) during spring 1997. At Mt Rax (1644 m a.s.l., Austria) during spring 1999 and 2000, the average number of bacteria in cloud water was about  $2.0 \times 10^4 \text{ cells mL}^{-1}$  with an air equivalent concentration estimated to be  $5.9 \times 10^3 \text{ cells m}^{-3}$  (Bauer et al., 2002). Amato et al. (2005) performing experiments at the Puy de Dôme Mountain (1465 m a.s.l., France) measured  $10^5 \text{ cells mL}^{-1}$  in cloud water corresponding to  $3 \times 10^4 \text{ cells m}^{-3}$  of cloud volume. Fuzzi et al. (1997), analyzing fog droplets sampled in the Pô Valley, identified three bacterial genera among the culturable fraction (*Pseudomonas*, *Bacillus* and *Acinetobacter*) whereas Amato et al. (2005, 2007a) observed a more diversified culturable population consisting of many bacterial genera, distributed among various phyla or sub-phyla ( $\alpha$ -,  $\beta$ - and  $\gamma$ -Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes), as well as an important diversity among the culturable fungal and yeast populations. Some isolates present high genetic similarities with strains isolated from cold regions and aquatic environments as well as with plant pathogens.

### 3 Influence of micro-organisms on cloud chemistry and multiphase processes

The atmospheric liquid phase is a very complex mixture undergoing numerous chemical and photochemical reactions (process 1 in Fig. 1), dissolving soluble molecules of the particulate phase (process 2 in Fig. 1) and exchanging chemical molecules with the gas phase and the condensed phase (process 3 in Fig. 1). Liquid phase chemistry has a potential impact on the oxidizing capacity of the atmosphere and on the greenhouse effect via an indirect effect of aerosol particles on the Earth's radiative budget. Chemical reactions in liquid phase modify the amount of radicals. These radicals drive the oxidizing power of the atmosphere. For example, iron in aerosols plays a major role in the concentration of radicals in cloud droplets (Deguillaume et al., 2005). As many organic compounds are complexing agents with an iron metal centre, the organo-metallic chemistry can be important in controlling the amount of radicals in clouds (Deguillaume et al., 2005; Parazols et al., 2006). During the lifetime of a cloud, cloud chemistry leads

**BGD**

5, 841–870, 2008

## Chemical interactions of PBA

L. Deguillaume et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

to the formation of new chemical molecules with relatively low volatility such as inorganic and organic acids, which can modify the physico-chemical properties of aerosol particles (Hegg, 2001) and lead to the formation of secondary organic aerosols (Gelençsér and Varga, 2005). Once the cloud droplet has evaporated, the resulting aerosol particle presents a different chemical composition compared to the initial CCN. It tends to be even more hygroscopic and, thus, more likely to take part in the formation of a new cloud droplet (Feingold and Kreidenweis, 2002).

Several studies have discussed the potential role of living bioaerosols in modifying the multiphase chemistry of the atmosphere via metabolic activity, by using labelled and non-labelled chemical species as nutrients (Ariya et al., 2002; Amato et al., 2005, 2007c; Fuzzi et al., 2006). Micro-organisms interact with nitrogen, sulfur and organic compounds in cloud water potentially modifying organic chemistry which is currently subject to high uncertainties. Organic compounds directly or indirectly interact with the aqueous chemistry of radicals, non metal and metal ions and oxidant pools (Ervens et al., 2003; Tilgner et al., 2005; Deguillaume et al., 2005). Organic chemistry inside clouds may consequently modify the chemical budget of both aqueous and gaseous phases (Herrmann et al., 2005). Finally, organic oxidation inside clouds can also be a potentially important source of SOA in the atmosphere after the evaporation of cloud droplets (Blando and Turpin, 2000; Ervens et al., 2004; Carlton et al., 2006). In particular, recent studies show that chemical reactivity in cloud droplets leads to the formation of dicarboxylic acids (DCA), such as oxalic acid which are commonly found in the particulate phase in the atmosphere (Ervens et al., 2004; Sorooshian et al., 2006). DCAs dominate in atmospheric cloud water representing between 10% and more than 70% of the total dissolved organic carbon (Marinoni et al., 2004).

Micro-organisms are incorporated into cloud droplets and raindrops by nucleation scavenging as they have CCN or IN potential (e.g., Lee et al., 2002; Bauer et al., 2003; Möhler et al., 2007) or by washout processes. Some investigations clearly show that most of these micro-organisms are able to develop at low temperatures (between  $-5$  and  $5^{\circ}\text{C}$ ) encountered in clouds. Furthermore, measurements of concentrations of

**BGD**

5, 841–870, 2008

---

## Chemical interactions of PBA

L. Deguillaume et al.

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

adenosine triphosphate (ATP) in cloud water indicate that most micro-organisms are still metabolically active (Amato et al., 2007d). Moreover, metabolic pathways involving micro-organisms can show some similarities with chemical reactions common to atmospheric radical chemistry as described below. However, the nature and the extent of such processes at molecular levels still have to be evaluated. Furthermore, biodegradation processes are expected to be sensitive to atmospheric conditions. The following discussion will focus on bacteria and fungi and on their potential influence on the chemistry of the atmosphere for which some studies are now available.

Herlihy et al. (1967) first studied the degradation of formic and acetic acid by bacteria in rainwater. Ariya et al. (2002) observed evidence for chemical reactions induced by bioaerosols in solutions containing DCA. They observed that several of the DCA detected in the atmosphere can efficiently be degraded by airborne bacteria and fungi. Estimated degradation lifetimes (few days) are also comparable to major atmospheric oxidants (OH, O<sub>3</sub> and HO<sub>2</sub>) but highly variable as a function of environmental conditions. They also found that the observed microbiological transformation varied as a function of the organic acid used and of the type of micro-organism. They analysed products of the reaction mixture which are non- or only slightly toxic compounds (acetamide, butanoic and propionic acids, etc.) as well as highly toxic and carcinogenic ones (kojic acid, aflatoxin B1). The isotopic <sup>13</sup>C NMR studies clearly demonstrated that airborne microbes can metabolise and chemically transform the DCA to other organic compounds. During these biodegradation processes, molecules can be transformed to more volatile compounds rendering the products available for recycling back into the atmosphere and also to more toxic/pathogenic chemicals. Amato et al. (2005) show that bacterial strains present in cloud water (Puy de Dôme Mountain) contain the enzymatic equipment required for the degradation of some atmospheric compounds. Cloud water was collected by a single-stage cloud impactor (Kruisz et al., 1993) over two hours of sampling for various cloud events. These bulk samples are representative of the bacterial community of the cloud system. In their more recent work (Amato et al., 2007c), they present an overview over the capacities of isolates from cloud water

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**Chemical  
interactions of PBA**L. Deguillaume et al.

---

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[I◀](#)[▶I](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

to transform numerous atmospheric compounds. From this study based on 60 microbial strains, they established that, depending on atmospheric conditions, the microbial component is likely to be a sink for carboxylic acids like acetate, formate, lactate and succinate, and also of methanol and formaldehyde. They also can represent a source, with example e.g. the transformation of succinate into fumarate, and of lactate into pyruvate. All those compounds can be found in relatively large concentrations in cloud water (Puxbaum et al., 1988; Grosjean et al., 1989; Granby et al., 1997; Suzuki et al., 1998; Löflund et al., 2002; Marinoni et al., 2004; Van Pinxteren et al., 2005) and play a major role in atmospheric chemistry (Ervens et al., 2005; Herrmann et al., 2005). Their results also indicate preferential metabolic routes for some microbial groups. For instance, *Staphylococcus* spp. are specialized in the transformation of C<sub>1</sub> compounds. This means that depending on the communities present in a given cloud, microbial participation in chemistry would be different. The authors suggest that the genus *Pseudomonas* would be a good model for further investigations, as this would represent an upper estimate of what can be expected, in terms of chemical impact, from microbes being active in cloud water. Figure 2 adapted from Amato (2006) illustrates the similarities between the radical chemistry within clouds and observed metabolic ways of bacteria collected in cloud water. The degradation of carboxylic acids leads to a final release of CO<sub>2</sub> with similar active intermediate molecules. Preliminary studies of Amato et al. (2008)<sup>4</sup> confront the kinetics of photochemical degradation vs. biodegradation of organic compounds. Their results show that during night-time conditions, the biodegradation rates for organic compounds are in the same order of magnitude as chemical degradation rates driven by NO<sub>3</sub> oxidation. During daytime conditions, their calculations suggest that abiotic chemistry driven by the OH radical is faster than biodegradation. It has to be noted that the degradation speeds (abiotic and biotic) are evaluated for ideal conditions and are different than for a complex mixture.

<sup>4</sup>Amato, P., Sancelme, M., Laj, P., and Delort, A.-M.: Is oxidation of organic compounds in clouds by micro-organisms an alternative route to radical chemistry?, Appl. Environ. Microb., submitted, 2008.

**BGD**

5, 841–870, 2008

---

## Chemical interactions of PBA

L. Deguillaume et al.

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

This preliminary result indicates that common radical chemistry considered in a numerical cloud model could compete with kinetic biological degradation as already shown by Ariya et al. (2002), but as they noted, there are substantial differences in the microbiological activity for different environmental conditions, and thus the direct observation without adjustments to relevant environmental conditions (such as T, pH, irradiation, nutrients and other existing chemicals) can not yield meaningful conclusions. Therefore, the contribution of biodegradation processes has to be evaluated in a cloud system where microphysical processes, photochemical reactivity, dissolution processes and biodegradation will compete and modify the general chemistry of the troposphere.

#### 4 Influence of atmospheric chemistry on the viability of micro-organisms and other PBA properties

For microbial cells, the atmosphere is a very stressful environment (Jones and Harrison, 2004; Adhikari et al., 2006). Low temperature is considered to be an important limiting factor for cell activity in the atmosphere and brutal shifts in temperature can stop metabolic activity. However, it has been demonstrated that bacterial activity can occur at subzero temperatures (Christner et al., 2003; Junge et al., 2004) notably due to several physiological processes such as increase in membrane fluidity (Graumann and Marahiel, 1996; Seshu Kumar et al., 2002) and the presence of enzymes active at low temperature (Groudieva et al., 2004). Microbes are shown to be capable of adopting several survival mechanisms; for instance, becoming dormant, changing size and forming spores (Price and Sowers, 2004). In cold temperatures they can adapt by reducing their cell size and the thickness of their capsular polysaccharide, by changing their fatty acid and phospholipids composition, or through energy releasing catalytic redox reactions in ice or in permafrost thereby leading to the creation of habitable microenvironments in ice crystals (Thomas and Dieckmann, 2002; Kos and Ariya, 2008<sup>2</sup>).

In addition to temperature, other environmental limiting factors for cell activity in the

**BGD**

5, 841–870, 2008

### Chemical interactions of PBA

L. Deguillaume et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

atmosphere exist. The atmosphere is a very oxidative medium (e.g., OH and HO<sub>2</sub>/O<sub>2</sub><sup>-</sup> radicals and ozone) which can oxidise cellular material and damage DNA. Cells have at their disposition enzymes (superoxidase, peroxydases etc.) which reduce or trap radicals such as β-carotene (Gourmelon et al., 1994; Ochsner et al., 2000). When nutrient concentrations (organic matter essentially) are not sufficient, the global cell metabolism can be reduced and cell development can be stopped. During cell division, irradiation, especially UV, can cause significant damage such as mutation of DNA which can lead to cell death (Sommagura et al., 1997). In response to this stressor, bacteria can also produce a diverse suite of pigments (scytonemins, carotenoids, phycobiliproteins, chlorophylls etc.) that absorb from the near UV-B to red wavelengths (Mueller et al., 2005). Sensitivity to irradiation strongly depends on the type of strains and also on altitude since intensity of the UV radiation increases with the altitude.

Micro-organisms in the atmosphere can suffer from dehydration and desiccation leading to the modification of vital structure and ultimately to death (Zentner, 1966). Cell aggregation can diminish sensitivity to desiccation as cells in aggregates protect each other. When incorporated into cloud or fog droplets, microbial cells would not suffer from desiccation and would be transported over greater distances than under clear sky conditions. Thus, clouds and fog represent relatively favourable environments for micro-organisms thereby sustaining their activity and viability. Consequently, fog and clouds represent media in which microbial cells can potentially divide as proposed by Dimmick et al. (1979), Fuzzi et al. (1997), Sattler et al. (2001), Bauer et al. (2002), Amato et al. (2007a, d).

In the atmospheric aqueous phase, the pH is a crucial parameter (Ariya et al., 2002) for cell viability, partially as cells have to adjust progressively to an acidic medium (Koutsoumanis and Sofos, 2004). The effect of pH is also linked to other parameters such as oxygenation; hence, the responses to oxidative and acidic stress are similar. The concentration of micro-organisms is also clearly linked to the chemical composition of cloud water. Recent investigations led by Amato et al. (2007d) show that the number of micro-organisms in cloud water increases with increasing oceanic contribution and

**BGD**

5, 841–870, 2008

---

## Chemical interactions of PBA

L. Deguillaume et al.

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

decreases with increasing anthropic influence. It suggests that micro-organisms originating from the ocean are preferably integrated in cloud droplets compared to those from continental sources. They also suggest that an eventual multiplication of cells in cloud droplets can be disturbed due to the toxicity of certain polluted clouds.

5 Thus, we have to consider positive and negative feed-backs amongst various environmental factors with regard to the viability and the metabolic state of airborne micro-organisms.

Apart from the viability of micro-organisms, multiphase chemical reactions in the atmosphere can also have influences on the effects of PBA on climate and public health. For example, protein molecules can be efficiently nitrated in polluted urban air under summer smog conditions or in mixtures of NO<sub>2</sub> and O<sub>3</sub> (Franze et al., 2005). The nitration reaction leads to the formation of 3-nitrotyrosine residues in the polypeptide chain of the protein molecule, i.e. to the introduction of an NO<sub>2</sub> group next to the OH group on the phenyl ring of the aromatic amino acid tyrosine (2-amino-3-(4-hydroxyphenyl)propanoic acid). This posttranslational modification of proteins is likely to trigger immune reactions and provides a molecular rationale for the promotion of allergies by traffic-related air pollution, which is confirmed by recent and ongoing biomedical investigations (Gruijthuijsen et al., 2006).

20 Nitration and other chemical modifications may not only influence the biological properties (allergenicity, toxicity, viability) of PBA. Chemical transformations can also influence the PBA physico-chemical properties which determine their effects on climate (optical parameters; hygroscopicity, surfactant activity, and CCN activity; crystallinity and INA (Ice Nucleation Active) activity). For example, nitration renders colourless proteins yellow (nitrotyrosine acts as a chromophore) thereby potentially modifying reflectivity of PBA. It may also change the molecular and crystalline structures of proteins which serve as active sites for ice nucleation on INA bacteria. The amino acid sequence at the water binding site on the bacterial IFN active protein contains tyrosine. Folding of the protein leading to the formation of  $\beta$ -sheets occurs at the gly-tyr-gly site in the amino acid sequence in the octapeptide repeated zone of this protein (Gazit,

**BGD**

5, 841–870, 2008

---

## Chemical interactions of PBA

L. Deguillaume et al.

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

2002). Formation of 3-nitrotyrosine residues as described above might reduce the water-binding capacity of this site or disrupt the protein tertiary structure necessary for INA.

## 5 Research perspectives and suggestions for future studies

5 Current knowledge regarding the relationship between atmospheric chemistry and bioaerosols is not very advanced. Bioaerosol chemistry and the reciprocal influence of environmental conditions on bioaerosols with regard to the effect on the atmosphere are very complex subjects at the early stages of their development. This young scientific issue gives rise to many questions.

10 Firstly, regarding the influence of bioaerosols in dry air, the major uncertainty concerns to the modification of the physico-chemical properties of aerosols: (1) Does the modification of chemical composition of organic aerosols due to contact/collision with bioaerosols along with particle size and density significantly influence their CCN efficiency?; (2) Do biopolymers present on carbonaceous aerosols undergo heterogeneous chemistry leading to possible formation of macromolecules such as HULIS substances?

15 Secondly, recent studies have clearly demonstrated that viable micro-organisms present in the atmosphere can contribute to atmospheric chemistry through degradation processes, as well as chemical change due to the release or desorption of molecules from microbiological entities (Cote et al., 2008). Clouds, fog and rain seem to represent media where the biological activity is significant due to the protection offered from desiccation. Micro-organisms present in fog and clouds use chemical compounds in the aqueous solution as nutrients and therefore perturb the radical chemistry of clouds.

25 Different approaches are available for studying the effect of bioaerosols on atmospheric chemistry. Laboratory studies allow characterizing (bio)chemical degradation processes but at a given temperature and/or at a given pressure for a given chemical

**BGD**

5, 841–870, 2008

---

### Chemical interactions of PBA

L. Deguillaume et al.

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

species. In-situ measurements aim at better understanding the chemical processes within clouds through biological, chemical, microphysical, and meteorological measurements and complement laboratory studies in providing more general information on the environmental conditions. The main difficulty is to coordinate the types of measurements in time and space. Process models are good tools to integrate laboratory measurements while transport models allow studying various reaction pathways, complex interactions between biological, microphysical, chemical, radiative, and dynamic processes in the framework of the whole cloud system. Then, models are also helpful in running sensitivity tests to study the influence of environmental parameters on multiphase chemistry. These approaches need to be used and developed in synergy in order to quantify the effect of bioaerosols in the frame of atmospheric chemistry:

(1) Further fundamental laboratory chemical-biological research is mandatory to provide answers concerning the kinetics and mechanisms of (bio)chemical transformation involving enzymatic and non-enzymatic processes.

Firstly, many future laboratory investigations are needed for studying the contribution of bioaerosols to organic aerosols and its role on climate. Most of the techniques for monitoring bioaerosols are offline and highly labour intensive (for example, cultivation, staining, fluorescence and electron microscopy, enzyme and immunoassays, DNA analysis etc.) (Maron et al., 2005). New challenging techniques such as aerosol mass spectroscopy (Noble et al., 2000; van Wuijckhuijse et al., 2005; Kleefsman et al., 2007) or aerodynamic sizing and fluorescence spectroscopy (Ho, 2002) allow online monitoring of bioaerosols but still improvements in capturing detailed chemical characteristics at sufficient detection limits are needed.

Secondly, further studies should also include understanding of kinetics of biological and chemical processes. Laboratory studies are needed to determine the kinetics and mechanisms of the chemical reactions involving organics and molecules such as nitrogenous compounds and trace metals that can potentially interact with microorganisms. Micro-organism strains isolated in the aqueous phase of clouds need to be selectively isolated or identified in terms of their ability to degrade chemical compounds

**BGD**

5, 841–870, 2008

---

**Chemical  
interactions of PBA**

L. Deguillaume et al.

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

as well as for their ability to be active under extreme conditions (bacteria with pigments, psychrotrophic bacteria, spore-discharging micro-organisms etc.). The degradation ability of these strains will be followed by the laboratory with the objective of determining kinetic constants which will be incorporated in numerical models. The enzymatic activity of micro-organisms has also to be evaluated as a function of numerous factors such as pH, concentrations of oxidizing agents ( $H_2O_2$ , iron) and actinic flux. Therefore, parameterization of the relationships between these sensitive parameters and the metabolic activity of micro-organisms has to be developed.

(2) As mentioned before, the biological activities of micro-organisms are very dependent on environmental conditions. Long-term and detailed observations of the microbial communities (total number, characterization of strains, metabolic activity etc.) are necessary to evaluate the diurnal and seasonal variations of structure and activity of cells as a function of environmental conditions such as humidity, light, temperature etc. Moreover, this climatology of the population of micro-organisms needs to be correlated with the chemical characterization of the sampled air mass.

More specifically, in atmospheric water, similar long-term observations need to be performed in order to characterize the living microbial biota as a function of encountered physico-chemical conditions. The laboratory investigations detailed above combined with these in-situ measurements of chemical composition of water will provide a real overall picture of the capacity of micro-organisms to develop under the nutritional conditions offered by atmospheric water.

(3) Finally, modelling will allow us to evaluate the relative contribution of bioaerosols to the chemistry and physics of the atmosphere, and their possible impact on climate. A complete treatment of this interaction requires a rather complex model concerning cloud microphysics (cloud droplets activation, ice activation and multiplication), cloud chemistry, tropospheric chemistry including transport, etc, involving the representation of different scales. For instance, cloud microphysics and chemistry are treated locally in a cloud while tropospheric chemistry and transport will be addressed by the way of 3-D meso-scale simulations. A possible approach to consider the effect of micro-organisms

**BGD**

5, 841–870, 2008

---

## Chemical interactions of PBA

L. Deguillaume et al.

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

on atmospheric chemistry, modelling could be the following:

(a) Numerical models describing multiphase cloud chemistry need to consider biological processes in order to evaluate the role of micro-organisms in cloud chemistry. Even though theoretical, models allow us to study – within the cloud system – the chemical pathways, the complex interactions between microphysics, chemistry and dynamics (i.e. transport) and the influence of environmental parameters on cloud chemistry. At the same time, the structure of numerical models offers the possibility to perform sensitivity analysis on uncertain parameters. Biological degradation processes have to be implemented in models in order to compare explicit photochemistry with biological degradation processes. The whole complexity of biological processes including all the metabolic pathways cannot be implemented in these models. The objective will be rather to evaluate the global potential of micro-organisms present in cloud water on the degradation of relevant chemical compounds. Laboratory studies, as indicated above, need to develop biodegradation parameterizations as a function of environmental conditions. Finally, simulations of various cloud events under different environmental conditions will allow us to generalize the effect of micro-organisms on cloud chemistry.

(b) Sensitivity analysis performed with models will allow development of parameterizations of the biological degradation of chemical compounds under specific environmental conditions. In 3-dimensionnal models, micro-organisms will be considered as one category of aerosol particles and kinetic constants of biological degradation will be introduced as a function of environmental conditions. These formulations will be first implemented in regional models such as the RAMS model (Cotton et al., 2003) or the Meso-NH model (Lafore et al., 1998) and afterwards in more global models such as the MOCAGE model (Josse et al., 2004) or the ECHAM5/MESSy1 model (Tost et al., 2007).

These past 15–20 years have produced a real boost in our knowledge regarding detailed chemistry occurring in the atmosphere. Recent investigations, summarized here, demonstrate that we also need to combine biological processes with classical chem-

**BGD**

5, 841–870, 2008

---

## Chemical interactions of PBA

L. Deguillaume et al.

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

istry. Diverse competences will need to be brought together to examine this interplay between bioaerosols and atmospheric chemical processes. These competences will represent a very broad spectrum of chemical and biological sciences including microbiology, agronomy, atmospheric chemistry, meteorology and modelling. Furthermore, because of the few scientists currently interested in this field and the wide range of competence needed, international and interdisciplinary collaboration is essential. Authors of this article are involved in various scientific fields and the combination of these competences represents a difficult but exciting challenge!

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**BGD**

5, 841–870, 2008

## Chemical interactions of PBA

L. Deguillaume et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

EGU

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**BGD**

5, 841–870, 2008

---

## Chemical interactions of PBA

L. Deguillaume et al.

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

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**BGD**

5, 841–870, 2008

---

## Chemical interactions of PBA

L. Deguillaume et al.

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

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**BGD**

5, 841–870, 2008

---

**Chemical  
interactions of PBA**L. Deguillaume et al.

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

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**BGD**

5, 841–870, 2008

---

## Chemical interactions of PBA

L. Deguillaume et al.

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

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**BGD**

5, 841–870, 2008

---

**Chemical  
interactions of PBA**

L. Deguillaume et al.

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

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5, 841–870, 2008

---

**Chemical interactions of PBA**L. Deguillaume et al.

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

I◀

▶I

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

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---

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L. Deguillaume et al.

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

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5, 841–870, 2008

---

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L. Deguillaume et al.

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

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**BGD**

5, 841–870, 2008

---

**Chemical  
interactions of PBA**

L. Deguillaume et al.

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

Turpin, B. J., Saxena, P., and Andrews, E.: Measuring and simulating particulate organics in the atmosphere: problems and prospects, *Atmos. Environ.*, 34, 2983–3013, 2000.

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**BGD**

5, 841–870, 2008

---

## Chemical interactions of PBA

L. Deguillaume et al.

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

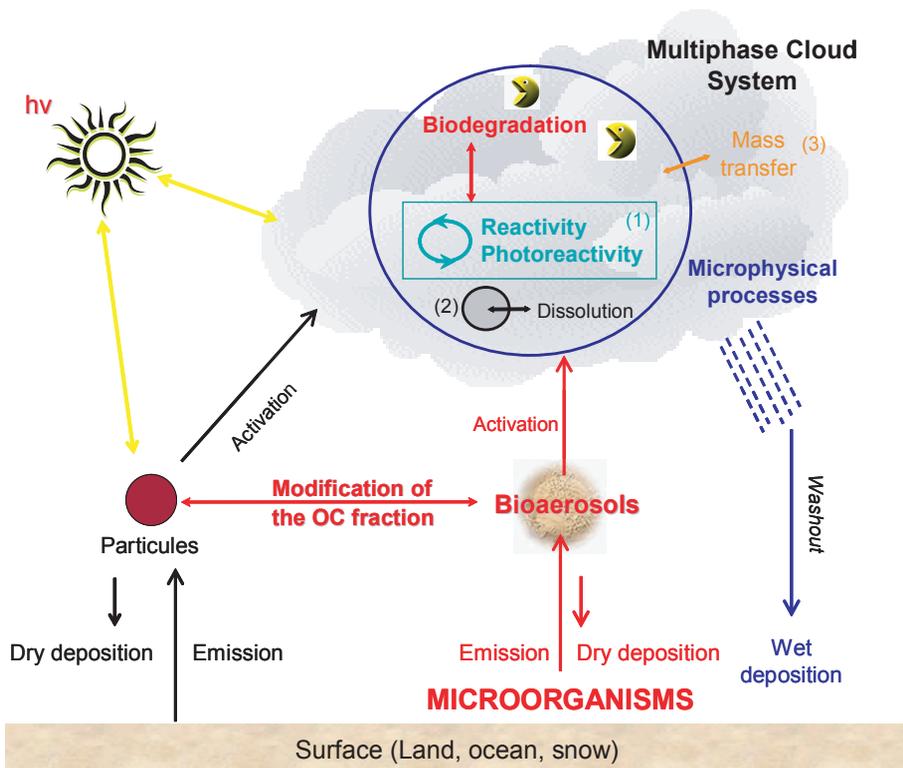


Fig. 1. Schematic representation of the effects of bioaerosols on atmospheric chemistry.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

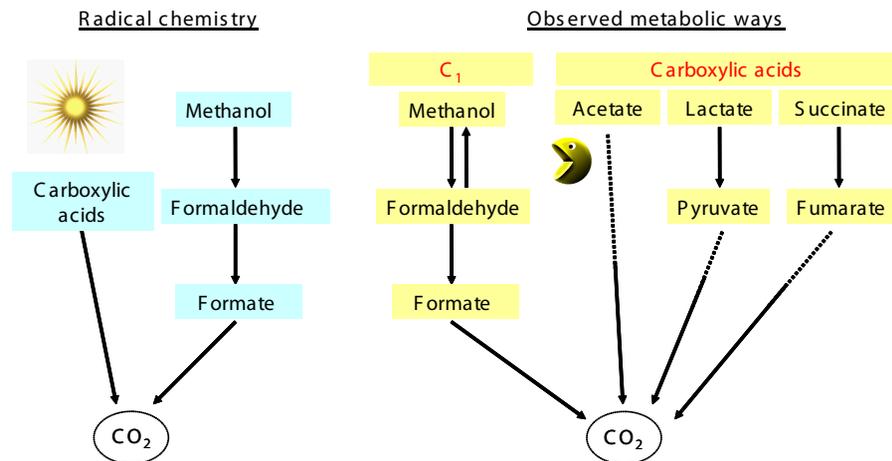
Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



**Fig. 2.** Transformations of organic compounds by photochemical pathways (on the left side) and by biochemical pathways (on the right side) observed in the laboratory for organic compounds with one carbon atom and for carboxylic acids (adapted from Amato, 2006).

Title Page	
Abstract	Introduction
Conclusions	References
Tables	Figures
◀	▶
◀	▶
Back	Close
Full Screen / Esc	
Printer-friendly Version	
Interactive Discussion	