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Microbial degradation of Sodium Trifluoroacetate under aerobic and anaerobic conditions

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INTRODUCTION:

The extensive use of hydrofluorocarbons (HFCs) and hydrochlorofluorocarbons (HCFCs) as environmental acceptable alternatives to chlorofluorocarbons (CFCs) has been responsible for the introduction in the environment of significant amounts of Trifluoroacetate (TFA). Specifically, TFA is produced by the atmospheric breakdown of HCFC-123, HCFC-124, HFC-134a and HFO-1234yf, which undergo oxidative, photolytic and hydrolytic reactions to generate the compound. TFA is a highly persistent, water soluble and extremely stable fluorinated compound, that tends to accumulate in low-streamed aquatic environments, with marine ecosystems as its ultimate environmental sink. Although it does not exert considerable toxicological effects neither in microbial communities, nor in aquatic organisms, it reveals a mild toxicity regarding plants (Boutonnet*et al.*, 1999). There are no reports on the aerobic biodegradation of TFA, but the mineralisation of the compound by anaerobic methanogenic bacteria was demonstrated in few studies (Visscher, *et al.*, 1994; Kim *et al.,* 2000).

OBJECTIVES:

This study aimed to investigate the biodegradation of sodium Trifluoroacetate (NaTFA) by microbial cultures obtained from an industrial contaminated site, microorganisms pre-exposed to other fluorinated compounds and aerobic activated sludge, under aerobic and anaerobic conditions. Biodegradation of NaTFA was studied as a sole carbon source or in co-metabolism with sodium acetate, the latter being only investigated in aerobic conditions. Under anaerobic conditions, this study assessed the TFA biodegradation potential of methanogenic, sulphate-reducing and denitrifying bacteria.

MATERIALS AND METHODS:

A variety of soil (wet and dry sediments), rhizosphere and water samples were collected from a contaminated drain in northern Portugal (Estarreja, Aveiro) and used as an environmental microbial source for the experiments. Two microbial cultures capable of degrading fluorinated compounds, samples of aerobic activated sludge from a local wastewater treatment plant were also used as inoculum.

The aerobic experiment was divided in two experimental setups: in setupA, biodegradation of NaTFA as a sole carbon source (20 ppm NaTFA) was investigated, while in the setup Bbiodegradation of NaTFA was studied in co-metabolism with sodium acetate (5 ppm NaTFA + 500 ppm sodium acetate fed daily). In the anaerobic experiments, biodegradation of TFA was only tested as a sole carbon source (0.7 ppm). Sterile controls supplemented with NaTFA were also included (5 ppm NaTFA).

The aerobic experiments were conducted in 250 mL Erlenmeyer flasks, containing 70 mL of a sterile minimal salts medium supplemented with NaTFA in concentrations according to the experimental setup, and sodium acetate for the co-metabolic experiments. Half of the culture volume was removed and replaced with fresh medium at a 10 days interval. Anaerobic experiments were performed in 100 mL serum vials, containing 50 mL of a culture medium which composition varied according to the type of the anaerobic bacteria to select. To ensure that cultures were free of oxygen, cysteine and sodium sulphide were added to reduce all the

oxygen remaining in the cultures, and resazurin (1 mg l^{-1}) was used as an oxygen indicator. Prior to incubation, anaerobic cultures were flushed with a N_2 stream and sealed with aluminum capsules.
All cultures were incubated on a rotary shaker at 100 rpm and 25ºC. Samples were taken every 10 days for the aerobic cultures and after a period of 15 days for the anaerobic cultures.

Defluorination of NaTFA was detected through the measurement of fluoride anions in the cultures' supernatants, with a fluoride-selective electrode. For every measurement, calibration curves were established by using freshly prepared standard solutions of sodium fluoride with concentrations ranging from 0.001 to 1 mM sodium fluoride.

RESULTS AND DISCUSSION:
Significant defluorination was not verified in any of the aerobic cultures, with the most prominent defluorination rates being well below 10% throughout the 118 days of the experiment. The aerobic cultures fed with NaTFA as a sole carbon source revealed non-existing or insignificant biodegradation patterns, and supplementation with sodium acetate as a co-metabolite was also found not to exert a significant effect in the aerobic biodegradation of this compound.

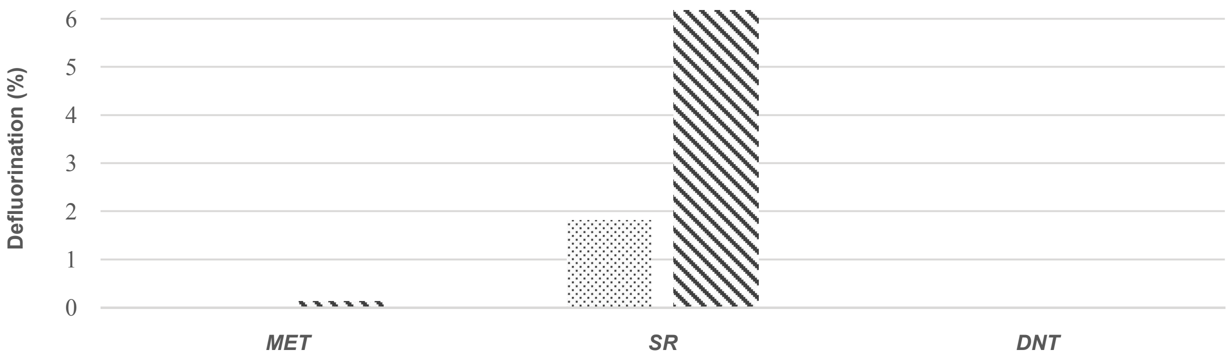

Figure 3 - Defluorination data, regarding the anaerobic cultures, after 15 days. MET, SR and DNT stand for methanogenic, sulphate-reducing and denitrifying bacteria, respectively. The striped bars correspond to the rhizosphere inoculum, while the dotted bar corresponds to the wet sediment inoculum.

Such results could be explained by the lack of a proper bacterial acclimation to the compound, since it has been suggested that a considerable pre-exposure period is needed before the microbial communities can develop and improve suitable catalytic mechanisms (Bott&Standley, 1999). This hypothesis is also supported by the fact that the microbial cultures capable of degrading fluorinated compounds, revealed higher microbial densities and slightly better defluorination rates in both experimental setups (data not shown).

Regarding the anaerobic experiments, from the three groups of bacteria tested, only sulphate-reducing bacteria showed some (although low) defluorination of NaTFA along a 15 days period, which is a much shorter period than the one tested in the aerobic conditions (Fig. 1). Both methanogenic and denitrifying bacteria revealed insignificant biodegradation patterns (Fig. 1). These results are not in agreement with the ones reported in the literature, which revealed a more active role of methanogenic bacteria on the biodegradation of NaTFA (Visscher*et al.*, 1994; Kim *et al.*, 2000). This incongruence may be due to the concentrations of NaTFA used in the anaerobic experiment, which both Visscher*et al.* (1994) and Kim *et al.* (2000) reported to be inhibitory to methanogenic activity. Although, the same authors also suggested that sulphate-reducing bacteria are only capable of TFA biodegradation in concentrations bellow 0.925 M TFA, which we found not to be the case.

CONCLUSION:
From the results obtained it is possible to state that biodegradation of NaTFA is not a direct mechanism. This compound seems to be highly recalcitrant under aerobic conditions, indicating that environmental occurring

aerobic bacteria do not possess efficient catalytic mechanisms necessary to attack the molecular structure of TFA. Under anaerobic conditions, the results obtained after a two weeks period indicate that biodegradation may occur, although at a very low rate.

Although TFA does not represent a major threat to the environment, its persistence and natural ability to accumulate in the environment may lead in the future to environmental hazardous concentrations. Such increase over time may be however balanced if the strong driving force posed by the presence of this compound in the environment is able to develop better biodegradation mechanisms.

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