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Degradation of mecoprop in polluted landfill leachate and waste water in a moving bed biofilm reactor

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ABSTRACT

Mecoprop is a common pollutant in effluent-, storm- and groundwater as well as in leachates from derelict dumpsites. Thus, bioremediation approaches may be considered. We conducted batch experiments with moving bed biofilm (MBBR)-carriers to understand the degradation of mecoprop. As a model, the carriers were incubated in effluent from a conventional wastewater treatment plant which was spiked to 10, 50 and 100 μg L^{-1} mecoprop. Co-metabolic processes as well as mineralization were studied. Initial mecoprop concentration and mecoprop degradation impacted the microbial communities. The removal of (*S*)-mecoprop prevailed over the (*R*)-mecoprop. This was associated with microbial compositions, in which several operational taxonomic units (OTUs) co-varied positively with (*S*)-mecoprop removal. The removal-rate constant of (*S*)-mecoprop was 0.5 d^{-1} in the 10 μg L^{-1} set-up but it decreased in the 50 and 100 μg L^{-1} set-ups. The addition of methanol prolonged the removal of (*R*)-mecoprop. During mecoprop degradation, 4-chloro-2-methylphenol was formed and degraded. A new metabolite (4-chloro-2-methylphenol sulfate) was identified and quantified.

1. Introduction

Mecoprop, or 2-(4-chloro-2-methylphenoxy)propanoic acid, is a mixture of two stereoisomers (i.e., enantiomers) where the (*R*)-(+)-enantiomer has the herbicidal activity (Smith et al., 1980). Mecoprop is an approved pesticide in a few countries of the European Union (European Commission, 2015) but it has been discontinued in Denmark since 2001 (SEGES, 2016) as excessive pollution of groundwater is connected to this compound. Still, mecoprop is approved in Denmark for biocidal purposes, such as in bituminous construction materials (roofing felts, sealants and insulations) to prevent root penetration (European Parliament and Council, 2012; Bucheli et al., 1998). The runoff water from these construction materials has proven to be the main path of entry of mecoprop into wastewater treatment plants (WWTPs) and surface waters (Bucheli et al., 1998; Bollmann et al., 2014). In addition, mecoprop has been documented at high concentrations (up to 600 μg L^{-1}) in contaminated plumes down-gradient of Danish landfills (Baun et al., 2003). In fact, many drinking-water wells are closed every year in Denmark because of mecoprop exceeding the threshold for pesticides concentration in drinking water (Frková et al., 2016). In WWTPs, the concentrations of mecoprop in wastewater influents and effluents are similar and range between 0.03 and 2 μg L^{-1} (Bollmann et al., 2014; Gerecke et al., 2002; Singer et al., 2010). This means that mecoprop is not degraded via conventional wastewater treatment and it is considered a trace organic contaminant.

Considering soil ecosystems, mecoprop is a fairly well-studied organic compound and its degradation is known to start with the formation of 4-chloro-2-methylphenol (Tett et al., 1994; Müller and Buser, 1997). At aerobic conditions, the degradation of mecoprop has been found to be enantioselective, which can be towards either of the enantiomers (Frková et al., 2016; Qiu et al., 2014; Romero et al., 2001) (Müller and Buser, 1997; Zipper et al., 1999). In relation to wastewater, aerobic incubations of mecoprop in sewage sludge (according to ISO 7827) showed that mecoprop was fully degraded within 200 h. This degradation happened in an enantioselective manner, usually with preference to degrade the (*S*)-(−)-enantiomer (Zipper et al., 1999). Furthermore, the enantiomer-specific catabolic genes *rdpA* and *sdpA* have been characterized in the bacterial isolates *Delftia acidovorans* MC1 (Schleinitz et al., 2004) (Westendorf et al., 2002, 2003), and *Sphingomonas*

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herbicidovorans MH (Muller et al., 2004) (Westendorf et al., 2003; Muller et al., 2006a, 2006b; Nickel et al., 1997; Zipper et al., 1998). The *sdpA* gene, associated with specific (*S*)-mecoprop degradation, was found indigenously in soil, whereas the *rdpA* gene could not be detected. However, following amendment of the phenoxypropionate herbicide (*R,S*)-dichlorprop, the expression of both genes was significantly upregulated, suggesting an innate potential for microbial degradation of racemic mixes of phenoxypropionate herbicides in natural settings (Paulin et al., 2010, 2011). This observation was recently supported in a study investigating phenoxypropionate herbicide degradation by natural bacterial populations in sand filters (Feld et al., 2016). Furthermore, it has been suggested that the alternative genes *tfdA* and *cadA* are involved in degradation of (*S*)-mecoprop, but not (*R*)-mecoprop, and that *sdpA*-encoded SdpA might also be involved in removal of (*R*)-mecoprop (Zakaria et al., 2007).

Nowadays, soil- or sand-filtration are the main solutions to remove mecoprop when producing drinking water from contaminated ground- and surface water (Hedegaard et al., 2014). These solutions involve sorption on the matrix and biodegradation by the biofilm. Our study aimed to specifically understand the role of biofilms in mecoprop degradation under low-nutrient settings such as typical for landfill leachate, effluent wastewater or stormwater in opposite to raw wastewater. For this purpose, the usage of Moving Bed Biofilm Reactors (MBBRs) was considered. MBBRs consist of biofilm which is located on plastic carriers (1–4 cm of diameter) that support the growth of biofilm while they are stirred inside a water container (Ødegaard et al., 1999). While MBBRs are currently used in wastewater treatment to eliminate easily degradable carbon and nitrogen from wastewater (Barwal and Chaudhary, 2014), recent studies have shown that MBBRs can remove trace organic compounds (Falås et al., 2012, 2013; Escolà Casas et al., 2015). As mecoprop is a subject of concern in low-nutrient waters, we decided to test an MBBR set-up that would mimic biofilm systems to clean landfill leachates that often contain up to mg/L concentrations as well as less contaminated groundwater and wastewater. The biodegradation of mecoprop was assessed in terms of kinetics, co-metabolism, transformation products, mineralization, shifts in microbial community structures and identification of gene homologs to known degradation genes.

2. Materials and methods

Two different experiments were performed: one biodegradation experiment, in which several metabolites were monitored, and one mineralization experiment, where the transformation of ^{14}C labelled mecoprop was observed by determining development of $^{14}CO_2$. Both experiments were conducted as batch tests.

2.1. Chemicals and instruments

Mecoprop and mecoprop D_3 were obtained from Dr. Ehrenstorfer, Augsburg, Germany. The mecoprop metabolite 4-chloro-2-methylphenol, was also obtained from Dr. Ehrenstorfer while 4-chloro-2-methylphenol sulfate was obtained from Toronto Research Chemicals. For HPLC-MS/MS analysis, methanol and formic acid were obtained from Merck, Darmstadt (Germany) and HPLC-grade water was obtained from Sigma-Aldrich. For the mineralization analyses, acetone (>99.7% purity) was obtained from Rathburn Chemicals (Walkerburn, Scotland, UK), water was obtained from a MilliQ in-house apparatus (Millipore Corporation, Billerica, MA, US).

HPLC-MS/MS was used to quantify mecoprop and its metabolites (see Section 2.2). The HPLC was an Ultimate 3000 from Dionex (Germeringen, Germany), and consisted in a dual low-pressure mixing ternary-gradient system. The system had a pump of the 3000 series (DGP-3600 M), a 3000 TSL autosampler (WPS 3000 TSL) and a column oven and degasser from the same Dionex 3000 series. The HPLC was equipped with two eight-port Valco valves. The mass spectrometer was an API 4000 (ABSciex, Framingham, MA, USA). The API 4000 was operated in ESI in negative mode with a capillary voltage of 4500 V.

For the enantioseparation and quantification of mecoprop enantiomers, a cyclodextrin-modified column (30 × 4 mm Nucleodex alpha-PM from Macherey-Nagel, Düren, Germany) was used with water and methanol (both containing 0.2% formic acid v/v) as mobile phases. The identification of the enantiomers was based on previous work (Frková et al., 2016). The elution gradient is specified in Table S2 of the Supplementary Information (SI). For the quantification of mecoprop metabolites a C18 column (Synergy polar from Phenomenex) was used with water and methanol (both containing 0.2% formic acid v/v) as mobile phases and the gradient specified in Tables S2 and SI.

For subsequent DNA extractions from biofilms, MBBR carriers were fully submerged in RNAlater® for later DNA analysis (Roggenbuck et al., 2014).

2.2. Biodegradation of mecoprop

The removal of mecoprop by the biofilms was assessed by incubating MBBR-carriers in effluent wastewater, obtained from Bjergmarken WWTP (Roskilde) the same day that the experiment started, spiked with mecoprop. The biofilm on the MBBR-carriers was previously grown in an effluent-wastewater reactor over three months. K5 type carriers from AnoxKaldness (Lund, Sweden), with a protected surface area of 800 m^2/m^3, were used for these experiments. Usually the biomass on these carriers is distributed very evenly between the different carriers, as it is controlled by the shear forces. Biomass was 19.58 mg per carrier or 2.18 g L^{-1}.

Different incubations corresponding to four different set-ups were performed (each of them in triplicates). The first three experiments consisted of effluent wastewater spiked with 10, 50 and 100 μg L^{-1} mecoprop. For this, 25, 125 and 250 μL of a mecoprop stock solution in methanol (10 μg/mL) were spiked on the bottom of Erlenmeyer flasks and the solvent was evaporated for one hour. Successively, 18 mL of effluent wastewater were added to the flasks. To test whether or not additional carbon sources might be beneficial or detrimental to the process in the fourth set-up effluent wastewater was spiked with 10 μg/mL of mecoprop and 25 μL of methanol. Additional carbon can boost codegradation processes and thus enhance degradation. On the other hand it can also lead to all efforts are directed towards the easy to degrade carbon and thus decrease degradation speed (Tang et al., 2017b; Torresi et al., 2017). For this set-up, 18 mL of effluent wastewater were put in the flasks and 25 μL of mecoprop stock solution in methanol (10 μg/mL or 10 mg/L TOC) were directly added to the water. After preparation, all the Erlenmeyer flasks were shaken for five minutes and, afterwards, two MBBR carriers with biofilm were added. Biological and chemical controls were also performed in triplicates. The biological control consisted of MBBR-carriers with biofilm incubated in non-spiked effluent wastewater (18 mL). The chemical control consisted of new MBBR-carriers, without biofilm, incubated in effluent water spiked to contain 10 μg L^{-1} of mecoprop and no methanol. The chemical control was used to consider degradation processes not linked to the biofilm on the MBBR-carrier. All the Erlenmeyer flasks had 100 mL capacity, were covered with tin foil to allow air but excluded sunlight and eventual dust contamination, placed in a shaker at 80 rpm, kept at room temperature and sampled simultaneously. Also, the ratio of water per carrier was kept at 7.8 ± 0.8 mL in all flasks during the whole experiment. The first