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Factors influencing the acclimation period preceding the mineralization of organic chemicals in seawater by microbial communities

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Abstract

An acclimated mixed culture of degrading bacteria and a degradable substance (4-Nitrophenol) were introduced into differently treated water samples.

It could be shown that in all cases where an acclimated inoculum was used, degradation took place almost immediately compared to not acclimated cultures, where it took at least 10 days. The rate and extent of mineralization was influenced by low temperature, the presence of other organic nutrients and especially protozoan grazing.

The data suggest that one of the main reasons for the acclimation period was the very small initial population of degrading bacteria. The role of other carbon sources is ambivalent. Low concentrations of organic chemicals which cannot sustain growth slow down acclimation and the degradation rate. Another important factor slowing down growth is grazing by protozoa, which can inhibit effective degradation.

Introduction

Many chemicals enter the natural environment. Often the onset of mineralization is delayed, and degradation rate constants cannot be obtained. If a prolonged period of time is necessary for the development of the ability for fast degradation of a chemical this is called acclimation period. Several factors may be important for the occurence of acclimation periods, which lead to increased persistence of chemicals in nature:

- A mutation has to take place, if the ability in question is not present in the bacterial population investigated.

- Degrading enzymes may have to be induced.

- If the initial degrader population is small, growth is necessary for degradation; growth in natural environments can be slowed down by low temperatures, low concentration of the chemical in question, missing nutrients or protozoan grazing.

Material and methods

The mineralization of ^{14}C-labelled 4-Nitrophenol in natural water samples from the Kiel Fjord was investigated. Triplicate samples were incubated at 20 or partly 10 °C with shaking. At first only 8 µg/l were used. Secondly 8 µg/l were added to two communities, one of which had already degraded 250 µg/l of unlabelled 4-NP is considered acclimated. The other one was only pre-incubated for the same interval of 18 days before the small dose of 4-Nitrophenol was added and a new incubation of 28 days length started. A third experiment was made, where an acclimated mixed culture was introduced into differently treated water samples. 5 vol% of this community were added to estuarine water samples containing 8 µg/l 4-NP. The water was either aged and autoclaved, or fresh and amended with cycloheximide, to inhibit protozoan growth. To promote the development of bacterial grazers it was either not treated at all, only the degrader population and 4-NP were added. Or it was pre-incubated at 10 °C (about *in situ* temperature) for 3 days, till a bloom of heterotrophic nanoflagellates was microscopically observed. Then the acclimated community and 4-NP were added and the last treatment was further incubated at 10 °C.

Results and discussion

With natural water samples from the Kiel Fjord about 19 days passed before substantial mineralization of 8 µg/l 4-NP occurred (Fig. 1). For an initial dose of 250 µg/l about 10 - 14 days passed before the onset of rapid degradation (data not shown).

Degradation of 250 µg/l 4-NP by a mixed bacterial community resulted in fast mineralization of a second dose of 8 µg/l by the now acclimated population (Fig. 2). When no 4-NP was given during the pre-incubation, no degradation was observed in 28 days following exposure to 8 µg/l 4-NP (Fig. 2). After acclimation to 1 µg/l 4-NP which took about 20 days, a second addition of 250 µg/l was only degraded after 16 more days (data not shown).

In the experiment where the acclimated population was diluted it could be shown that degradation started fast (Fig. 3). The highest mineralization rate was found in the treatment with aged autoclaved seawater, which contained only very little organic carbon and no protozoa (Fig. 3). A lower initial degradation rate was observed but the same final percentage was reached, when protozoan activity was suppressed in fresh seawater (Fig. 3). Where untreated seawater was used the initial rate was similar to the cycloheximide treatment. After 4 days the CO_2-production became stagnant for about two days. A second plateau was observed during the last 2 days of the experiment. Therefore the extent of mineralization after 19 days was about 15 % less than in the other treatments.

The treatment with pre-incubation at 10 °C showed a slower rate of 4-NP degradation in the beginning, probably due to the lower incubation temperature (Fig. 4). Then a stagnation for 8 days with only about 25 % mineralization followed, which coincided with elevated numbers of heterotrophic nanoflagellates during most parts of the incubation (4 - 21 x 10^3/ml). After 32 days only about 50 % were mineralized.

The rate and/or extent of mineralization was negatively influenced by low temperatures, other organic nutrients and especially protozoan grazing. The plateau in the treatment with fresh seawater incubated at 20 °C after 4 days was presumably due to grazing, as this is the regular time span for protozoa to develop

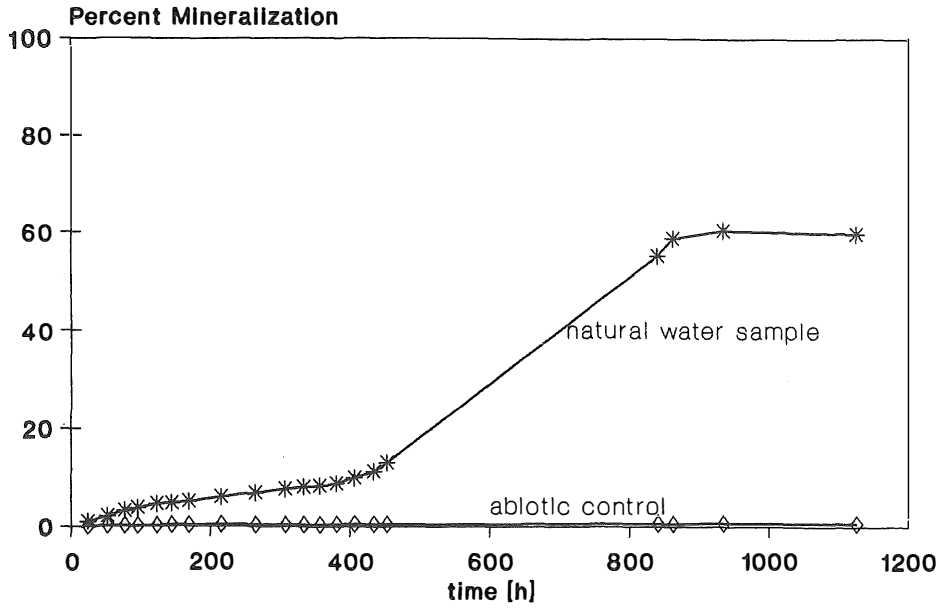

Fig. 1. Mineralization of 4-Nitrophenol: natural water sample from Kiel Fjord. Addition of 8 µg/l labelled 4-NP.

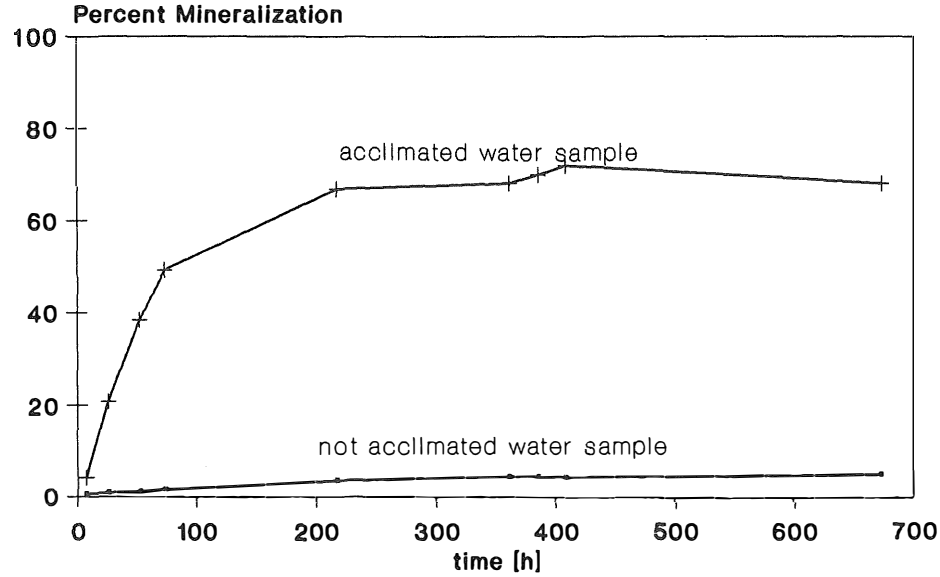

Fig. 2. Mineralization of 4-NP after acclimation: influence of pre-incubation. Incubation for 18 days with/without 250 µg/l 4-NP. Addition of 8 µg/l 4-NP.

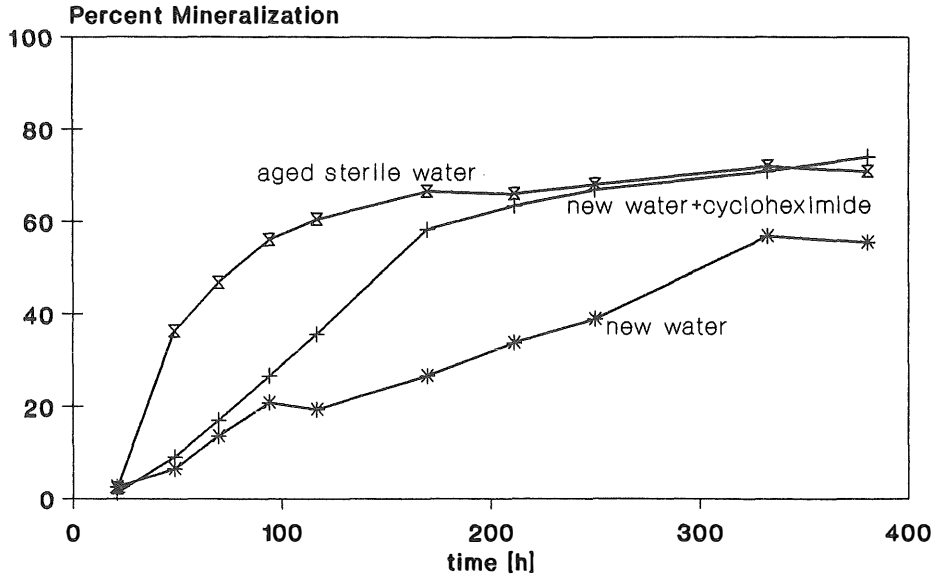

Fig. 3. Mineralization of 4-NP after acclimation: influence of differently treated water. Acclimation of a mixed culture to 4-NP. Dilution to 5 vol%. Addition of 8 µg/l.

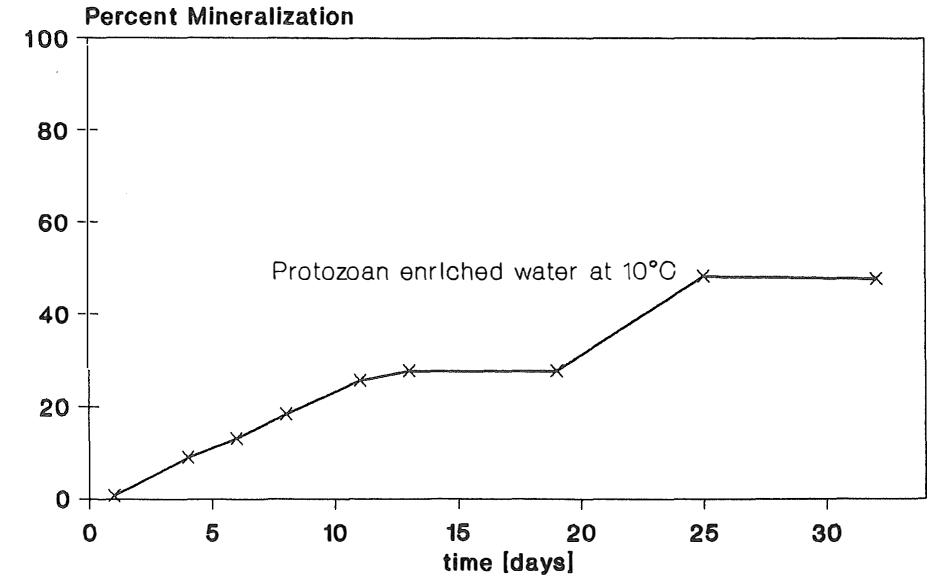

Fig. 4. Mineralization of 4-NP after acclimation: influence of low temperature and grazing. Pre-incubation for three days for protozoan growth, incubation at 10 °C.

(GALVAO, pers. comm.) and there were between 0.7 and 1.4 x 10^3/ml protozoa in the incubation vessels during the whole experiment. Organic nutrients slowed down degradation in all treatments with fresh seawater. Grazing had the biggest effect in combination with low temperature in the treatment with pre-incubation.

The data of experiments with differing concentrations show, that when a higher amount is used in the first incubation, enough degrading bacteria can develop for a fast mineralization of 4-NP after a second addition. The increase of degrading bacteria on low doses of 4-NP seems to be marginal. This could be verified with MPN counts (WESNIGK 1990). Without any 4-NP added in the first incubation the potential degrading bacteria cannot be activated any more with a later addition, even after prolonged incubation (Fig. 2).

The data suggest that one of the main reasons for the occurence of acclimation periods for the mineralization of xenobiotics in natural waters may be the very small initial degrader population. The role of other carbon sources seems to be ambivalent. They can lower the degradation rate but can also promote growth of the small population, especially when the concentration of the chemical in question is low. Concentrations of organic chemicals below about 10 µg/l cannot sustain growth (SPAIN and van VELD 1983). Therefore acclimation may take a long time and the rate of degradation may be slow. Additionally a second dose will not be degraded in a shorter time interval.

Another important factor impeding degradation of organic chemicals and leading to prolonged acclimation periods can be grazing by protozoa (WIGGINS et al. 1987). Especially slow growing and big bacteria can be grazed very efficiently (SINCLAIR and ALEXANDER 1989, KRAMBECK 1988). This phenomenon is of great importance in natural aquatic environments where low water temperatures and low concentrations of nutrients and chemicals prevail.

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