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Biodegradation of chloro- and bromobenzoic acids: Effect of milieu conditions and microbial community analysis

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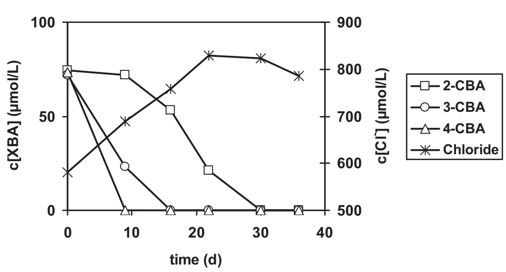
HIGHLIGHTS

- Enrichment cultures degraded mixtures of chloro- and bromobenzoic acids.
- A distinct order of degradation was observed in mixtures of isomers.
- Biodegradation of 4-CBA occurred at pH 5–9 and elevated salt concentrations.
- Alphaproteobacteria represented an important group of bacteria.
- *Afipia* sp. was detected most frequently on genus level.

GRAPHICAL ABSTRACT



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ABSTRACT

Monohalogenated benzoic acids often appear in industrial wastewaters where biodegradation can be hampered by complex mixtures of pollutants and prevailing extreme milieu conditions. In this study, the biodegradation of chlorinated and brominated benzoic acids was conducted at a pH range of 5.0–9.0, at elevated salt concentrations and with pollutant mixtures including fluorinated and iodinated compounds. In mixtures of the isomers, the degradation order was primarily 4-substituted followed by 3-substituted and then 2-substituted halogenated benzoic acids. If the pH and salt concentration were altered simultaneously, long adaptation periods were required. Community analyses were conducted in liquid batch cultures and after immobilization on sand columns. The Alphaproteobacteria represented an important fraction in all of the enrichment cultures. On the genus level, *Afipia* sp. was detected most frequently. In particular, Bacteroidetes were detected in high numbers with chlorinated benzoic acids.

1. Introduction

Globally, insufficient wastewater treatment, improper handling and disposal of halogenated organic compounds have led to serious contaminations of soil, groundwater and surface water and pose a threat to human health. Due to batch production processes, industrial wastewater effluents are often discharged discontinuously. Furthermore, industrial wastewaters often imply a very

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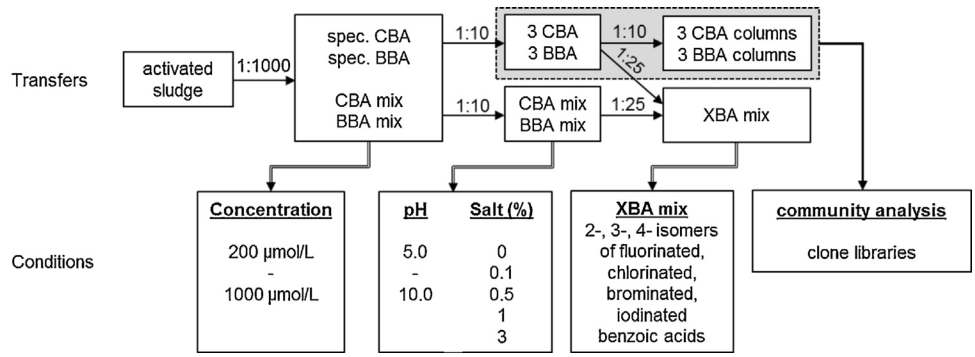

Fig. 1. Experimental set-up.

heterogeneous composition with varying milieu conditions (i.e., pH and salt concentrations). For example, at the Ramat Hovav industrial site in Israel, increased salinities and pH values between 2 and 10 have been measured [1]. The chemicals found in the wastewater of the Ramat Hovav industrial site consist of over 50 different halogenated hydrocarbons including chlorinated and brominated benzoic acids. Chlorobenzoic acids (CBAs), in particular, are found not only in wastewaters but also in the environment. CBAs are often dead-end metabolites, for example, during degradation of polychlorinated biphenyls (PCBs) or pesticides, and CBAs can negatively affect the degradation of PCBs [2–5].

Since the early 1980s, monohalogenated benzoic acids (XBAs) have often been studied as halogenated-model compounds with the prevalent purpose of isolating pure cultures and investigating degradation capabilities and pathways. However, pure cultures show very characteristic degradation patterns limited to specific isomers and are often not able to degrade mixtures of monohalogenated benzoic acids [6–11].

Furthermore, it has been shown repeatedly that degradation patterns of pollutant mixtures can differ from single-compound studies (e.g., [12]). Zhao et al. [13] showed that there are inhibiting effects on the aerobic biodegradation of chloroethenes within mixtures of different chloroethenes. However, co-metabolic degradation of poorly degradable compounds in the presence of growth substrates can improve overall degradation (e.g., in the case of vinyl chloride and dichloroethene) [14]. Additionally, pH and salt concentrations affect biodegradation [15,16], and high pollutant concentrations can result in toxic effects [17].

The objective of this study was to examine the occurrence of bacteria in activated sludge that are capable of aerobic degradation of chlorinated and brominated benzoic acids as single compounds and as compound mixtures (Fig. 1). Biodegradation was studied under varying pH and salt concentrations simulating wastewater conditions in industrial batch production processes. The community composition of the enriched bacteria was analyzed in batch cultures and after immobilization on flow-through columns.

2. Materials and methods

All of the chemicals used in this study were analytical grade or higher from Merck (Darmstadt, Germany) or Sigma–Aldrich (St. Louis, USA). Abbreviations of the monohalogenated benzoic acids (XBAs) are defined as shown by the following examples: 2-chlorobenzoic acid (2-CBA) and 3-bromobenzoic acid (3-BBA).

2.1. Degradation experiments

2.1.1. Batch experiments

Microbial consortia capable of XBA degradation were enriched using activated sludge of a municipal wastewater treatment plant. The XBAs were provided as a sole source of carbon under aerobic conditions. After initial enrichment, the effects of compound concentration, pH and salt concentration, and monohalogenated benzoic acids mixtures were studied. The transfer of cultures and the experimental conditions are illustrated in Fig. 1. More detailed information is provided in the figure captions.

Batch experiments were conducted in 1-liter-laboratory glass bottles with a mineral medium containing the following mineral salts per liter of demineralized water: 1.05 g of $K_2HPO_4{\cdot}3H_2O$, 0.2 g of KH_2PO_4, 0.1 g NH_4NO_3, 0.04 g of $MgSO_4{\cdot}7H_2O$, 0.023 g of $CaSO_4{\cdot}2H_2O$ and 2 mL of trace element solution (10 mg of $ZnSO_4{\cdot}7H_2O$, 200 mg of $FeSO_4{\cdot}7H_2O$, 3 mg of $MnCl_2{\cdot}4H_2O$, 30 mg of H_3BO_3, 20 mg of $CoCl_2{\cdot}6H_2O$, 10 mg of $CuSO_4{\cdot}2H_2O$, 6 mg of $NiCl_2{\cdot}6H_2O$, 3 mg of $Na_2MoO_4{\cdot}2H_2O$, and 2 mL of concentrated phosphoric acid per liter).

The pH was adjusted to 7.1 unless otherwise stated. In experiments addressing extreme milieu conditions, the pH was adjusted between 5 and 10 by modulation of the phosphate buffer, and NaCl was added at concentrations of 0.1, 0.5, 1.0 and 3%. The glass bottles were equipped with a separate small opening with a Teflon-coated septum, which allowed sampling using a glass syringe and stainless steel needles. After sampling, the septum was exchanged to avoid losses due to volatilization at low pH. Sterile controls were prepared by the addition of 1 g/L NaN_3 and showed no degradation.

2.1.2. Flow-through column experiments

For column experiments, the MSM used was the same as in the batch experiments except that NH_4NO_3 was replaced by 100 mg/L $NaNO_3$ to prevent oxygen consumption due to ammonium oxidation.

Bacteria capable of 3-CBA and 3-BBA degradation were immobilized on sand columns. Two borosilicate columns with a length of 24 cm and an internal diameter of 3.5 cm were filled with sterile silica sand (porosity 0.26), and the organisms were immobilized by recirculation of the enrichment cultures with 100 µmol/L of XBA. The flow rate was 0.7 L/d, resulting in a hydraulic retention time of 2.06 h. The samples for chemical analysis were collected periodically from the influent and the effluent of the columns. The samples for community analysis were taken from the batch cultures used to inoculate the columns and then collected again from the columns after 5 months of flow through operation.

The degradation experiments were incubated at room temperature and in the dark.

2.2. Analytical methods

2.2.1. Chemical analysis

The quantification of monohalogenated benzoic acids was performed by high-pressure liquid chromatography (HPLC, Agilent Technologies 1200 Series, Waldbronn, Germany), with a Gemini