A Study on the Aerobic Biodegradability of Fluorophenols

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Abstract By measuring the respiratory oxygen consumption, a study on the aerobic biodegradability of 2-fluorophenol, 3-fluorophenol and 4-fluorophenol was conducted using activated sludge acclimated by themselves respectively. The experimental results showed that bio-oxidation ratio of 2-fluorophenol, 3-fluorophenol and 4-fluorophenol was 25.30%, 35.28% and 36.60% respectively, and the oxygen consuming rate constant was 0.0093 L/gSS·h, 0.0133 L/gSS·h and 0.0145 L/gSS·h respectively, so the aerobic biodegradability of 4-fluorophenol was the best, and that of 2-fluorophenol was the worst among the three isomerises.

Key words: Aerobic biodegradability, Bio-oxidation ratio, Fluorophenols,
Oxygen consuming rate constant

1. Introduction

The production and the use of fluorinated substances have been increased enormously in the recent years (Edwards, 1994；Cociglio，1996). 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 of fluorinated organics, their bioactivity and their potential for accumulation in the environment, it is important to understand their environment fate and the mechanism by which they might be degraded. The usage of fluorinated compounds, such as fluorophenols, in agricultural or industrial processes, has led to their accumulation in the environment. The aerobic biodegradability of an organic chemical is a critical factor in assessing its environmental fate and impact. Therefore, our interest has been focused on the aerobic biodegradability of fluorinated aromatics, especially on the metabolism of mono-fluorophenols by the acclimated activated sludge.

Biodegradability of chemicals is one important criterion in ecotoxicological risk assessment. Persist chemicals with a low toxicity at applied concentrations may accumulate in the environment and reach concentration causing toxic effects. As biodegradability is a basic prerequisite for chemicals, a number of biodegradation tests have been developed and standardized in the last 25 years in order to predict the ultimate fate of chemicals in the environment(Pagga，1997). Here, the aerobic biodegradability of the fluorophenols was determined using the respirometric method based on oxygen consumption, which have been successfully used for many years. OxiTop BOD detector was used to measure the amount of oxygen required by the bacteria to oxidize the organic matter. The aerobic biodegradability of mono-fluorophenols was evaluated by bio-oxidation ratio and the oxygen consuming rate constant.

2. Materials and Methods

Chemicals

2-fluorophenol, 3-fluorophenol and 4-fluorophenol, used in the degradation studies were obtained from Xieshi Chemical Company (Shanghai, China), the purity of these chemicals was 99.9%. The other chemicals were got from Shanghai Chemical Company (Shanghai, China)

Analytic Methods

A pH electrode was used to measure the pH value (model: pHS-3C). 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 ℃ for 2 h and reweighing them.

Microorganism and Growth Condition

The specific fluorophenols degrading mixed culture was obtained through acclimating activated sludge (AS) to a synthetic wastewater with fluorophenol as the sole carbon source for about six months. The activated sludge used as the seed source for enriching fluorophenols degrading special culture was the mixture of AS from Quyang wastewater treatment plants in Shanghai(China). During the whole experiment, DO was kept at 2-3 mg/L and temperature was set at 25 ℃.

Experiment procedure

Fluorophenol biodegradation experiments were performed in closed 500 mL WTW bottle containing 250 mL of minimal medium and certain mono-fluorophenol as sole carbon source. The pH of the medium was adjusted to 7.0. The biomass mixture in bioreactor was centrifuged at 5000 rpm 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). OxiTop BOD detector (Weilheim, German) was used to measure the mount of oxygen required by the bacteria to oxidize the organic matter. In this case, the test was conducted over a 5 days period and the consumed oxygen measured manometrically. Carbon dioxide was removed from the samples by placing the NaOH pellets in each bottle cap. The influence of possible nitrification processes was considered by adding 0.5 g/L allyl sulfourea in the reaction bottle. The reaction bottles were incubated in an orbital shaker at 150 rpm at 25 ℃. Uninoculated bottled were incubated in parallel. The tests were conducted in duplicate. Results of all analysis represent the mean values of replicate trial degradations.

3. Results and discussions

Bio-oxidation ratio of mono-fluorophenol

Theoretical oxygen demand (ThOD) includes oxygen consumed by the biodegradation of the chemicals and oxygen used in the assimilation. The level of biodegradation is determined by comparing the biochemical oxygen demand (BOD) with the theoretical oxygen demand and expressed in percent. The biodegradability of fluorophenols (bio-oxidation ratio, *E*) was expressed as maximum percent of BOD compared with the theoretical oxygen demand.

$$E=\frac{(Qbiochemical-Qendogenous)\times SS}{ThOD}\times 100\% \quad (1)$$

Where E is bio-oxidation ratio, $Q_{biochemical}$ is consumed oxygen demand by the biodegradation, $Q_{endogenous}$ is endogenous oxygen demand, SS is the activated sludge, $ThOD$ is the theoretical oxygen demand.

Relative accumulated consumed oxygen was expressed as the difference between the biochemical consumed oxygen demand and endogenous oxygen demand. If the value of relative accumulated consumed oxygen was more than zero, it was considered that the test chemical could be biodegraded, or else the chemical was considered to inhibit the endogenous respiration. The bigger the bio-oxidation ratio is, the higher the biodegradability of the test chemical is.

In order to obtain the average bio-oxidation ratio, five tests with different original concentration was conducted. As illustrated in Fig 1-3, O_2 consumption data were expressed as relative accumulated consumed oxygen versus time.

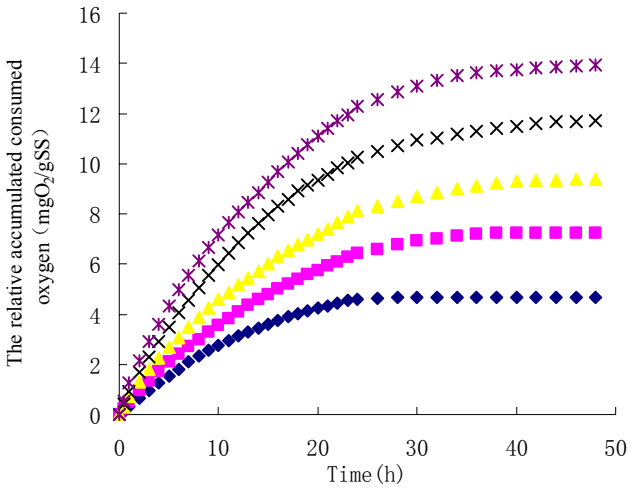

Fig 1 Oxygen consumption of 2-fluorophenol

(◆ 20mg/L, ■ 30mg/L, ▲ 40mg/L, × 50mg/L, ∗ 60mg/L)

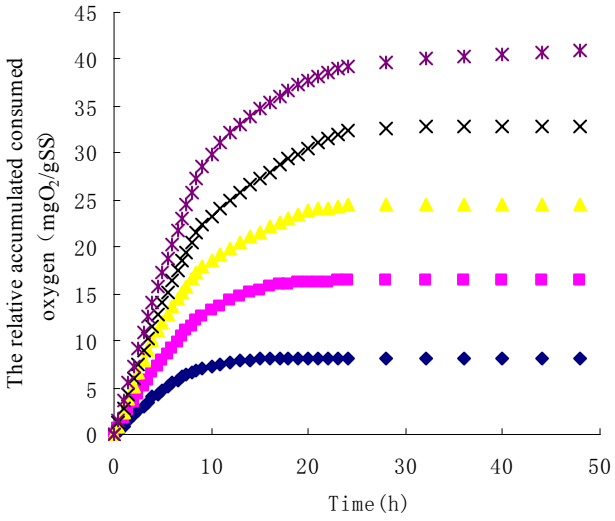

Fig 2 Oxygen consumption of 3-fluorophenol

(◆ 25mg/L, ■ 50mg/L, ▲ 75mg/L, × 100mg/L, ∗ 125mg/L)

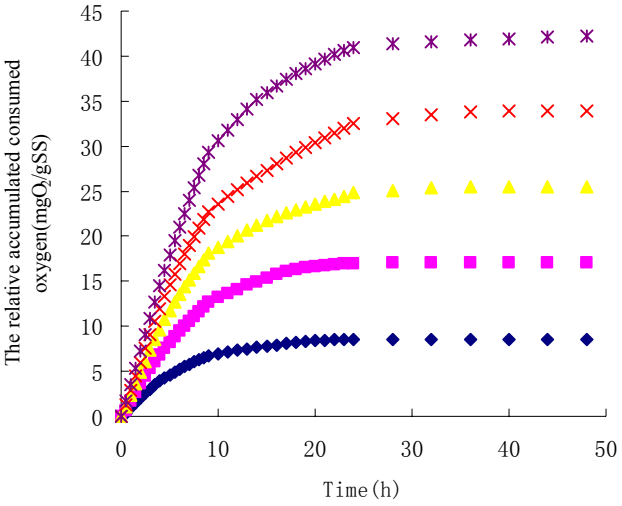

Fig 3 Oxygen consumption of 4-fluorophenol

(◆ 25mg/L, ■ 50mg/L, ▲ 75mg/L, × 100mg/L, ∗ 125mg/L)

The relative accumulated consumed oxygen of the mono-fluorophenol (shown in Fig 1-3) was higher than zero illustrated that all of mono-fluorophenols could be degraded by the microbial community. The slope of the respiration curve (oxygen consumed rate) decreased accompanying with the biodegradation until 48 hours after the biodegradation reaction. After 48 hours the relative accumulated consumed oxygen curve was paralleled to the X axis, namely the biodegradation of fluorophenol finished. Bio-oxidation ratio of the fluorophenol was calculated by the relative accumulated consumed oxygen at 48 hour, as shown in table 1.

Table 1 Bio-oxidation ratio of mono-fluorophenol

concentration of 2-fluorophenol mg/L	**bio-oxidation ratio %**	**concentration of 3-fluorophenol mg/L**	**bio-oxidation ratio %**	**concentration of 4-fluorophenol mg/L**	**bio-oxidation ratio %**
20	25.15	25	35.37	25	36.37
30	25.92	50	35.20	50	36.54
40	25.20	75	35.11	75	36.65
50	25.22	100	35.46	100	36.79
60	25.00	125	35.26	125	36.66
average *E*	25.30	average *E*	35.28	average *E*	36.60

The bio-oxidation ratio of fluorophenol(shown in table 1) was smaller than that of phenol (54.7%). This was in line with the expected effect of fluorine substituents, decreasing the nucleophilic reactivity of the л-electrons in the fluorophenol, a characteristic that was of importance for their rate of conversion by phenol hydroxylase (Peelen， 1995). From the fact that the bio-oxidation ratio of 2-fluorophenol, 3-fluorophenol and 4-fluorophenol was 25.30%, 35.28% and 36.60% respectively, the conclusion that the biodegradability of the mono-fluorophenol decreased in the order of 4-fluorophenol>3-fluorophenol>2-fluorophenol was obtained.

Oxygen consuming rate constant of mono-fluorophenol

If the biodegradation of mono-fluorophenol followed the first-order kinemics, the following equation could be obtained.

$$\frac{dO_2}{dt} = KXSThOD \qquad (2)$$

where $\frac{dO_2}{dt}$ is oxygen consuming rate, K is oxygen consuming rate constant，X is concentration of activated sludge，S is concentration of chemical，$ThOD$ is the theoretical oxygen demand. When the concentration of activated sludge (X) was fixed, $dO_2/(dt \cdot X)$ would be in direct proportion to the concentration of the chemicals (S), and the slope of fit line would be K×ThOD, so the K could be got from the value of slope/ ThOD. The graph of original oxygen consuming rate versus concentration of mono-fluorophenol was shown in Fig 4-6.

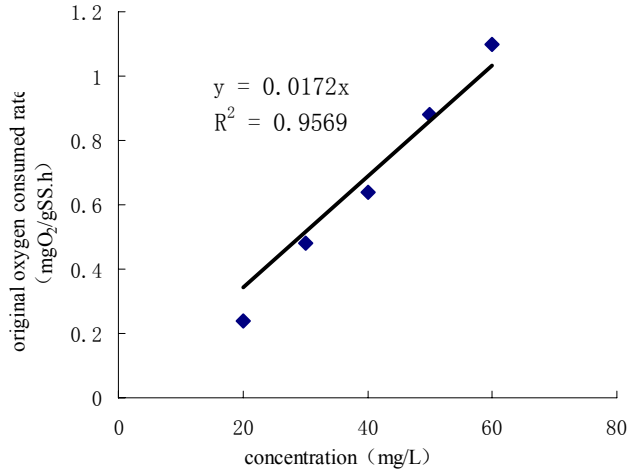

Fig 4 Original oxygen consuming rate versus concentration of 2-fluorophenol

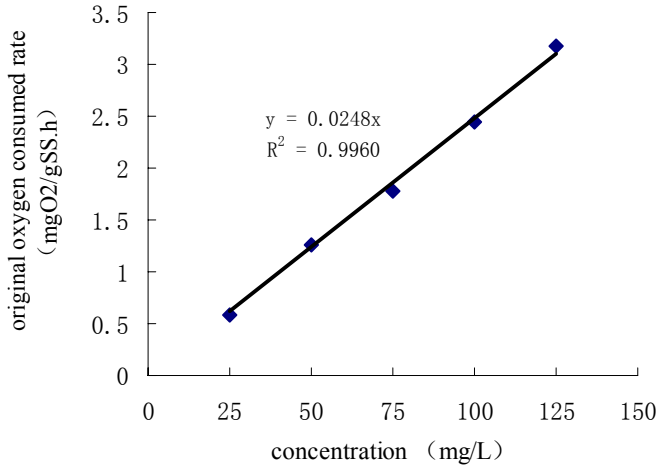

Fig 5 Original oxygen consuming rate versus concentration of 3-fluorophenol

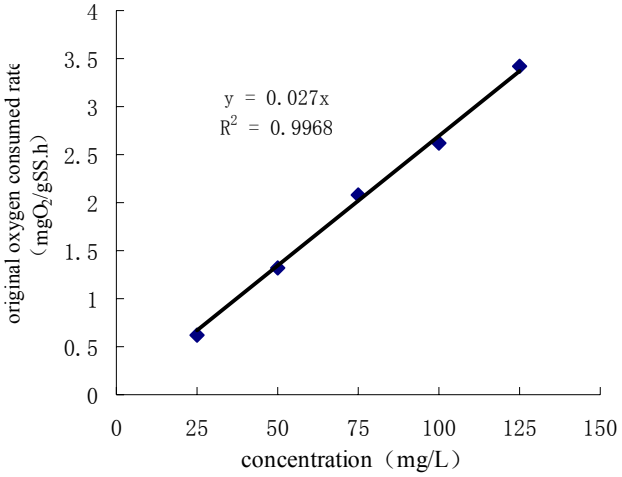

Fig 6 Original oxygen consuming rate versus concentration of 4-fluorophenol

Since the correlation coefficient of fit line in Fig 4-6 was bigger than 95%, the first order kinemics fitted successfully, and the biodegradation of mono-fluorophenol followed the first-order kinemics. The biodegradation dynamics of mono-fluorophenol was listed in table 2. The oxygen consuming rate constant of 2-fluorophenol, 3-fluorophenol and 4-fluorophenol was 0.0093 L/gSS·h, 0.0133 L/gSS·h and 0.0145 L/gSS·h respectively, so the aerobic biodegradability of 4-fluorophenol was the best, and that of 2-fluorophenol was the worst among the three isomerises

Table 2 Biodegradation dynamics of mono-fluorophenol（25℃）

substrate	differential equation	oxygen consuming rate constant （L/gSS.h）	integral equation
2-fluorophenol	$\frac{dO_2}{dt \cdot X} = 0.0712S$	0.0093	$S=S_0e^{-0.0093Xt}$
3-fluorophenol	$\frac{dO_2}{dt \cdot X} = 0.0248S$	0.0133	$S=S_0e^{-0.0133Xt}$
4-fluorophenol	$\frac{dO_2}{dt \cdot X} = 0.027S$	0.0145	$S=S_0e^{-0.0145Xt}$

Biodegradation of a chemical in the aquatic environment is predominantly through microbial attack, and thus, we may presume, *via* enzymatic process. In general, the factor determining the rate of biodegradation can be divided into two kinds: the uptake rates and transport rates (the uptake rates by microbial cells or transport rates within the cell to the relevant enzymes); the rates binding to the active site of an enzyme, and/or by 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 cell membrane belongs to partition process, the diffusion coefficient should be a direct proportion to log *k*ow. Therefore, biodegradation rates should be related macroscopic hydrophobic parameter if diffusion and uptake are rate-limiting step of 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 compound coinciding with the active site of enzyme. So, if binding to enzyme or transformation is rate-limiting step, biodegradation rate of compounds should be related to the factors influencing the binding or reacting with enzyme (electronic and/or steric parameters). Hydrophobic, electric and steric parameters may be three main quantum-chemical factors which affect the biodegradation of substrate.

The parameter values which have proven useful in earlier investigation modeling the quantitative relationship of structure and biodegradability (Wang， 1992) of mono-fluorophenols were listed in Table 1. The correlation coefficient between quantum-chemical descriptors and biodegradability showed that the biodegradation of mono-fluorophenol was related mainly to n-octanol/water partition coefficient which could describe the hydrophobicity of the compound (transformation through the cell wall). The larger the octanol/water partition coefficient, the easier the molecule 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 can affect the biodegradation of mono-fluorophenol. ^{1}X could describe the size of the molecule. The smaller the molecule is, the easier the molecule passes though the membrane of the cell. So the smaller the ^{1}X, the bigger the oxygen-consumed rate constant of mono-fluorophenol. The differences between the degradation of the fluorophenols resulted from the differing octanol/water partition coefficients and ^{1}X which can affect the pass of fluorophenol into cell membrane. Poor correlation between biodegradation rate constant demonstrated that the rate-limiting step within the overall biodegradation process was the rates of uptake and transport instead of the rates of binding with the active site of an enzyme and enzymatic reaction.

Table 3 The experimental biodegradation and structure parameters of mono-fluorophenol

		2-fluorophenol	3-fluorophenol	4-fluorophenol	R^2
hydrophobic parameter	log *Kow*	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 consuming rate constant	L/gSS·h	0.0093	0.0133	0.0145	

4. Conclusions

（1） Three mono-fluorophenols could be aerobic biodegraded efficiently by the acclimated activated sludge and be utilized as sole carbon and energy source.

（2） The bio-oxidation ratio of 2-fluorophenol, 3-fluorophenol and 4-fluorophenol is 25.30%, 35.28% and 36.60% respectively，and oxygen consuming rate constant is 0.0093 L/gSS·h, 0.0133 L/gSS·h and 0.0145 L/gSS·h. So the aerobic biodegradability of the mono-fluorophenols decreases in the order of 4-fluorophenol >3-fluorophenol >2-fluorophenol.

（3） The differences between the biodegradability of the fluorophenols resulted from the differing octanol/water partition coefficients and ^{1}X which can affect the penetration of fluorophenol into cell membrane. The rate-limiting step within the overall biodegradation process was the rates of uptake and transport instead of the rates of binding with the active site of an enzyme and enzymatic reaction.

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