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Biodegradation of *meta*-fluorophenol by an acclimated activated sludge

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Abstract

An acclimated activated sludge was examined for its ability to degrade *meta*-fluorophenol as sole carbon source in aerobic batch cultures. The mechanism study revealed that the initial step in the aerobic biodegradation of *meta*-fluorophenol was their transformation to fluorocatechol. Following transformation of the fluorophenol to fluorocatechol, ring cleavage by catechol 1,2-dioxygenases proceeded *via* an *ortho*-cleavage pathway, then defluorination occurred.

Keywords: Aerobic biodegradation; *ortho*-Cleavage pathway; Defluorination; Fluorophenol

1. Introduction

Halogenated compounds are important environmental pollutants of soil, water and air. Research investigating the environmental fate of halogenated compounds has largely focused on brominated and chlorinated organics [1]. Fluorinated organics have received less attention because they are perceived to be more inert biologically and therefore less likely to have an impact on human health or the environment. But the perception of inertness and its environmental significance are debatable: inert molecules tend to persist and accumulate, and they are more difficult to remediate. Moreover, organofluorine molecules actually do exhibit significant biological effects, as inhibitors of enzymes, cell–cell communication, membrane transport, and processes for energy generation [2].

In addition, the production and the use of fluorinated substances have been increased enormously in the recent years [3,4]. These compounds are used as propellants, surfactants, agrochemicals, adhesives, refrigerants, fire retardants and medicines. A large number of fluorinated compounds are intermediates or end products in the synthesis of agrochemicals. Because of the apparent stability, the bioactivity and the potential for accumulation in the environment of fluorinated organics, it is important to understand their environment fate and their biodegradation mechanism. The usage of fluorinated compounds, such as fluorophenols, in agricultural or industrial processes, has led to their accumulation in the environment. Therefore, the interest has been focused on the microbial degradation of fluorinated aromatics, especially on the metabolism of mono-fluorophenols by the acclimated activated sludge.

Several studies have described the oxidative degradation of mono-fluorinated aliphatics and aromatics by pure bacteria. Monofluoroacetate is the most investigated fluoroaliphatic compound since it is produced and stored by certain plants [5,6]. The bacterial metabolism of *p*-fluorophenylacetic acid, fluorobenzoic acid has been reported in detail [7,8]. A partial defluorination was observed. However, there is no report on the aerobic biodegradation of fluorinated phenols by acclimated activated sludge. Thus, it is the purpose of this paper to study the degradation of *meta*-fluorophenol by acclimated activated sludge and its mechanism.

2. Materials and methods

2.1. Chemicals

meta-Fluorophenol, used in the degradation studies were obtained from Xieshi Chemical Company (Shanghai, China), the purity of these chemicals was 99.9%. 3-fluorocatechol was purchased from ACROS Organics (New Jersery, USA). HPLC grade acetonitrile was obtained from Merk Company (Darm-

Abbreviations: AS, activated sludge; DO, dissolved oxygen; MLSS, mixed liquor suspended solids

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stadt, Germany). Ethyl acetate was got from Shanghai Chemical Company (Shanghai, China).

2.2. Microorganism and growth condition

The fluorophenol utilizing culture was obtained through acclimated activated sludge (AS) to a synthetic wastewater with fluorophenol as the sole carbon source for about 6 months. The seed activated sludge was got from Quyang wastewater treatment plants in Shanghai (China). The sequencing batch reactor (SBR) was used to acclimate sludge for *meta*-fluorophenol. In brief, the reactor had an active volume of 5 L, and were mixed and aerated by stirrers and aerators, with a hydraulic retention time (HRT) of 24 h. In the first stages of acclimation (2 months), the domestic sewage was taken as the assistant carbon source. Along with the improvement of the biodegradability of sludge, the concentration of fluorophenol in influent was increased. When the biodegradability of sludge became stable, acclimation turned to the second stage. In the second stage (4 months), the concentration of fluorophenol was 100 mg/L. The DO and temperature was kept at 2–3 mg/L and 25 °C, respectively, in all of experiment.

The biomass mixture in bioreactor was centrifuged at 5000 rpm/min for 10 min, the activated sludge was then washed twice with 50 mL 0.01 M sodium phosphate buffer (pH 7.0), removing any additional growth substance contained in the mixed culture, and used to inoculate fluorophenol (3 g/L MLSS). Fluorophenol biodegradation experiments were performed in 500 mL conical bottle containing 250 mL of minimal medium and certain *meta*-fluorophenol as sole carbon source. The pH of the medium was adjusted to pH 7.0. The flasks were incubated in an orbital shaker at 150 rpm/min at 25 °C. Uninoculated control flasks were incubated in parallel. All values were corrected to account for evaporation loss. The tests were conducted in duplicate. Results of all analysis represent the mean values of replicate trial degradations.

The pH of the solution was adjusted to 7.5, 7.0 and 6.5 and 6.0 with buffer when the experiment about pH effect on the biodegradation was carried out. And the ratio of different metabolites was got from the peak area of chromatograph.

2.3. Enzyme analysis

Cells grown on the fluorophenol were harvested by centrifugation (5000 rpm/min, 10 min), and washed twice with 0.33 M Tris–HCl buffer (pH 7.6). The cells were broken by sonication and centrifuged at 20,000 rpm/min at 0–4 °C for 15 min. The cell extract was kept on ice and assayed for catechol dioxygenase activity.

Catechol 1,2-dioxygenase activity (*ortho*-cleavage activity) was measured by following the formation of 2-fluoromuconic acid, the *ortho*-cleavage product of 3-fluorocatechol. The following reagents were added to a quartz cuvette: 2 mL 50 mM Tris–HCl buffer (pH 8.0), 0.7 mL distilled water, 0.1 mL 100 mM 2-mercaptoethanol and 0.1 mL cell extract. The cuvette was mixed by inversion and 0.1 mL 3-fluorocatechol (1 mM) was then added and mixed again. 2-Fluoromuconic acid formation was followed by an increase in the absorbance at 260 nm over a period of 5 min.

Catechol 2,3-dioxygenase activity (*meta*-cleavage activity) was measured by following the formation of 2-hydroxymuconic semialdehyde, the *meta*-cleavage product of 3-fluorocatechol. The following reagents were added to a plastic cuvette: 2 mL 50 mM Tris–HCl buffer (pH 7.5), 0.6 mL distilled water, and 0.2 mL cell extract. After mixing 0.2 mL catechol (100 mM) was added and mixed again. 2-Hydroxymuconic semialdehyde production was followed by an increase in the absorbance at 375 nm over a period of 5 min.

2.4. Analytic methods

A pH electrode was used to measure the pH value (model: pHS-3C). Fluoride anion release was followed with a fluoride anion sensitive electrode (model: pF-1-01). Fluoride anion concentrations were calculated with reference to a standard curve constructed with NaF standards. All optical density measurements were carried out using spectrophotometer (model: 752N). Dry weight measurements were determined by filtering a specific volume of suspended culture through preweighed 0.45 μm pore size filters, drying the cells at 105 °C for 2 h and reweighing them. This method is based on the procedure described in Standard Methods for the Examination of Water and Wastewater.

2.5. Qualitative analysis of fluorophenol and fluorocatechol

Qualitative analysis of fluorophenol was made by using a 4-aminoantipyene colorimetric method based on the procedure described in Standard Methods for the Examination of Water and Wastewater [9]. Samples were centrifuged at 5000 rpm/min for 10 min and the resulting supernatants were diluted to bring the concentration into the range 0–5 mg/L. The samples were treated by placing 10 mL in a test tube and adding 0.25 mL 0.5N NH_4OH. The pH was then adjusted to 7.9 ± 0.1 with approximately 200 μL potassium phosphate buffer (pH 6.8). Hundred microliters of 2% (w/v) 4-aminoantipyene solution was added and the tubes were allowed to stand for 15 min at room temperature. The absorbance was read at 500 nm.

Fluorocatechol was qualified by the method of Arnow [10]. Samples were centrifuged at 5000 rpm/min for 10 min to remove sludge. One milliliter of sample was placed in the test-tube. To each test-tube 1 mL 0.5N HCl was added. Tubes were mixed well and to this 1 mL nitrite–molybdate reagent was added resulting in a yellow colour. Nitrite–molybdate reagent was prepared by dissolving sodium nitrite and sodium molybdate in water to a concentration of 0.1 g/mL. After mixing, 1 mL of 1N NaOH was added resulting in a red colour. To this 1 mL distilled water was added. Following mixing, the absorbance was read at 510 nm.

2.6. Quantitative analysis of fluorophenol and fluorocatechol

Analysis of fluorophenols and fluorocatechols was made using a Hewlett-Packard 1050 high performance liquid chromatograph with a reverse phase column (4.6 mm × 250 mm,