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Degradation of mono-fluorophenols by an acclimated activated sludge

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Abstract

Acclimated activated sludge was examined for its ability to degrade mono-fluorophenols as the sole carbon source in aerobic batch cultures. The acclimated activated sludge degraded fluorophenol efficiently. It degraded 100 mg/l 3-fluoropheno and 4-fluorophenol in 16 h with, respectively, 99.85% and 99.91% fluoride anion release and it degraded 50 mg/l 2-fluorophenol in 15 h with 99.26% fluoride anion release. The aerobic biodegradability of the mono-fluorophenols decreased in the order: 4-fluorophenol > 3-fluorophenol > 2-fluorophenol, resulting mainly from a different octanol/water partition coefficient and different steric parameter of the fluorophenols. The mechanism study revealed that the initial step in the aerobic biodegradation of mono-fluorophenols by the activated sludge 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.

Abbreviations: AS – activated sludge; DO – dissolved oxygen; GC-MS – gas chromatography–mass spectrometry; HPLC – high-performance liquid chromatography; K_{ow} – *n-octanol/water partition coefficient;* MLSS – mixed liquor suspended solids; ^{1}X – molecular connectivity index; ^{2}X – molecular connectivity index; $^{3}X_{p}$ – molecular connectivity index; $^{4}X_{p}$ – molecular connectivity index; $^{4}X_{pc}$ – molecular connectivity index

Introduction

Halogenated compounds are important environmental pollutants of soil, water and air. Research on the environmental fate of halogenated compounds has largely focused on brominated and chlorinated organics (Van Pée and Unversucht 2003). 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 (Key 1997).

In addition, the production and the use of fluorinated substances have increased enormously in recent years (Cociglio et al. 1996; Edwards 1994). These compounds are used as propellants, surfactants, agrochemicals, adhesives, refrigerants, fire retardants and drugs. A large number of fluorinated compounds are intermediates or end-products in the synthesis of agrochemicals. Because of their apparent stability, bioactivity and potential for accumulation in the environment of fluorinated organics, it is important to understand their environmental fate and their biodegradation mechanism. The use of fluorinated compounds,

such as fluorophenols, in agricultural or industrial processes has led to their accumulation in the environment. Therefore, this study focused on the microbial degradation of fluorinated aromatics, especially the metabolism of mono-fluorophenols by an 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 (Twigg and Socha 2001). The bacterial metabolism of fluorobenzoic acid has been reported in detail (Boersma et al. 2004). However, there is no report on the aerobic biodegradation of fluorinated phenols by acclimated activated sludge. Thus, the purpose of this paper is to study the degradation of mono-fluorophenols by acclimated activated sludge and the mechanism.

Materials and methods

Chemicals

2-Fluorophenol, 3-fluorophenol and 4-fluorophenol used in the degradation studies were obtained from Xieshi Chemical Co. (Shanghai, China); and the purity of these chemicals was 99.9%. 3-Fluorocatechol and 4-fluorocatechol were purchased from ACROS Organics (New Jersery, USA). HPLC-grade acetonitrile was obtained from Merck Company (Darmstadt, Germany). Ethyl acetate was obtained from Shanghai Chemical Co. (Shanghai, China).

Microorganism and growth condition

The fluorophenols utilizing culture was obtained through the addition of acclimated activated sludge (AS) to a synthetic wastewater, with each fluorophenol as the sole carbon source for about 6 months. The seed-activated sludge was obtained from the Quyang Wastewater Treatment Plants in Shanghai (China). The acclimation experiment carried out in a bioreactor. The bioreactor was made of borosilicate glass and had a volume of 4 l. The flow rate was controlled by peristaltic pump (Model PP 10; M/S Miclins, Chennai, India). The hydraulic retention time was 24 h. The DO and temperature were kept at 2–3 mg/l and 25 °C respectively, in all experiments.

The bioreactor feed was synthetic wastewater. In addition to fluorophenol, the composition of the synthetic wastewater (feed) was as follows (per liter): KH_2PO_4 50 mg, K_2HPO_4 50 mg, $CaCl_2$ 100 mg, $MgCl_2$ 25 mg, NH_4Cl 150 mg, $FeCl_3$ 5 mg, $NiCl_3$ 0.05 mg, $CoCl_2$ 0.01 mg and $ZnCl_2$ 0.005 mg.

The activated sludge taken from bioreactor was centrifuged at 5000 r/min for 10 min, was then washed twice with 50 ml 0.01 mol/l sodium phosphate buffer (pH 7.0) to remove any additional growth substance contained in the activated sludge and was used to inoculate fluorophenol (3 g/l MLSS). Fluorophenol biodegradation experiments were performed in a 500 ml conical bottle containing 250 ml minimal medium and monofluorophenol 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 r/min at 25 °C. The flasks with $HgCl_2$ were incubated in parallel. The tests were conducted in duplicate. Results of all analyses represent the mean values of replicate trial degradations.

Enzyme analysis

Cells in the medium containing fluorophenol were harvested by centrifugation (5000 r/min, 10 min) and washed twice with 0.33 mol/l Tris-HCl buffer (pH 7.6). The cells were broken by sonication and centrifuged at 20 000 rpm 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 testing for 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 mmol/l Tris-HCl buffer (pH 8.0), 0.7 ml distilled water, 0.1 ml 100 mmol/l 2-mercaptoethanol and 0.1 ml cell extract. The cuvette was mixed by inversion and 0.1 ml 3-fluorocatechol (1 mmol/l) 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 (Farrell and Quilty 1999).

Catechol 2, 3-dioxygenase activity (*meta*-cleavage activity) was measured by testing for 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 mmol/l Tris-HCl buffer (pH 7.5), 0.6 ml distilled water and 0.2 ml cell extract. After mixing, 0.2 ml catechol (100 mmol/l) 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 (Farrell and Quilty 1999).

Analytic methods

Fluoride anion release was detected by a fluoride anion sensitive electrode (model pF-1-01; Shanghai Apparatus Co., China). Fluoride anion concentrations were calculated with reference to a standard curve constructed with NaF standards. All optical density measurements were carried out using a spectrophotometer (model 752N; Shimadzu Co., Japan). 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 the standard methods for the examination of water and wastewater (ASTM 2002).

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 the standard methods for the examination of water and wastewater (2002).

Fluorocatechol was qualified by the method of (Arnow 1937).

Quantitative analysis of fluorophenol and fluorocatechol

Fluorophenols and fluorocatechols were analyzed using a Hewlett–Packard 1050 high performance liquid chromatograph (HPLC) with a reverse phase column (4.6×250 mm, packed with KR100-5C18). The sample was filtered through a 0.45 μm filter before analysis by HPLC. The mobile phase consisted of distilled–deionized water and acetonitrile and was used over a step gradient with a flow rate of 1 ml/min. Acetonitrile in the mobile phase began at 15% (v/v), increased to 30% in 5 min and then to 70% in 20 min; 90% acetonitrile (10% water) was reached at 30 min and was sustained until 35 min post injection. The injection volume was 10 μl. Detection of fluorophenols and fluorocatechols was at 220 nm using a diode array detector. The peaks were identified on the basis of added reference compounds; and the detection data were compared with calibration plots of peak areas of standard.

Identification of metabolites

Identification of fluorocatechols was carried out using gas chromatography–mass spectrometry (GC-MS). After centrifugation to remove cells, the resulting supernatant (30 ml) was acidified to pH 2.0 with 2 mol/l HCl and extracted with ethyl acetate in three successive extractions. The extract obtained was dried with anhydrous Na_2SO_4 and concentrated to 1 ml by gently blowing nitrogen over the surface. Then, the sample was assayed immediately or stored at 4 °C for 2–3 days. When the metabolites contain –COOH, the concentrated extract should be derivatized by bis(trimethylsilyl) tri-fluoroacetamide. Derivatization was used to decrease the polarity of compounds to make them more accessible to GC.

Separation and identification of the extracts were carried out by Hewlett–Packard 5890 series II fitted with a HP 5 capillary column (30 m length, 0.25 mm diameter, 0.25 μm film thickness); and a 1 μl extract was injected. The oven temperature was programmed: (1) when the extract was not derivatized, to hold at 90 °C for 3 min, then increase to 280 °C at 10 °C/min and hold constant at 280 °C for 5 minand (2) when the extract was derivatized, to ramp at 15 °C/min to 150 °C from an initial temperature of 70 °C (hold 2 min), increase to 200 °C at 2 °C/min, then increase to 280 °C at 15 °C/min and finally hold at 280 °C for 5 min. Helium was used as the carrier gas at the constant flow of 1 ml/min. Effluents from the GC column were transferred to a 70 eV electron impact source held at 200 °C. The injection temperature was kept at 250 °C. Identification was obtained by probability-based matching with mass spectra in a NIST library as well as by matching with the mass spectra and retention time of the standard reference compounds used.

Results

Biodegradation of mono-fluorophenol

The transformation of mono-fluorophenols by the acclimated activated sludge is shown in Figure 1. About 50 mg/l 3-fluorophenol and 50 mg/l 4-fluorophenol were degraded completely within 3.75 h and 3.5 h respectively, while 2-fluorophenol was less readily degraded than the other two mono-fluorophenols, with 50 mg/l 2-fluorophenol being degraded within 8.5 h. Growth of the acclimated activated sludge on the higher concentration of the mono-fluorophenol (125 mg/l 3-fluorophenol, 4-fluorophenol, 75 mg/l 2-fluorophenol), resulted in a decrease in the activated sludge despite metabolism of the substrates. About 75 mg/l 2-fluorophenol was not transformed completely, but the acclimated activated sludge degraded 75 mg/l 3-fluorophenols and 75 mg/l 4-fluorophenol within 5 h. These results indicated that the biodegradabilty of mono-fluorophenols was in the order: 4-fluorophenol > 3-fluorophenol > 2-fluorophenol.

The biodegradation of mono-fluorophenols was accompanied by concurrent release of fluoride anion (Figure 2). The activated sludge degraded 100 mg/l 3-fluoropheno and 4-fluorophenol in 16 h, with 99.85% and 99.91% fluoride anion release, respectively, and degraded 50 mg/l 2-fluorophenol in 15 h, with 99.26% fluoride anion release.

Conversion of mono-fluorophenol by acclimated activated sludge, as determined by GC-MS and HPLC

The peak at 6.33 min and 8.80 min in Figure 3 could be identified as 3-fluorocatechol and 4-fluorocatechol, respectively. This suggested that 3-fluorophenol could be hydroxylated to give catechol-type intermediates by the phenol hydroxylase. From the HPLC traces in Figure 4, the same results could be derived. 3-Fluorocatechol was also detected as the metabolite of 2-fluorophenol biodegraded by the acclimated activated sludge, and 4-fluorocatechol for 4-fluorophenol. Fluorinated biodegradation metabolites of fluorophenols were mainly fluorocatechols. In addition to the fluorocatechol, no other intermediate metabolites were observed.

3-Fluorocatechol and 4-fluorocatechol could be detected during biodegradation of 3-fluorophenol. But the ratio of 3-fluorocatechol to 4-fluorocatechol was different at different pH values. At pH 7.5, 3-fluorophenol was preferentially hydroxylated at the C2 *ortho*-position, resulting in no 4-fluorocatechol detected (C6 hydroxylated intermediate). When the pH value was 7.0, the C2/C6 hydroxylation ratio was 7.9. Along with decreasing pH value, the C6 *ortho*-hydroxylation increased relative to C2 hydroxylation, yielding a C2/C6 hydroxylation ratio of 1.7 at pH 6.0.

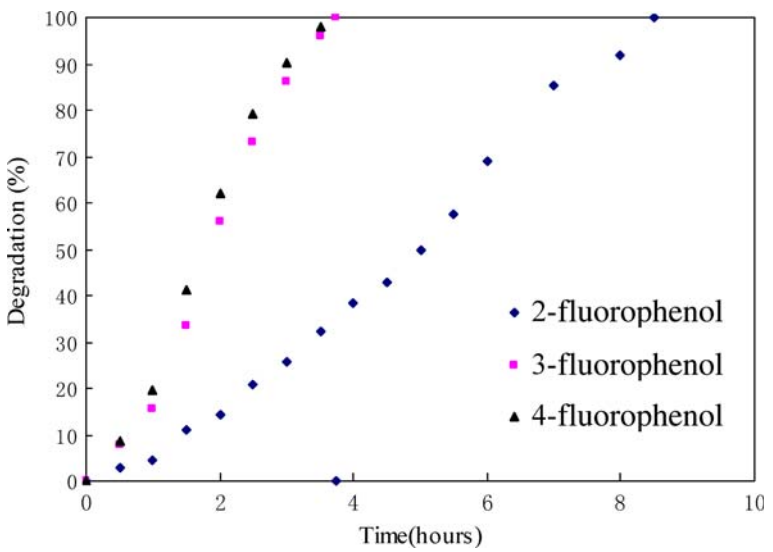

Figure 1. Degradation of mono-fluorophenol by the acclimated activated sludge: ♦ 2-fluorophenol, ■ 3-fluorophenol, ▲ 4-fluorophenol.

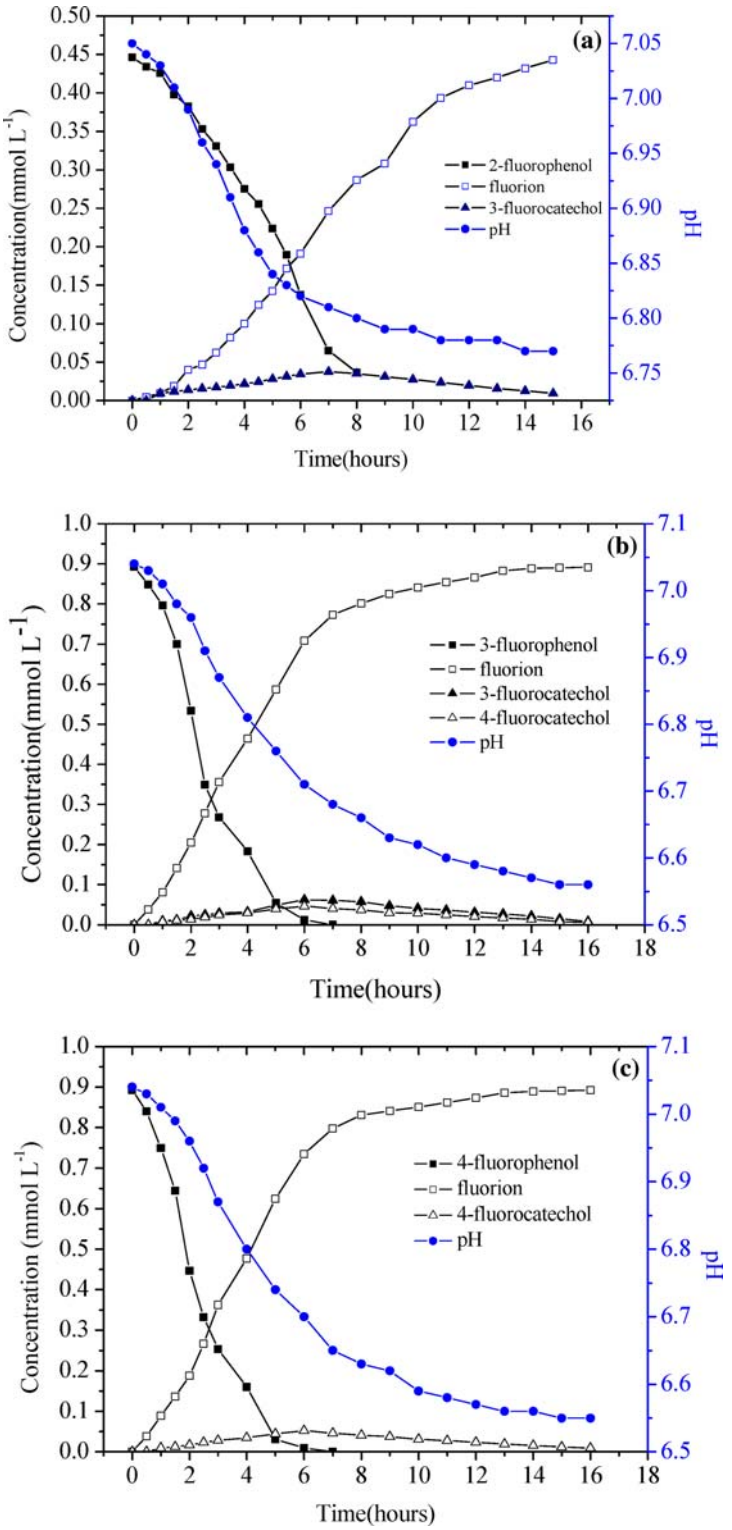

Figure 2. Biodegradation of 2-fluorophenol (a), 3-fluorophenol (b) and 4-fluorophenol (c) by the acclimated activated sludge. ■ mono-fluorophenol, □ fluoride anion, ▲ 3-fluorocatechol, Δ 4-fluorocatechol, ● pH.

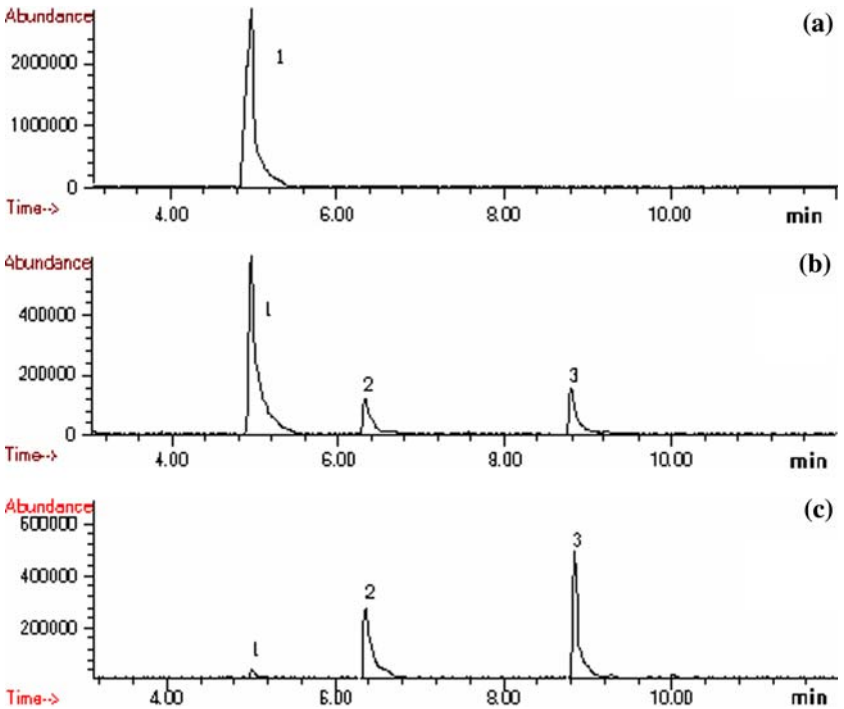

Figure 3. GC-MS total ion chromatogram of metabolites of 100 mg/l 3-fluorophenol biodegraded by the acclimated activated sludge at different times (a 0 h, b 4 h, c 6 h). *Peak 1* 3-fluorophenol (Rt 4.97 min), *peak 2* 3-fluorocatechol (Rt 6.33 min), *peak 3* 4-fluorocatechol (Rt 8.80 min).

Data for fluoride anion release and the metabolites identified during the degradation of the fluorophenols by the acclimated activated sludge are shown in Figure 2. Fluorocatechol measured by HPLC was formed gradually when fluorophenol metabolism continued. When the initial concentration of 2-fluorophenol was 50 mg/l, the amount of accumulated 3-fluorocatechol reached a maximum at 7 h and then began to drop, accompanied by a further increase in fluoride anion, as expected. For 100 mg/l 3-fluorophenol and 4-fluorophenol, the concentration of accumulated fluorocatechol at 6 h was the highest, then it began to decrease.

The initial step in the aerobic degradation of phenolic compounds was their transformation to catechols by the enzyme phenol hydroxylase, following which ring cleavage occurred via either the *ortho*- or *meta*-cleavage pathway (Farrell 2000; Sung et al. 1996). Because the same activated sludge should biodegrade the pollutants in the same way, the following experiment was carried out in order to study the ring cleavage pathway of the fluorocatechol by the acclimated activated sludge in detail. About 128 mg/l 3-fluorocatechol (intermediate of 2-fluorophenol) was degraded by the same acclimated activated sludge. The metabolite extract of 3-fluorocatechol was derivatized with bis(trimethylsilyl) tri-fluoroacetamide. The metabolites were identified as 2-fluoromuconates and dienelactone. These results suggested that 2-fluorophenol was transformed mainly via an *ortho*-cleavage pathway. As was shown for 2-fluorophenol, degradation of 3-fluorophenol and 4-fluorophenol by the acclimated activated sludge was also mainly via the *ortho*-cleavage pathway.

Experiments carried out on the degradation of fluorophenol by the activated sludge indicated that ring cleavage was mainly via the *ortho*-cleavage pathway. In order to confirm this, the key enzymes involved in catalyzing ring fission (catechol 1, 2-dioxygenase, catechol 2, 3-dioxygenase) were assayed (Farrell 2002). When adding fluorocatechol into the enzyme-containing solution, the absorbance at 260 nm increased from 0.05 to 0.21 over a

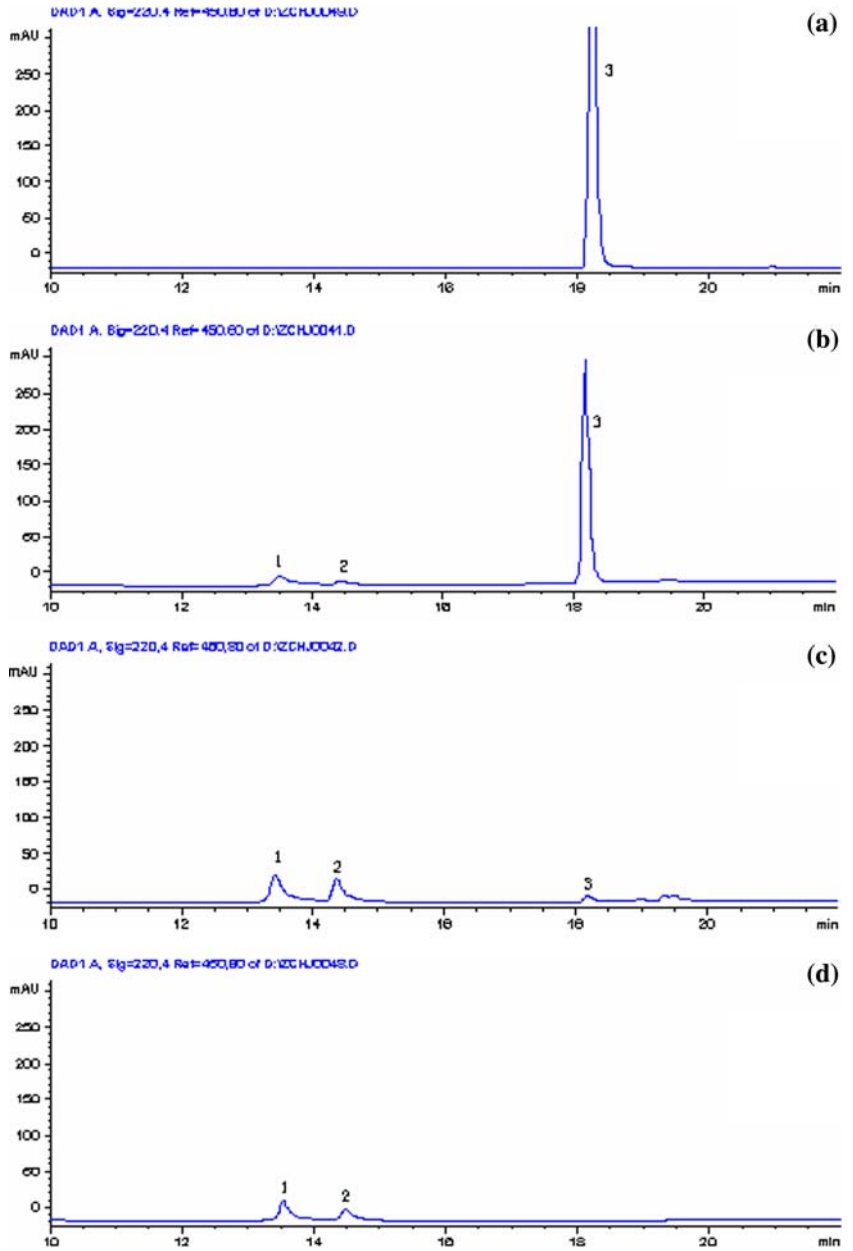

Figure 4. Liquid chromatogram of metabolites of 100 mg/l 3-fluorophenol biodegraded by the acclimated activated sludge at different time (a 0 h, b 2 h, c 6 h, d 9 h). *Peak 1* 3-fluorocatechol (Rt 13.370 min), *peak 2* 4-fluorocatechol (Rt 14.327 min), *peak 3* 3-fluorophenol (Rt 18.133 min).

period of 5 min. The result indicated the activated sludge contained catechol 1, 2-dioxygenase. No catechol 2, 3-dioxygenase activity was detected, indicating the absence of *meta*-cleavage capability in the mixed culture. Enzyme assays confirmed that degradation was mainly via the *ortho*-cleavage pathway.

Discussion

Aerobic biodegradability of fluorophenols by acclimated activated sludge

In the present study, the acclimated activated sludge was shown to be capable of degrading

mono-fluorophenol under aerobic conditions. The mono-fluorophenol could serve as the sole carbon and energy source for the acclimated activated sludge because mono-fluorophenol was biodegraded efficiently and the MLSS of bioreactor was constant for more than 6 months (3 g/l). There are several articles on the biodegradation of mono-fluorophenol, but they have been carried out using pure bacteria and fungi (Kramer 2004). Because acclimated activated sludge can be obtained more easily than pure bacteria and fungi, fluorophenol-containing wastewater can be treated using activated sludge rather than pure bacteria and fungi.

The research demonstrated that the aerobic biodegradability of mono-fluorophenols was in the order of 4-fluorophenol > 3-fluorophenol > 2-fluorophenol. Biodegradation of a chemical in the aquatic environment is predominantly through microbial attack via an enzymatic process. In general, the factors determining the rate of biodegradation can be divided into two kinds: the uptake rates and the transport rates (the uptake rates by microbial cells, the transport rates within the cell by the relevant enzymes; i.e. the rate of binding to the active site of an enzyme and/or the rate at which they undergo enzymatic transformation). In the absence of a specific uptake mechanism, organic compounds are probably transported into bacterial cells by passive diffusion through the liquid membrane. If the diffusion coefficient in a cell membrane belongs to a partition process, the diffusion coefficient should be a direct proportion to $\log K_{ow}$ (partition coefficient for *n*-octanol/water). Therefore, biodegradation rates should be related to a macroscopic hydrophobic parameter if diffusion and uptake are rate-limiting steps for biodegradation. The enzyme-catalyzed transformation of a compound occurs by its binding to the site of the enzyme through the formation of hydrogen or covalent bonds. The strength of this interaction is influenced by the electronic structure of compound and the steric structure of the compound coinciding with the active site of enzyme. Thus, if binding to enzyme or transformation is a rate-limiting step, the biodegradation rate of compounds should be related to the factors influencing the binding or reacting with enzyme (electronic and/or steric parameters). So, the hydrophobic, electric and steric parameters may be the three main chemical factors which affect the substrate biodegradation.

The parameter values of mono-fluorophenols, which have proven useful in an earlier investigation modeling the quantitative relationship of structure and biodegradability (Gamberger et al. 1996), are listed in Table 1. The correlation coefficient between chemical descriptors and biodegradability showed that the biodegradation of mono-fluorophenol by activated sludge under our acclimation conditions was related mainly to the *n*-octanol/water partition coefficient, which described the hydrophobicity of the compound (transport through the cell wall). The larger the octanol/water partition coefficient is, the easier it is for the molecules to penetrate into the cell through its cell membrane and arrive at the active site of an enzyme. The steric parameter was the second factor that could affect the biodegradation of mono-fluorophenol under our experimental conditions. The differences between the biodegradability of the fluorophenols resulted from the different octanol/water partition coefficients and ^{1}X, which can affect the passage of fluorophenol into cell membrane. Poor correlation between biodegradation rate constant and electric parameter demonstrated that the rate-limiting step within the overall

Table 1. The experimental biodegradation and structure parameters of mono-fluorophenol

		2-Fluorophenol	3-Fluorophenol	4-Fluorophenol	R^2
Hydrophobic parameter	$\text{Log}K_{ow}$	2.3852	2.6990	2.7875	0.999
Electronic parameter	E_{HOMO}	−9.1950	−9.3730	−9.0930	0.0042
	Dipole moment	2.7640	2.5360	1.6540	0.6518
Steric parameter	^{1}X	2.2399	2.2340	2.2340	0.9514
	^{2}X	1.4514	1.4799	1.4764	0.8919
	$^{3}X_{p}$	0.8525	0.8082	0.8266	0.6353
	$^{4}X_{p}$	0.4517	0.4761	0.4411	–
	$^{4}X_{pc}$	0.1863	0.1482	0.1588	0.7743
Oxygen-consumed rate constant (Zhang 2004)	l/gSS.h	0.0093	0.0133	0.0145	

biodegradation process was the rate of uptake and transport instead of the rate of binding with the active site of an enzyme and enzymatic reaction.

Ortho-hydroxylation of fluorophenols by acclimated activated sludge

When the fluorine was in the *ortho*-position with respect to the hydroxyl moiety of the phenolic substrate, such as 2-fluorophenol, the phenol hydroxylase could catalyze C2 hydroxylation, accompanied by dehalogenation in addition to hydroxylation at the non-fluorinated C6 position (Bondar et al. 1999). Hydroxylation at the fluorinated C2 position of the substrates represented a so-called oxidative or oxygenolytic dehalogenation in which the insertion of an oxygen atom derived from molecular oxygen was accompanied by dehalogenation. There was some tendency that the accumulation of the catechols was observed when oxidative defluorination in the first step was favored over hydroxylation at C6. In addition to 3-fluorocatechol, no other catechol-type metabolite peaks were observed during the biodegradation of 2-fluorophenol. Furthermore, HPLC analysis of the reaction did not reveal the accumulation of catechol. This suggested that hydroxylation at the non-halogenated *ortho*-position, leading to 3-fluorocatechol, was favored over oxidative dehalogenation of 2-fluorophenol, leading to catechol. From the fact that fluorocatechol was the only intermediate accumulating, it was concluded that the key step in the enzyme-catalyzed transformation of fluorophenol by the acclimated activated sludge was related to the enzyme catalyzing the conversion of fluorocatechol.

Since 3-fluorophenol has two unsubstituted *ortho*-positions, the phenol hydroxylase has two possible positions to attack. So, 3-fluorocatechol and 4-fluorocatechol could be detected during the biodegradation of 3-fluorophenol. Along with a decreasing pH value, the C6 *ortho*-hydroxylation increased relative to C2 hydroxylation. These results suggest that the regioselective hydroxylation depends on the pH value. Molecular orbital calculations in combination with frontier orbital theory could provide an insight into the reactivity of the C2 and C6 positions in 3-fluorophenol. The electron density of C2 and C6 of 3-fluorophenol was calculated using *Hyperchem* software. The result indicated that the C2/C6 electron density of 3-fluorophenol was 0.1364, while that of the fluorophenolate anion was 0.9565. Thus, as the pH value increased, the C2/C6 hydroxylation ratio increased.

Aromatic ring cleaved via ortho-cleavage pathway

Cleavage of the aromatic ring may occur using either the *ortho*- or the *meta*-cleavage pathway. Aromatic compounds found naturally in the environment, such as phenol and benzene, were typically broken down via the *meta*-pathway. While methyl-substituted aromatic compounds were also successfully degraded via the *meta*-cleavage pathway, chlorinated aromatic compounds were generally broken down via the *ortho*-cleavage pathway. The *ortho*-cleavage pathway of mono-fluorophenol biodegraded by this acclimated activated sludge was proposed on the basis of the following results.

1. Mono-fluorophenol was transformed completely with approximately 100% fluoride anion release. *Meta*-cleavage by catechol 2, 3-dioxygenase was known to generally result in incomplete metabolism due to the production of dead-end or suicide metabolites (Farrell and Quilty 1999).
2. Fluoromuconate was indentified in the biodegradation of fluorocatechol by the same acclimated activated sludge, which was the typical metabolite of the halogenated phenol via an *ortho*-cleavage pathway (Marelle et al. 1998).
3. The key enzyme (catechol 1, 2-dioxygenase) involved in catalyzing *ortho*-cleavage was detected in this activated sludge. No apparent biodegradation by the *meta*-cleavage pathway was found in the test, so it was concluded that the fluorophenol was transformed by the acclimated activated sludge mainly via the *ortho*-cleavage pathway.

Biodefluorination of fluorophenols by the activated sludge

The increase in fluoride anion indicated that biodefluorination had occurred. According to literature data on the conversion of 2-chloro-, 3-chlorophenol by (chloro-) muconate cycloisomerases (Blasco et al. 1995; Vollmer et al. 1994), a possible pathway for the defluorination of fluoromuconate by muconate cycloisomerase can be proposed. Figure 5 schematically presents these possible

pathways. Cyclization of fluoromuconate to fluoromuconolactone cannot lead to dehalogenation. Fluoromuconolactone is not stable; and defluorined then results in the formation of dienelactone. This reaction would be analogous to the dechlorination of 3-chloromuconate proceeding by its initial conversion to 4-chloromuconolactone catalyzed by chloromuconate cycloisomerase, followed by chloride elimination and resulting in the dienelactone (Vollmer and Schlöman 1995).

From Figure 2, it can be seen that the degradation of fluorophenols resulted in a drop in pH. This drop was due not only to the release of fluorine, but also to the production of organic acids which were

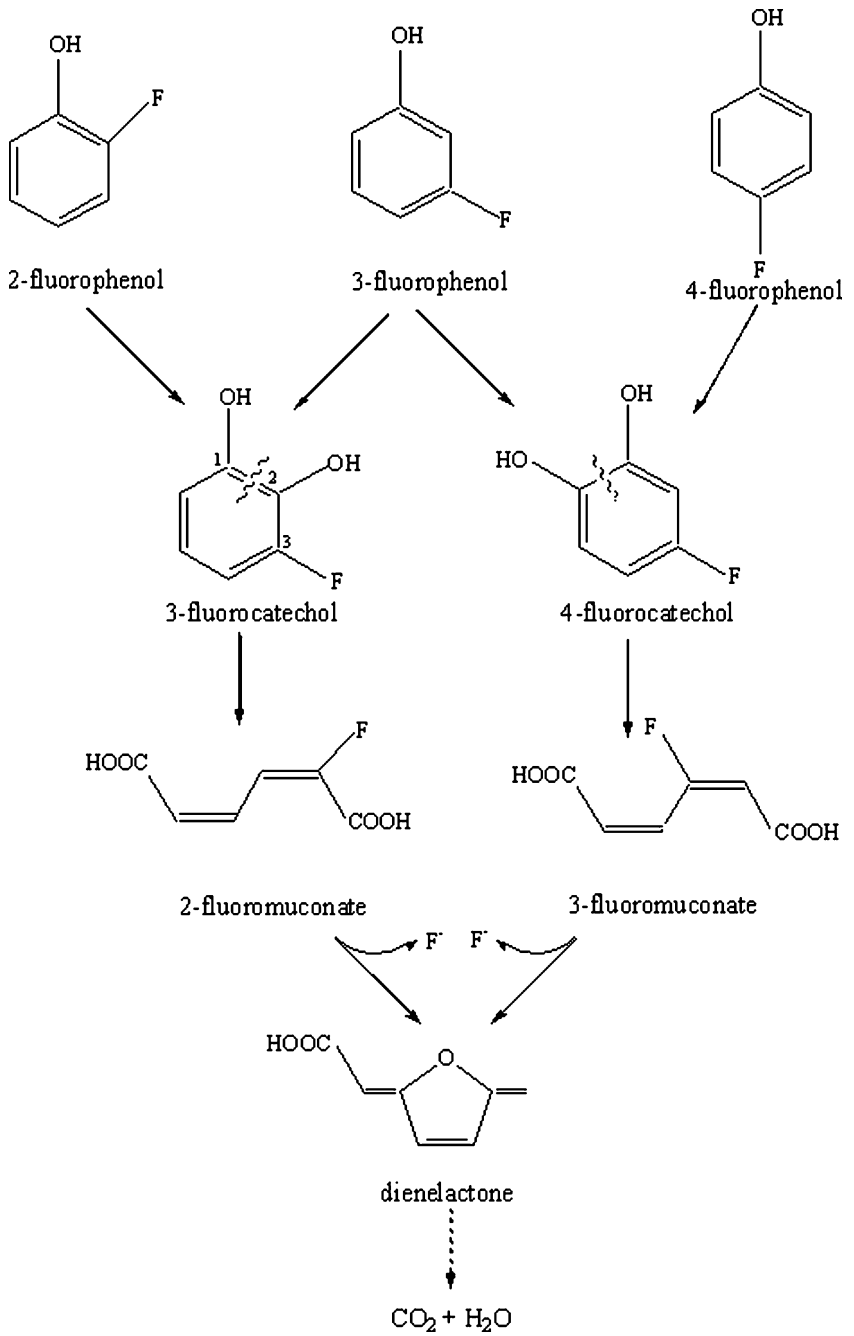

Figure 5. The proposed degradation pathway of mono-fluorophenol by acclimated activated sludge.

described as being intermediates in the degradation of halophenols (Bondar et al. 1999). The initial fluoride anion release went with a drop in pH, which reached a plateau as fluoride anion release was also completed. The pattern of fluorine release could mirror the pH drop, which suggested that the pH drop resulted mainly from the fluoride anion release.

During the biodegradation of all fluorophenols, nearly no accumulation of fluorinated muconates occurred, indicating that these non-aromatic intermediates were further degraded with the concomitant formation of the fluoride anion. The proposed pathway for biodegradation of mono-fluorophenol by the activated sludge is shown in Figure 5.

For complete degradation of halogenated phenols to occur, two steps are necessary: cleavage of the aromatic ring and removal of the halogen. This study revealed that the initial step in the aerobic biodegradation of mono-fluorophenol by this acclimated sludge is their transformation to fluorocatechol. Fluorocatechols are central metabolites in the aerobic degradation of fluorophenol. Following transformation of the fluorophenol to fluorocatechol, *ortho*-ring cleavage by 1, 2-dioxygenases proceeds. Defluorination occurs after the ring is cleaved.

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