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Biodegradation of mono-, di- and trifluoroacetate by microbial cultures with different origins

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ABSTRACT

This work focused on the biodegradation of three structurally related fluoroacetates (FAs), mono- (MFA), di- (DFA) and trifluoroacetate (TFA), using as microbial inocula samples collected from a site with a long history of industrial contamination and activated sludge obtained from a municipal wastewater treatment plant. Biodegradation experiments were carried out under different modes of substrate supplementation, which included (i) FAs fed as sole carbon sources; (ii) FAs (only for DFA and TFA) fed in co-metabolism with sodium acetate; and (iii) mixtures of MFA with DFA or TFA. Biodegradation of the target compounds was assessed through fluoride ion release. Defluorination was obtained in the cultures fed with MFA, while DFA and TFA were recalcitrant in all tested conditions. When present in mixture, DFA was shown to inhibit biodegradation of MFA, while TFA had no effect. A total of 13 bacterial isolates obtained from MFA degrading cultures were found to degrade 20 mgL^{-1} of this compound, as single strains, when supplemented as a sole carbon source. Sequencing of the 16S rRNA gene indicated that among these degrading bacteria only *Delftia acidovorans* had been previously reported to be able to degrade MFA. This work shows that, despite their similar chemical structures, biodegradation of the three tested FAs is very distinct and draws attention to the unknown impacts that the accumulation of DFA and TFA may have in the environment as a result of their high recalcitrance.

1. Introduction

Due to the valuable properties that fluorine confers on organic molecules, the use of synthetic organofluorines in industrial, medical and agricultural applications has increased significantly in recent decades [1]. As a result of their wide applications, fluoroorganic molecules are becoming pollutants in several environmental compartments, where they may persist for long periods due to the recalcitrant nature of many of these molecules [2,3]. The degradation of organofluorine compounds constitutes a challenge to microorganisms, not only because the environmental pollution originated by these compounds is a relatively recent problem, causing microorganisms to be exposed to compounds so far unknown, but also because the C-F bond of organofluorines has one of the highest known energies [4].

Fluoroacetates (FAs) are a family of carboxylic aliphatic organofluorines composed of mono- (MFA), di- (DFA) and trifluoroacetate (TFA), that are non-volatile and highly soluble in water. They are important building blocks and intermediary reagents for the chemical synthesis of various fluorinated compounds with different applications [5–11], and also result from the abiotic breakdown of fluorinated polymers [12–15]. MFA is also used in some countries as a pesticide to control mammalian pests and is known to be naturally produced by a number of plants and a few *Streptomyces* species [16–22]. In addition, TFA is a derivative of the tropospheric degradation of several hydrochlorofluorocarbons (HCFCs) and hydrofluorocarbons (HFCs) [23].

Due to the different applications of FAs and various entry routes in the environment, pollution caused by these compounds may be either diffuse or point source. FAs have been reported to occur in several environmental compartments, with the aquatic media being their major

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environmental sink [24]. TFA has been detected in seasonal wetlands, marine environments, rainwater and lotic environments, in concentrations ranging from 30 to 600 ngL^{-1} [25–29]. The environmental occurrence of MFA is mainly linked with its application, aerially or in baits, for pest control, though release through discharges from chemical industries may also occur [30], being likely to reach groundwater streams and even surface waters due to its mobility in the environment. The environmental dynamics of DFA are poorly explored in the literature but its structural similarity to the other FAs, namely regarding its physicochemical properties, suggests a similar environmental behaviour.

MFA was found to be biodegraded by a number of soil microorganisms [31]. Kelly et al. [32] first reported the bacterial degradation of MFA, and since then other MFA-degrading bacteria have been isolated from different environmental sources, including bovine rumen, MFA-accumulating plants, anaerobic sludge and aquatic sediments [21,33,34]. Microbial degradation of this compound is mediated by fluoroacetate dehalogenase, which catalyses the cleavage of the C-F bond, yielding glycolate [35–37]. More recently, MFA has been also found to be defluorinated by L-2-haloacid dehalogenases [38]. Biodegradation of TFA has been reported to occur under anaerobic conditions by microbial communities from freshwater and saltmarsh sediments, although its aerobic conversion to fluoroform by the same communities was also described [13,39]. DFA has been identified as a metabolite resultant from the anaerobic biodegradation of TFA, being further converted into MFA and then acetate by a reductive dehalogenation mechanism [13]. Although the data suggest degradation of DFA under anaerobic conditions, no studies on its aerobic biodegradation are available in the literature (as far as we are aware). Moreover, due to their wide industrial applications, FAs may occur simultaneously in the environment and thus it is important to understand how the biodegradation of each compound is affected by the presence of its analogues. In this context, the work reported here aimed to investigate the aerobic biodegradation of MFA, DFA and TFA as sole carbon sources, as well as in mixtures of two FAs which to the best of our knowledge have not been studied previously. In addition, co-metabolic degradation of DFA and TFA in the presence of their non-fluorinated analogue, acetate, was also explored. Biodegradation was investigated using microbial inocula from different origins.

2. Materials and methods

2.1. Reagents

All chemicals used were of the highest purity grade available (Sigma- Aldrich Chemie, Steinheim, Germany; Merck, Darmstadt, Germany). DFA (97%), TFA (98%) and acetate (99%) were bought to Sigma-Aldrich and MFA (> 95%) was obtained from Supelco (Bellefonte, USA).

2.2. Microbial inocula

Sediment samples and rhizosphere of *Phragmites australis* (Cav.) Trin. ex Steud., were collected from a site in Estarreja (Portugal) with a long history of industrial chemical contamination [40], and used as an environmental source of microorganisms. For each sample, approximately 100 g were collected in triplicate and then pooled and homogenised in sterile zip bags. An activated sludge (AS) consortium obtained from a municipal wastewater treatment plant (Gondomar, Porto) was also used as inoculum for this study. AS samples (100 mL) were collected in triplicate from the aeration tank and pooled and homogenised in a sterile flask. Sediment, rhizosphere and AS samples were transported to the laboratory at room temperature and used in the same day of sampling. AS inoculum was obtained by centrifuging 40 mL of AS (5000 rpm for 15 min at 4 °C), washing twice the resultant pellet with a minimal salts medium (MM) and resuspending it in the same medium to one tenth of its original volume.

2.3. Biodegradation experiments

Biodegradation experiments were performed in batch mode in 250 mL flasks with 70 mL of sterile MM. MM contained (per litre of ultra-pure water): $Na_2HPO_4{\cdot}2H_2O$ 2.7 g, KH_2PO_4 1.4 g, $(NH_4)_2SO_4$ 0.5 g, $MgSO_4{\cdot}7H_2O$ 0.2 g and 10 mL of a trace elements solution with the following composition, per litre: $Na_2EDTA{\cdot}2H_2O$ 12.0 g, NaOH 2.0 g, $MnSO_4{\cdot}4H_2O$ 0.4 g, $ZnSO_4{\cdot}7H_2O$ 0.4 g, H_2SO_4 0.5 mL, Na_2SO_4 10.0 g, $Na_2MoO_4{\cdot}2H_2O$ 0.1 g, $FeSO_4{\cdot}7H_2O$ 2.0 g, $CuSO_4{\cdot}5H_2O$ 0.1 g and $CaCl_2$ 1.0 g. Flasks were inoculated with 5 g of fresh sediment or rhizosphere samples and for the AS inoculum, flasks were inoculated in order to have an initial optical density (OD) at 600 nm of 0.1. Cultures were fed with FAs individually, in mixtures of two FAs and, for DFA and TFA, in co-metabolism with acetate. When fed individually, FAs were supplemented at a concentration of 20 mgL^{-1} (0.20, 0.17 and 0.15 mM for MFA, DFA and TFA, respectively), while in the binary mixtures of FAs, each compound was fed at the concentration of 10 mgL^{-1} (0.10, 0.085 and 0.074 mM for MFA, DFA and TFA, respectively). Cultures in co-metabolism with acetate were supplemented with DFA or TFA at the concentration of 5 mgL^{-1} (0.042 and 0.037 mM for DFA and TFA, respectively) and fed three times a week with 500 mgL^{-1} of sodium acetate. In this latter condition, cultures were weekly transferred to new sterilised flasks in order to ensure sufficient oxygen for the aerobic degradation of the target compounds. Biodegradation of FAs was followed during a three week period, after which half of the cultures were transferred to new flasks containing the same proportion of MM and refed with the respective carbon sources. Culture flasks were maintained closed throughout the experiments and incubated in a rotary shaker (130 rpm) at 25 °C in the dark. Abiotic controls consisting in MM supplemented individually with each of the FAs (5 mgL^{-1}) and incubated under the same conditions were also included. Experiments were conducted in duplicate. FAs biodegradation was followed by measuring bacterial growth and fluoride ion release at the beginning and at the end of each three week period.

2.4. Microbial characterization of MFA-degrading cultures

At the end of the biodegradation experiments, the microbial diversity of MFA degrading cultures was analysed by spreading several tenfold dilutions of culture samples onto minimal salts agar plates supplemented with MFA (20 mgL^{-1}) as sole carbon source and Plate-Count Agar (PCA). The plates were incubated at 25 °C until growth was detected. Bacterial diversity was analysed by visual inspection and morphologically distinct colonies were purified by streaking the different colonies in new agar plates.

2.5. Biodegradation capacity of microbial isolates obtained from MFA-degrading cultures

The capacity of the different isolates retrieved from the MFA-degrading cultures to degrade this compound in axenic cultures was investigated by inoculating single strains into 30 mL sterile flasks, filled to two thirds of their volume with MM and supplemented with MFA at 20 mgL^{-1}. The initial OD (600 nm) of the cultures was 0.1. Flasks were incubated in a rotary shaker (130 rpm at 25 °C), in the dark. Biodegradation was followed along a three week period by monitoring bacterial growth and fluoride ion release. A microbial culture consisting of a mixture of all MFA-degrading isolates was also created and used as inoculum for investigating its capacity to degrade DFA and TFA fed individually as sole carbon source (20 mgL^{-1}) and co-metabolism with MFA (20 mgL^{-1} of MFA and 5 mgL^{-1} of DFA or TFA).The experiment was conducted under the same conditions described above.