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Atmospheric Chemistry and Physics Discussions

Interactive comment on "A fate for organic acids, formaldehyde and methanol in cloud water: their biotransformation by micro-organisms" *by* P. Amato et al.

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The authors addressed the question of the fate of organic substrates in cloudwater by biotransformation using two types of experiments. Only the first approach used cloudwater. The second approach was basically a study of pure bacterial cultures under artificial laboratory conditions.

In summary, I found the demonstration of microbial growth in cloudwater samples the most interesting result, albeit being a snapshot. However, the remainder of the study is, to my opinion, not useful. From that I did not learn anything relevant about microbial transformation processes in cloudwater. This is mainly due to the focus on microbial cultures under arbitrary laboratory conditions, rather than on the environment

itself with its peculiar conditions of substrates and concentrations and physicochemical background conditions. Unfortunately, the results also do not provide any novel aspect concerning biotransformation in bacteria.

1. In the first experiment, a bulk cloud water sample was incubated for about 4 days at 17C with shaking. During this time both DAPI-stained microbial cells and concentrations of ATP increased. This experiment demonstrated that microbial growth in non-supplemented cloud water was possible. The obtained data allow calculation of the average ATP content per cell. This was about 6 times 10ˆ-18 mol ATP per cell or 100 000 ATP molecules per cell, which is a reasonable value in comparison to literature data. Assuming a cellular mass of about 1 pg dry weight, the final yield of about 106 bacteria per ml is equivalent to about to 1 mg bacterial dry mass per liter. Assuming a growth yield of 50%, about 2 mg substrate had to be consumed per liter, or in case of acetate the initial concentration would have had to be about 40 micromolar. This figure is about 10 time higher than the acetate concentration (4 micromolar) that had actually been measured in the cloud water. Availability of substrates with a higher molecular weight than acetate would decrease the discrepancy, but even when using succinate still 20 micromolar would be required initially. Therefore, I assume that the average bacterial cell mass was less than 1 pg. Assuming 0.1 pg per cell would make the data consistent. Such a value, or even lower ones, is conceivable for natural microbial populations. The authors could evaluate the microscopic cell counts with respect to cell volume, to further constrain the calculation.

In conclusion, microbial growth in cloud water resulted in a very low cell biomass since the concentrations of the growth substrates were probably extremely low. Microbial growth not only requires substrates as energy and carbon source, but in addition as essential nutrients in sufficient stoichiometric amounts, in particular N, P, S and Fe. It remains unclear which element limited microbial growth in the cloudwater sample studied. I assume that the substrate for energy and carbon was the most likely one, simply because C/N/P is required at a ratio of about 100:10:1, but nevertheless, it remains un-

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clear. A shortcoming of the present study is that it represents only a snapshot on one particular cloudwater sample. It is therefore not yet possible to generalize the results.

2. The second type of experiment was substrate utilization tests using pure bacterial cultures. These cultures had previously been isolated from cloudwater samples. However, the assays were not conducted under conditions that mimic the situation in cloudwater systems, but under arbitrarily chosen laboratory conditions. Therefore, they are not relevant for cloudwater systems. From a microbiological perspective, the results are also not interesting, since they were basically substrate utilization tests in phosphate buffer with high millimolar concentrations of substrates, which is routine assay in bacterial taxonomy. There are plenty of similar experiments. Since they are trivial for microbiologist, they are usually only reported in diagnostic tables. Nowadays such substrate utilization assays are mostly done using commercial test kits. The reporting on whether or not a particular substrate is utilized by a bacterial isolate is thus not really exciting, even when the percentage utilization has been measured or substrates are tested that, by coincidence, happen to be ubiquitous in cloudwater samples.

In principle, a substrate can be used by microorganisms in several different ways. (1) the substrate may be used as energy substrate. Its usage is then stoichiometrically related to cell yield or to maintenance requirement. Synthesis of cell biomass can constrain catabolism. For example, the inability of utilizing acetate may be due to the absence of the glyoxylate shunt that is required for synthesis of C3 from C2 compounds. (2) The substrate may be assimilated into microbial biomass while a second substrate (or light) supplies the energy. Still there would be stoichiometric relationships between the substrates utilized and the biomass produced. (3) The substrate may be used fortuitously, simply because suitable degradation enzymes are present or since enzymes are unspecific enough to transform also a foreign substrate besides the natural one. Utilization is then not principally connected to biomass formation. It may even happen that enzymes are excreted that transform the substrate, such as, for example hydrolysis of cellulose or depolymerization of lignin.

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A substrate utilization test in phosphate buffer does not allow much biosynthesis, mainly since a nitrogen source is missing. It is probably a test of the enzyme machinery that had been synthesized during previous growth. In the present study this previous growth was on highly complex media containing glucose, yeast extract, peptone, etc. It would have been important to see whether substrate utilization was affected by the previous growth conditions of the bacteria, since utilization of specific substrates, in particular acetate and methanol, may not be expressed on complex media. The substrate utilization tests therefore do not tell much about the actual capabilities of the bacteria.

Rather than percentage of substrate consumed the determination of kinetic parameters (Vmax, Km, threshold) might have been more interesting. These parameters would at least illustrate whether the physiological capability of the microbes is compatible with the cloudwater situation that is characterized by very low substrate concentrations, in fact almost 4 orders of magnitude lower than those tested. Kinetic studies would need to consider also the pH conditions besides temperature, since cloudwater can have a pH much lower than pH 7 used in the test, and this would be crucial for bacterial activity. Also, the buffer capacity of the cloudwater would be much much lower than that of the 100 mM phosphate buffer used for the assays.

For elucidating the mechanisms of substrate utilization the authors applied NMR analysis, which is certainly a step into the right direction, since NMR analysis is one (among several others) useful technique for assessing metabolic pathways. In fact, the data give some useful hints, but have not been conducted in sufficient detail for definitive answers. Again, the data give only hints for the conditions in the pure bacterial cultures at extremely high substrate concentrations. Transformation mechanisms in-situ may be different.

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