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Research Article

Cometabolism of Fluoroanilines in the Presence of 4-Fluoroaniline by *Ralstonia* sp. FD-1

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A strain of *Ralstonia* sp. FD-1 capable of using 4-fluoroaniline (4-FA) as the sole carbon and nitrogen source was investigated for its ability to utilize 4-FA isomers (2-FA, 3-FA) and homologs (2,4-DFA, 3,4-DFA, and 2,3,4-TFA). Both 4-FA and 3-FA could be mineralized as the sole carbon and nitrogen source by FD-1. 2-FA, 2,4-DFA, 3,4-DFA, and 2,3,4-TFA could not be degraded by FD-1, respectively, and were selected as secondary substrates for cometabolism with 500 mg/L 4-FA as growth substrate. Bacterial growth (OD_{600}), F^- concentrations, and fluoroanilines contents were measured to determinate the degradation ability of 4-FA isomers and homologs by FD-1. FD-1 growth was inhibited by 2,4-DFA, 3,4-DFA, and 2,3,4-TFA at higher concentrations (400 mg/L), except for 2-FA. Complete fluoroanilines degradation was achieved while incomplete defluorination was characterized by the stoichiometric fluoride release indicating partial degradation but not total mineralization. When fluoroaniline was supplied to the resting cells of strain FD-1, a relatively effective removal was showed. Strain FD-1 had broadened application prospect of toxicity and low nutrition fluoroanilines wastewater.

1. Introduction

With the recognition of the novel characteristics of fluorine and the development of the fluorine industry, fluorinated compounds are playing increasingly important roles in numerous industries and our daily life. Since most fluorides are highly stable and relatively safe, fluorinated chemicals are extensively used in the marketplace for various functions, such as agriculture, pharmacy, textiles, and military manufacturing [1–4]. However, the increasing application of fluorinated chemicals has resulted in unavoidable contamination, which has led to accumulation in the environmental matrix, making them an increasing risk to human health and living things [5, 6]. Inadequate disposal of these pollutants can cause significant toxicity on organisms, such as inhibition of enzyme activities, interruption of cell-to-cell communication, disruption of membrane transportation, and reduction of energy generation [7]. Therefore, the remediation on fluorine contamination should be paid more attention.

The major reported fluorinated compounds remediation techniques containing electrochemical and electrokinetic methods are expensive and likely to produce secondary pollutants in the environment [8, 9]. To overcome these problems, microbial degradation of contaminants is firmly favored because of its thorough degradation, effective cost, and eco-friendly pathway [10–12]. Microorganisms are ubiquitously filled in the nature and have tremendous metabolic ability to utilize most toxic compounds as sources of energy and nutrient for growth. Some of them possess specifically characteristic degrading enzymes for biodegradation of pollutants into nontoxic substances [13]. However, owing to the distinct properties of fluorine such as a large van der Waals radius (1.45 Å, which is between that of hydrogen and oxygen), a high electronegativity (4.0 is the strongest of all atoms), and a strong carbon–fluorine (C–F) bond energy (C–F, 485 kJ/mol; C–H, 414 kJ/mol; C–OH, 359 kJ/mol; C–C, 345 kJ/mol; C–Cl, 339 kJ/mol) [14], only a small number of bacteria capable of degrading fluorinated compounds have been isolated

[15, 16]. Since most of the organofluorinated compounds are biorefractory [7, 17], all isolated fluorine-degrading bacteria are precious to fluorine-contaminated bioremediation.

Though many refractory organisms could not be utilized by isolated bacteria as the sole source of carbon, nitrogen, and energy, these pollutants could be transformed by some isolating bacteria as non-growth supporting substrate, generally in the presence of a growth supporting substrate, a process termed cometabolism [18]. Cometabolism was proved to be useful for degrading a variety of minimally degradable compounds, especially isomers and homologs of an identified parent compound [13, 19]. Definitely, cometabolism broadens the application of microbial degradation on fluorine contamination remediation.

Because of chemical synthesis and biotransformation, a considerable number of nondegradable and notorious fluorine pollutants exist in the environment [20]. According to "Integrated Wastewater Discharge Standard" in China (GB 8978-1996), fluoride concentration was limited under 10 mg/L. To our knowledge, 50–500 mg/L of 4-fluoroaniline (4-FA), 2-fluoroaniline (2-FA), 3-fluoroaniline (3-FA), 2,4-difluoroaniline (2,4-DFA), 3,4-difluoroaniline (3,4-DFA), and 2,3,4-trifluoroaniline (2,3,4-TFA) partially or together exist in industrial wastewater (such as the Juhua Group Corporation (Quzhou, China)). To so many refractory fluorides, cometabolism has great potential in their treatment.

As reported by our previous study [21], a strain of *Ralstonia* sp. FD-1 was isolated. This strain can totally degrade 500 mg/L 4-fluoroaniline (4-FA) within 24 h. The highest content of 4-FA that was tolerated and degraded by strain FD-1 was 1250 mg/L. Thus, strain FD-1 probably has great potential to metabolize or cometabolize other varieties of fluoroanilines besides 4-FA.

Therefore, in order to tap the potential of 4-FA degrading bacteria *Ralstonia* sp. FD-1 on the fluoroaniline pollutant treatment, 4-FA isomers and homologs, including 2-FA, 3-FA, 2,4-DFA, 3,4-DFA, and 2,3,4-TFA, were selected for degradation by *Ralstonia* sp. FD-1. In particular, the cometabolism biodegradation of these isomers and homologs by FD-1 with 4-FA as growth substrate was investigated. Finally, resting cell catalysis and crude enzyme degradation of fluoroanilines were conducted to understand the preliminary mechanism responsible for the cometabolism reactions carried out by strain FD-1 [22].

2. Materials and Methods

2.1. Bacterium, Medium, and Reagents. *Ralstonia* sp. FD-1, a 4-FA-degrading strain, was isolated from Linhai wastewater treatment plant (Zhejiang, China) and conserved in our lab [21].

Mineral salt medium (MSM) was used for culturing strain FD-1 [23]. The components were K_2HPO_4 0.200 g, KH_2PO_4 0.800 g, $Na_2MoO_4{\cdot}2H_2O$ 0.0033 g, $CaSO_4$ 0.100 g, $MgSO_4$ 0.200 g, and $FeSO_4{\cdot}7H_2O$ 0.005 g in 1 L of distilled water. The initial pH was 7.0. Depending on the experiments, 4-FA and its isomers and homologs were added to the MSM.

Five kinds of 4-FA isomers and homologs were selected. 2-FA (99%, purity), 3-FA (99%), 4-FA (99%), 2,4-DFA (99%), and 3,4-DFA (99%) were purchased from J&K CHEMICA (J&K Chemical Ltd.); 2,3,4-TFA (98%) was purchased from Matric Scientific and Apollo Scientific Ltd. (Denton, Manchester, UK). Other reagents used in this study were of analytical reagent grade and purchased from Huipu Inc. (Hangzhou, China).

2.2. Growth of Strain FD-1 Using Fluoroaniline as the Sole Carbon and Nitrogen Source. 200 mg/L of each fluoroaniline (2-FA, 3-FA, 4-FA, 2,4-DFA, 3,4-DFA and 2,3,4-TFA) was added to different MSMs as the sole resource of carbon, nitrogen, and energy for FD-1, respectively. Then 1% (v/v) of logarithmic phase strain FD-1 was inoculated into 50 mL of sterilized MSM with pH 7.0, which was contained in a 150 mL Erlenmeyer flask. Afterward, these samples were cultured at 30°C and 130 rounds per min (rpm). The cultures were sampled at 72 h and the produced fluorine ion (F^-) concentration was measured.

2.3. Cometabolic Degradation of Fluoroanilines by FD-1 with 4-FA as the Growth Substrate. Five concentrations of secondary substrate fluoroanilines (0, 50, 100, 200, and 400 mg/L) were used for the cometabolism experiments. The growth substrate was 500 mg/L of 4-FA, which was the optimal concentration for FD-1 growth as determined in our previous study [21]. Cultivation was carried out in a 150 mL Erlenmeyer flask with 50 mL MSM. The cultures were sampled for analysis of FD-1 growth and fluoroaniline degradation and defluorination extent. The defluorination extent was evaluated by the concentration of fluorine ion in the culture.

2.4. Degradation of Fluoroanilines by Resting Cells and Crude Enzyme. Strain FD-1 was cultured in MSM with 500 mg/L 4-FA at 30°C and pH 7 for 20 h. The cells in logarithmic growth phase were collected by centrifugation at 8640 g for 10 min and then washed three times with phosphate buffer solution (PBS, pH 7.4). Afterward, the strain FD-1 was collected as the resting cells. Resting cells were inoculated into 50 mL MSM with 200 mg/L fluoroaniline at 30°C and pH 7. The initial OD_{600} of resting cells in MSM was adjusted to 0.38 for degradation analysis.

2.5. Analytical Methods. Bacterial growth was measured by optical density at 600 nm (OD_{600}) using an UNICO 2000 spectrophotometer.

The fluoroaniline concentrations were determined using a Waters high-performance liquid chromatography (HPLC) system equipped with a Waters e2695 spectrophotometer and Waters 2489 UV/visible detector. Detection conditions were as follows: mobile phase of methanol/water at a ratio of 70 : 30, detection wavelength 230 nm, temperature 35°C, flow rate 1 mL/min, and injection volume 10 μL on an XBridge C18 column (5.0 μm and 4.6 mm × 250 mm column).

The F^- concentration was measured by a Metrohm 882 compact ion chromatography plus with a flow rate of 0.7 mL/min and sodium carbonate/sodium bicarbonate as the effluent.

3. Results and Discussion

3.1. Bacterial Growth with Fluoroanilines as the Sole Carbon and Nitrogen Source. Our previous study [21] had demonstrated that *Ralstonia* sp. FD-1 can use 4-FA (even to 1250 mg/L) as the sole carbon and nitrogen source. In addition to 4-FA, other fluoroanilines, 2-FA, 3-FA, 2,4-DFA, 3,4-DFA, and 2,3,4-TFA, were also tested individually as the sole carbon and nitrogen source for strain FD-1. The dynamic growth of FD-1 was shown in Figure 1. When cultured in 4-FA containing MSM, the growth of strain FD-1 reached a maximum OD_{600} within 24 h at 30°C and pH 7. While in 3-FA containing MSM, a maximum OD_{600} was reached at 60 h under 30°C and pH 7. However, no apparent bacterial growth was observed when 2-FA, 2,4-DFA, 3,4-DFA, or 2,3,4-TFA was employed as the sole carbon and nitrogen source.

After 72 h of incubation, the F^- concentration in the culture using each fluoroaniline as the sole carbon and nitrogen source was measured. If the fluoroaniline was totally defluorinated, the F^- concentration should be equal to the theoretical F^- concentration. Instead, if the detected F^- concentration was lower than the theoretical one, it is suggested that the fluoroaniline was partially defluorinated. As shown in Figure 2, compared to the theoretical F^- concentration, 200 mg/L of either 4-FA or 3-FA was nearly 100% defluorinated within 3 d by strain FD-1. However, other fluoroanilines including 2-FA, 2,4-DFA, 3,4-DFA, and 2,3,4-TFA were only slightly defluorinated by FD-1.

These results indicated that 3-FA as well as 4-FA can be employed by strain FD-1 as the sole carbon and nitrogen source and energy supply. However, 2-FA, 2,4-DFA, 3,4-DFA, and 2,3,4-TFA were difficult to be biodegraded by strain FD-1. These were corresponding to the limited reports on the microbial degradation of fluoroanilines [24].

3.2. Cometabolism Biodegradation of Fluoroanilines with 4-FA as the Growth Substrate. Since the first report by Leadbetter and Foster [25] and its bioremediation application by J.-T. Wilson and B.-H. Wilson [26], cometabolism has been used for degradation of reluctant organic pollutants [27, 28]. Due to nearly no degradation of 2-FA, 2,4-DFA, 3,4-DFA, and 2,3,4-TFA by strain FD-1, all of these were selected as secondary substrates, while 4-FA was added as the growth substrate.

3.2.1. Bacterial Growth. Bacterial growth under different cometabolism systems is illustrated in Figure 3. In the 2-FA cometabolism system, there were no differences of bacterial growth among the various concentration treatments. After 24 h of incubation, the maximum OD_{600} was achieved. However, bacterial growth in the presence of 400 mg/L of 2,4-DFA, 3,4-DFA, and 2,3,4-TFA was significantly lower than that in nonpresence of these three kinds of secondary substrates. The results suggested that higher than 400 mg/L of 2,4-DFA, 3,4-DFA, and 2,3,4-TFA would likely inhibit the growth of strain FD-1.

3.2.2. 4-FA Degradation. As the growth substrate, 4-FA is served as the main source of carbon, nitrogen, and energy

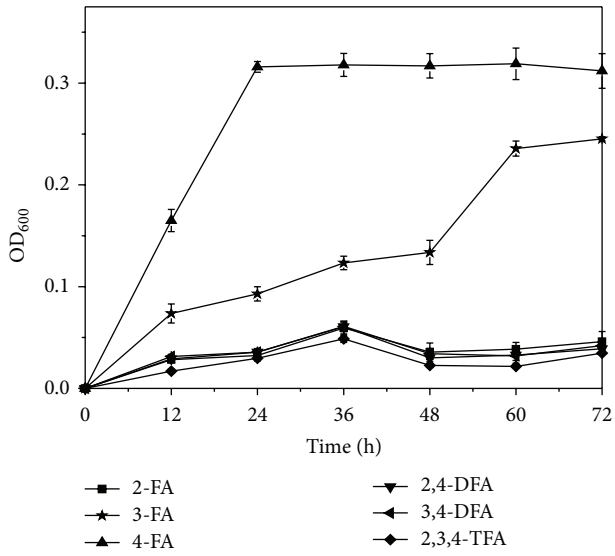

Figure 1: Bacterial growth in the presence of different fluoroanilines (200 mg/L) as the sole carbon and nitrogen source.

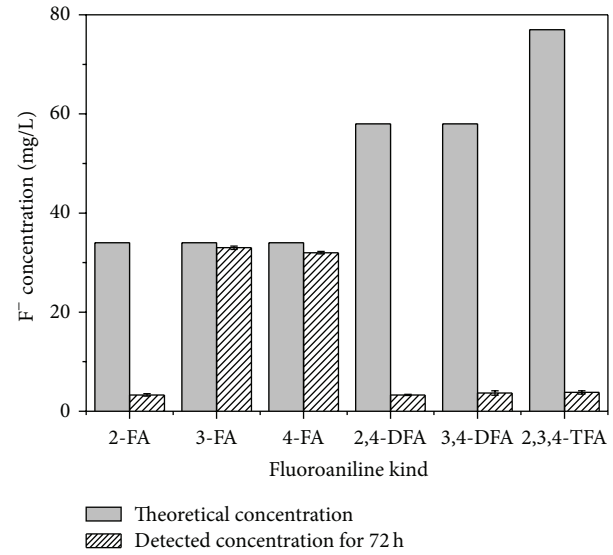

Figure 2: Fluorine ion produced from fluoroanilines as the sole carbon and nitrogen source for FD-1.

for strain FD-1 in the cometabolism systems. Thus, its degradation by strain FD-1 was analyzed, which was shown in Figure 4. In the 2-FA cometabolism system, 500 mg/L of 4-FA was completely degraded within 24 h in the presence of different concentrations of 2-FA (Figure 4). By comparison, 4-FA in other cometabolism systems was not completely degraded by strain FD-1 within 24 h, when 2,4-DFA, 3,4-DFA, and 2,3,4-TFA were present at concentrations higher than 200 mg/L. In the 2,4-DFA cometabolism system, 72.4% of 4-FA was degraded in the presence of 200 mg/L of 2,4-DFA within 24 h, while 36.1% of 4-FA was degraded in the

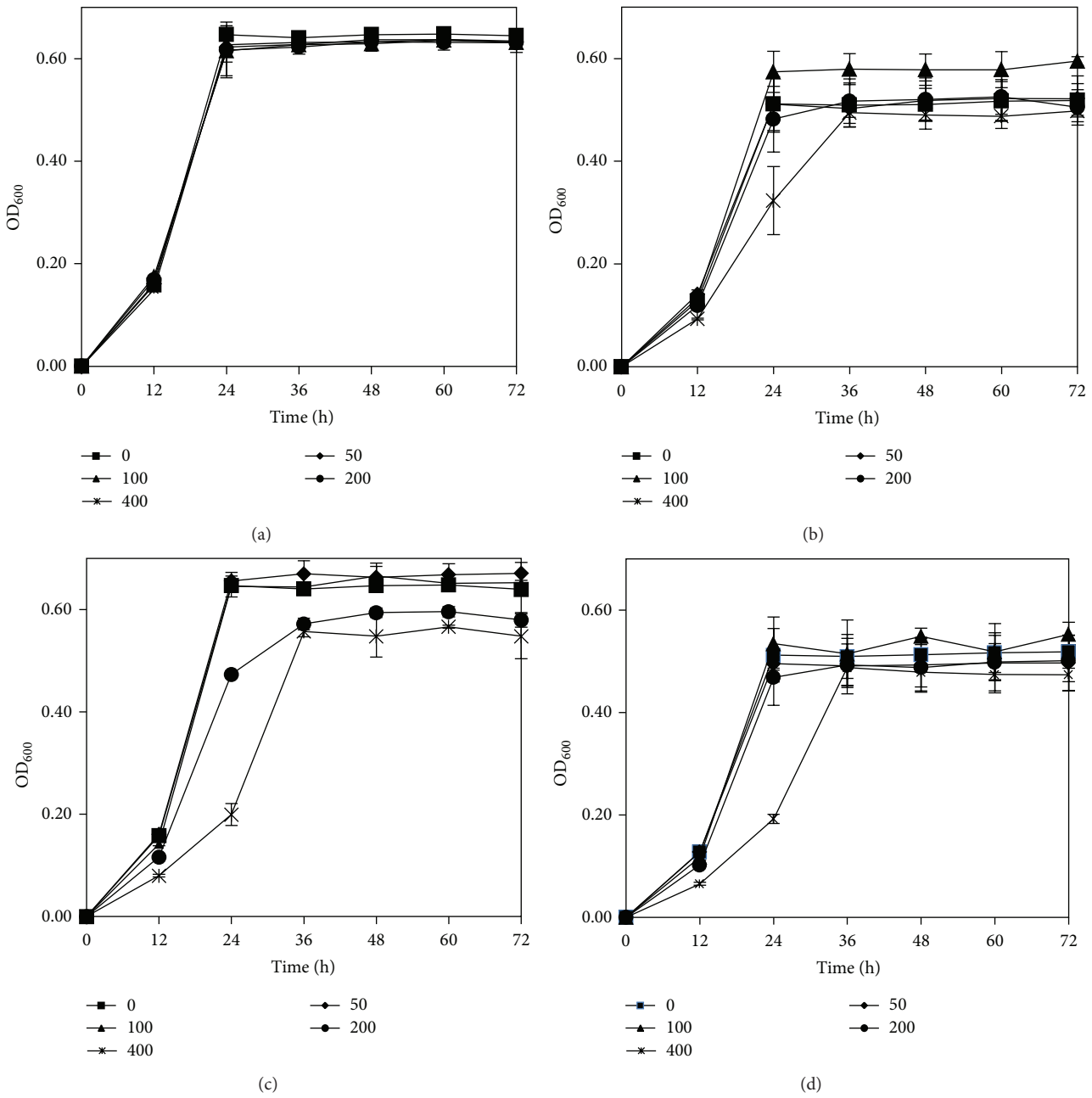

Figure 3: Bacterial growth of strain FD-1 in cometabolism systems. 4-FA served as the first-class substrate (also named growth substrate), while 2-FA (a), 2,4-DFA (b), 3,4-DFA (c), and 2,3,4-TFA (d) were the secondary substrates. Unit for legends is mg/L.

presence of 400 mg/L 2,4-DFA. Similar trends were observed for the 3,4-DFA and 2,3,4-TFA cometabolism systems. 4-FA was totally degraded after 48 h of incubation in all different cometabolism systems. This suggested that 500 mg/L of 4-FA was optimal for the cometabolism systems, while higher than 200 mg/L of 2,4-DFA, 3,4-DFA, and 2,3,4-TFA could disrupt the degradation of 4-FA by strain FD-1.

3.2.3. Fluoroaniline Cometabolism. Many fluoroanilines were employed as the secondary substrates by strain FD-1; their degradation drew our significant attention. This will affect the applied potential of strain FD-1 on fluorine contamination bioremediation. As shown in Figure 5, all four tested fluoroanilines (2-FA, 2,4-DFA, 3,4-DFA, and 2,3,4-TFA) could be efficiently cometabolically degraded by strain FD-1. After 72 h of cultivation, 79.65%, 80.27%, 78.28%, and 74.09% of 2-FA were degraded in the presence of 50, 100, 200, and 400 mg/L of 2-FA, respectively. Likewise, similar amounts of degradation were observed for 2,4-DFA cometabolic degradation by FD-1.

In addition, effective cometabolic degradation of 3,4-DFA and 2,3,4-TFA was also observed. Taking 72 h as an

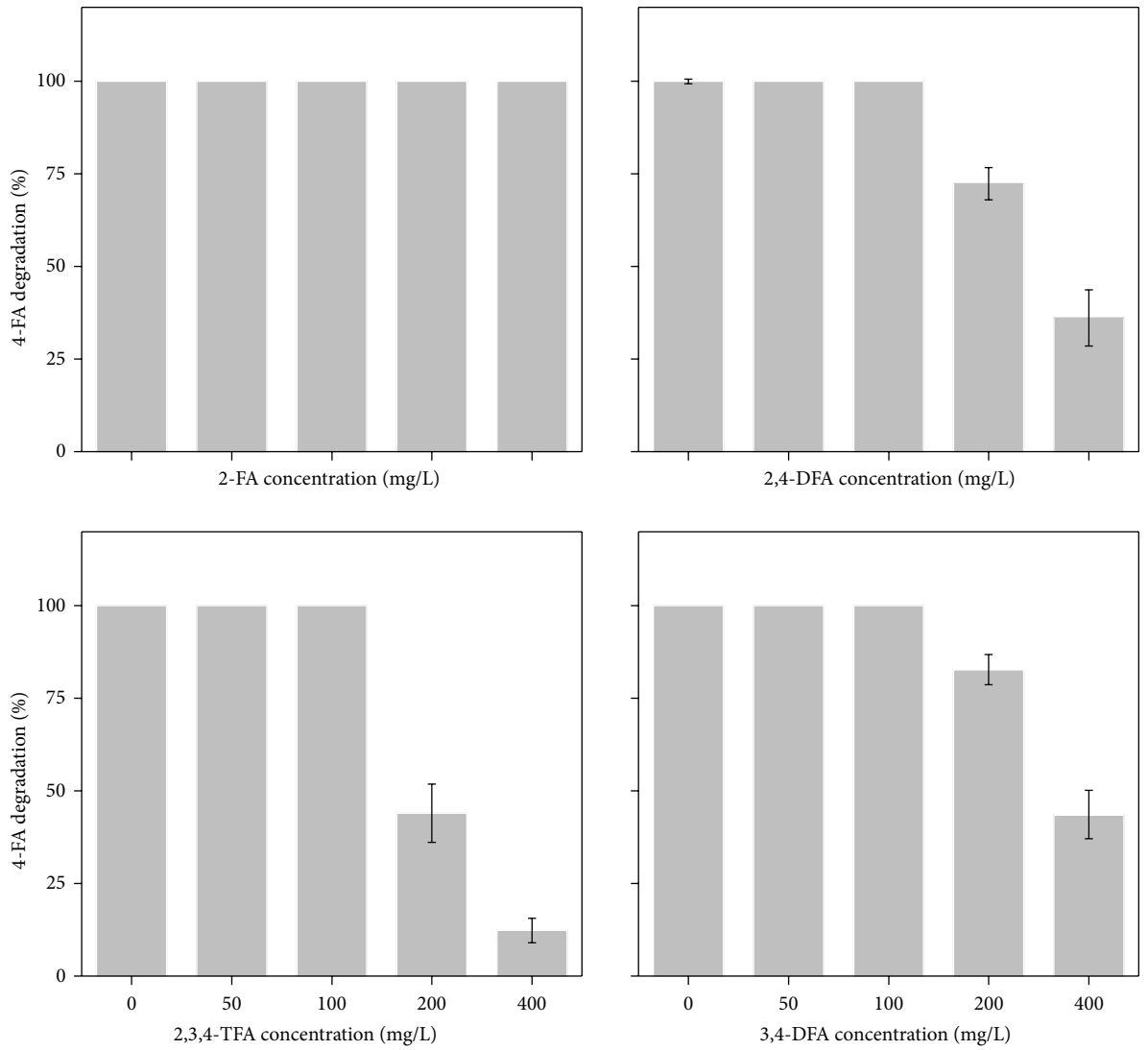

Figure 4: 4-FA degradation by strain FD-1 in cometabolism systems at 24 h.

example, 3,4-DFA degradation at 50, 100, 200, and 400 mg/L cometabolism treatments was 100.0%, 72.7%, 66.3%, and 48.9%, respectively. In the 2,3,4-TFA cometabolism system, 100%, 81.2%, 73.6%, and 71.3% of 2,3,4-TFA were degraded by strain FD-1 in the presence of 50, 100, 200, and 400 mg/L cometabolism treatments, respectively. Notably, with the increasing of 3,4-DFA and 2,3,4-TFA initial concentrations, their degradation decreased. This is possibly due to the varying toxicities of the different fluoroanilines toward strain FD-1 [29].

Taking the fluoroanilines removal degradation into account, strain FD-1 is very useful for fluoroaniline bioremediation by cometabolism with 4-FA as the growth substrates.

3.2.4. Defluorination. Cometabolism of most complicated organisms can be divided into two kinds: partial degradation or complete mineralization. The higher F^- concentrations detected generally mean easier subsequent treatment to these metabolites. Thus, the degree of defluorination was evaluated. As shown in Figure 6, all detected F^- concentration at 72 h was higher than the one only existence of 4-FA, but lower than the theoretic ones. It suggested that strain FD-1 could metabolize other fluoroanilines by partial degradation but not total mineralization. In addition, with the increasing of initial concentrations of 2-FA and 2,4-DFA, the detected F^- concentrations increased. Meanwhile, there were nearly no differences among different treatments in both 3,4-DFA and 2,3,4-TFA cometabolic systems. It was also suggested that the toxicity from 3,4-DFA and 2,3,4-TFA was higher than that from 2-FA and 2,4-DFA on strain FD-1, which was consistent in with a study about toxicity of fluoroanilines [30]. Notably, partial defluorination was detected. It means the fluorine substitution of the certain fluoroanilines was reduced into the solution. The more halogen substitutions a fluoroaniline has, the more toxic it usually is; the less halogen substitutions a fluoroaniline have, the easier it could be degraded [31].

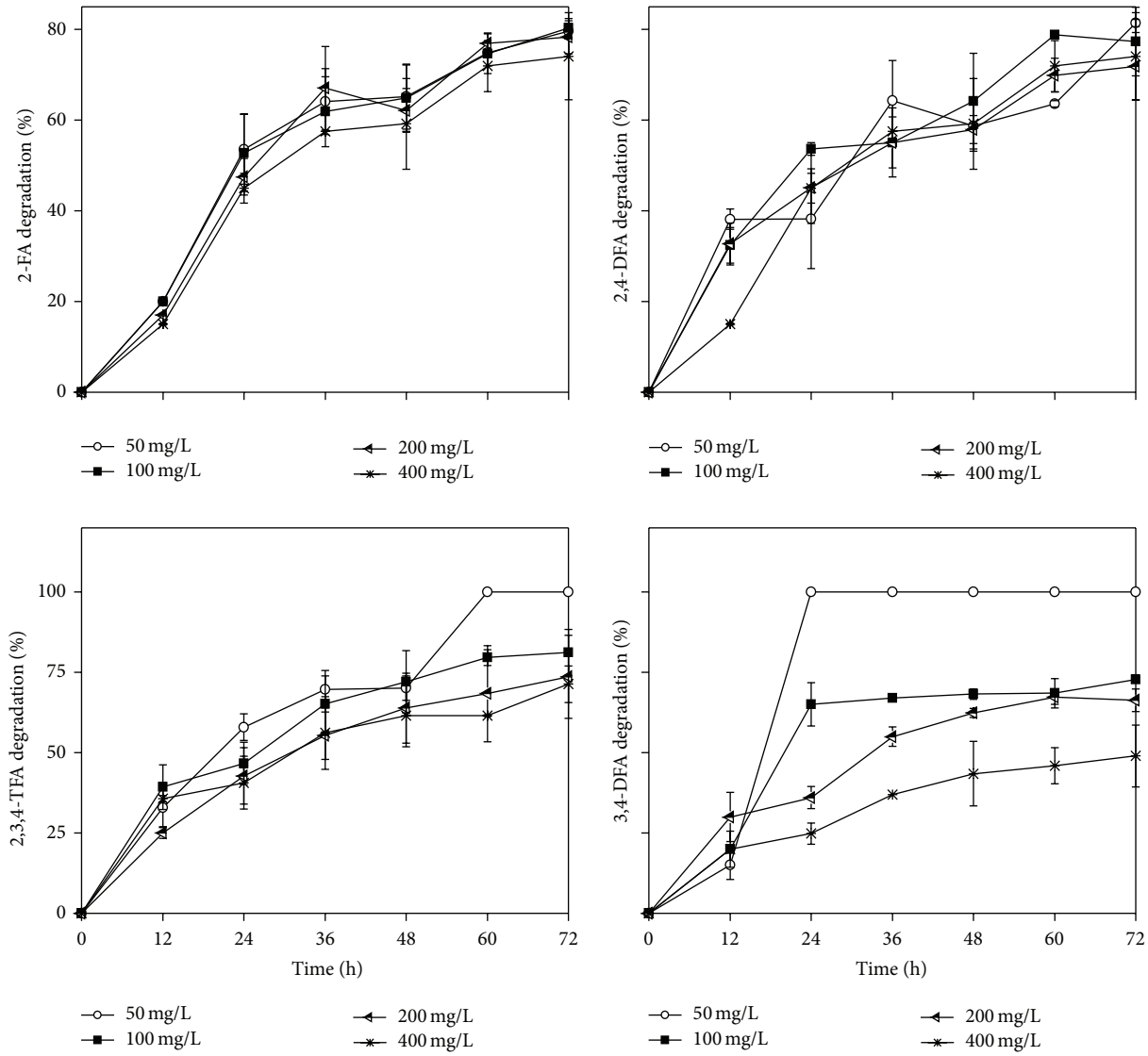

Figure 5: Second-class substrate fluoroaniline degradation in cometabolism systems.

Thus, taking the subsequent degradation of fluoroanilines into account, it was useful for strain FD-1 applied to the fluoroanilines bioremediation, even just biotransformation.

3.3. Resting Cells Removal of Fluoroanilines. In cometabolism systems, versatile bacteria usually synthesize enzymes in the presence of a growth substrate, and then the secondary substrates are degraded by the induced enzymes [32, 33]. Thus, strain FD-1 was incubated in 4-FA culture; after entering into logarithmic growth phase, resting cells were prepared for other fluoroanilines' removal. As shown in Figure 7, the degradation efficiency of 2-FA, 2,4-DFA, 3,4-DFA, and 2,3,4-TFA was 3,4-DFA < 2-FA < 2,4-DFA < 2,3,4-TFA in order. All fluoroanilines could be removed efficiently by resting cells. It suggested that, similar to other cometabolic systems, some induced enzymes played an important role in these cometabolic ones. In our previous study, catechol 2,3-dioxygenase was detected to be essential to 4-FA degradation [21]. 2,3-Dioxygenase was also found to play an important role in fluorobenzoic acid degradation by other bacteria [34]. According to Cardy et al. [35], most induced enzymes oxygenases play critical roles in the cometabolic process in aerobic cometabolism systems. It implied catechol 2,3-dioxygenase is the key induced enzyme for fluoroanilines degradation, which would be studied further in our lab.

Resting cells were a special kind of cells, which could process energy metabolism even on serious shortage of nutrition. It is known to us all that fluorine-containing wastewater was lacking nourishment and the period of cultivating bacteria was inefficient. Thus, the effective degradation of resting cells had broadened application prospect.

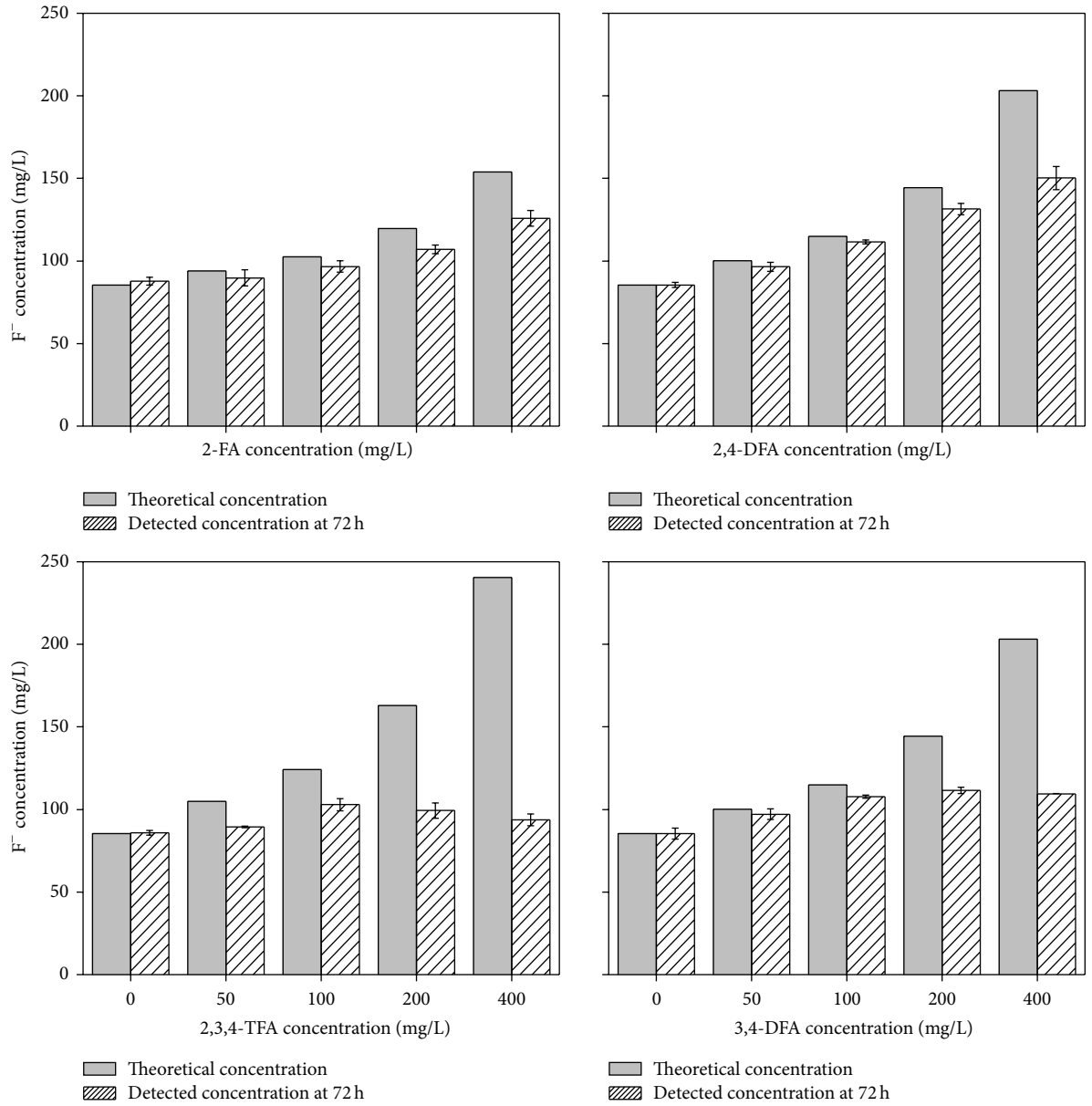

Figure 6: Fluorine ion concentration in cometabolism systems with 500 mg/L of 4-FA as the growth substrates.

4. Conclusions

The isolated strain FD-1 could use 4-FA and 3-FA as the sole carbon and nitrogen source and energy supply. However, the fluoroanilines, 2-FA, 2,4-DFA, 3,4-DFA, and 2,3,4-TFA, could not be used as the growth substrates but could be cometabolized as secondary substrates by *Ralstonia* sp. FD-1 with 4-FA as the growth substrate. With 4-FA as the growth substrate, 2-FA, 2,4-DFA, 3,4-DFA, and 2,3,4-TFA could be removed by strain FD-1. In addition, these fluoroanilines could be also removed by resting cells of strain FD-1 efficiently. Taking the toxicity of fluoroanilines and low nutrition in the fluoroanilines wastewater into account, strain FD-1 has great potential to fluoroanilines wastewater treatment.

Abbreviations

4-FA: 4-Fluoroaniline
2-FA: 2-Fluoroaniline
3-FA: 3-Fluoroaniline
2,4-DFA: 2,4-Difluoroaniline
3,4-DFA: 3,4-Difluoroaniline
2,3,4-TFA: 2,3,4-Trifluoroaniline.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

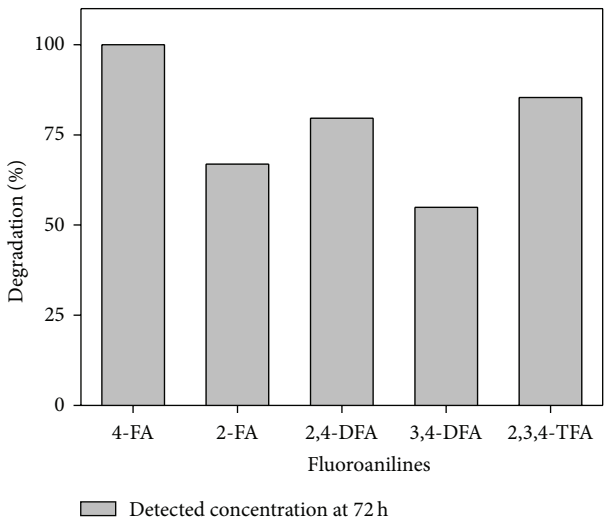

Figure 7: Fluoroanilines (200 mg/L) degradation by resting cells of strain FD-1.

Acknowledgment

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References

[1] R.-C. Buck, P.-M. Murphy, and M. Pabon, "Chemistry, properties, and use of commercial fluorinated surfactants," in *Polyfluorinated Chemicals and Transformation Products*, T. P. Knepper and F. T. and Lange, Eds., pp. 1–24, Springer, Berlin, Germany, 2012.

[2] D. O'Hagan, "Fluorine in health care: organofluorine containing blockbuster drugs," *Journal of Fluorine Chemistry*, vol. 131, no. 11, pp. 1071–1081, 2010.

[3] I. Ericson, R. Martí-Cid, M. Nadal, B. Van Bavel, G. Lindström, and J. L. Domingo, "Human exposure to perfluorinated chemicals through the diet: intake of perfluorinated compounds in foods from the Catalan (Spain) market," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 5, pp. 1787–1794, 2008.

[4] C.-A. Huset, A.-C. Chiaia, D.-F. Barofsky et al., "Occurrence and mass flows of fluorochemicals in the Glatt Valley Watershed, Switzerland," *Environmental Science and Technology*, vol. 42, no. 17, pp. 6369–6377, 2008.

[5] T. Frömel and T.-P. Knepper, "Biodegradation of fluorinated alkyl substances," *Reviews of Environmental Contamination and Toxicology*, vol. 208, pp. 161–177, 2010.

[6] J.-P. Giesy, J.-E. Naile, J.-S. Khim, P.-D. Jones, and J.-L. Newsted, "Aquatic toxicology of perfluorinated chemicals," *Reviews of Environmental Contamination and Toxicology*, vol. 202, pp. 1–52, 2010.

[7] B. D. Key, R. D. Howell, and C. S. Criddle, "Fluorinated organics in the biosphere," *Environmental Science and Technology*, vol. 31, no. 9, pp. 2445–2454, 1997.

[8] D.-H. Kim, C.-S. Jeon, K. Baek, S.-H. Ko, and J.-S. Yang, "Electrokinetic remediation of fluorine-contaminated soil: conditioning of anolyte," *Journal of Hazardous Materials*, vol. 161, no. 1, pp. 565–569, 2009.

[9] J. Liu and S. Mejia Avendaño, "Microbial degradation of polyfluoroalkyl chemicals in the environment: a review," *Environment International*, vol. 61, pp. 98–114, 2013.

[10] M.-M. Sun, Y.-M. Luo, Y. Teng, Z.-J. Jia, Z.-G. Li, and S.-P. Deng, "Remediation of polycyclic aromatic hydrocarbon and metal-contaminated soil by successive methyl-β-cyclodextrin-enhanced soil washing-microbial augmentation: a laboratory evaluation," *Environmental Science and Pollution Research*, vol. 20, no. 2, pp. 976–986, 2013.

[11] J.-F. Wu, C.-Y. Jiang, B.-J. Wang, Y.-F. Ma, Z.-P. Liu, and S.-J. Liu, "Novel partial reductive pathway for 4-chloronitrobenzene and nitrobenzene degradation in *Comamonas* sp. strain CNB-1," *Applied and Environmental Microbiology*, vol. 72, no. 3, pp. 1759–1765, 2006.

[12] B. Erable, I. Goubet, S. Lamare, M.-D. Legoy, and T. Maugard, "Bioremediation of halogenated compounds: comparison of dehalogenating bacteria and improvement of catalyst stability," *Chemosphere*, vol. 65, no. 7, pp. 1146–1152, 2006.

[13] K. Baek, M. Wang, R. McKeever, K. Rieber, C. Park, and K. Nüsslein, "Biodegradation of low concentrations of 1,2-dibromoethane in groundwater is enhanced by phenol," *Applied Microbiology and Biotechnology*, vol. 98, no. 3, pp. 1329–1338, 2014.

[14] D. O'Hagan, "Understanding organofluorine chemistry. An introduction to the C-F bond," *Chemical Society Reviews*, vol. 37, no. 2, pp. 308–319, 2008.

[15] M.-F. Carvalho, R.-F. Jorge, C.-C. Pacheco, P. de Marco, and P. M.-L. Castro, "Isolation and properties of a pure bacterial strain capable of fluorobenzene degradation as sole carbon and energy source," *Environmental Microbiology*, vol. 7, no. 2, pp. 294–298, 2005.

[16] B. Heffernan, C. D. Murphy, E. Syron, and E. Casey, "Treatment of fluoroacetate by a *Pseudomonas fluorescens* biofilm grown in membrane aerated biofilm reactor," *Environmental Science and Technology*, vol. 43, no. 17, pp. 6776–6785, 2009.

[17] M. B. Murphy, E. I. H. Loi, K. Y. Kwok, and P. K. S. Lam, "Ecotoxicology of organofluorous compounds," *Topics in Current Chemistry*, vol. 308, pp. 339–363, 2012.

[18] O. Suttinun, R. Müller, and E. Luepromchai, "Trichloroethylene cometabolic degradation by *Rhodococcus* sp. L4 induced with plant essential oils," *Biodegradation*, vol. 20, no. 2, pp. 281–291, 2009.

[19] Y. Morono, H. Unno, Y. Tanji, and K. Hori, "Addition of aromatic substrates restores trichloroethylene degradation activity in *Pseudomonas putida* F1," *Applied and Environmental Microbiology*, vol. 70, no. 5, pp. 2830–2835, 2004.

[20] A. H. Kwon, J. A. Conklin, M. Makhinson, and R. B. Kaner, "Chemical synthesis and characterization of fluoro-substituted polyanilines," *Synthetic Metals*, vol. 84, no. 1–3, pp. 95–96, 1997.

[21] E.-X. Song, M.-Z. Wang, and D.-S. Shen, "Isolation, identification and characterization of a novel *Ralstonia* sp. FD-1, capable of degrading 4-fluoroaniline," *Biodegradation*, vol. 25, no. 1, pp. 85–94, 2014.

[22] H.-Z. Tang, S.-N. Wang, L.-Y. Ma et al., "A novel gene, encoding 6-hydroxy-3-succinoylpyridine hydroxylase, involved in nicotine degradation by *Pseudomonas putida* strain S16," *Applied and Environmental Microbiology*, vol. 74, no. 5, pp. 1567–1574, 2008.

[23] M. Wang, G. Yang, X. Wang, Y. Yao, H. Min, and Z. Lu, "Nicotine degradation by two novel bacterial isolates of *Acinetobacter*sp. TW and *Sphingomonas* sp. TY and their responses

in the presence of neonicotinoid insecticides," *World Journal of Microbiology and Biotechnology*, vol. 27, no. 7, pp. 1633–1640, 2011.

[24] J. Zeyer, A. Wasserfallen, and K. N. Timmis, "Microbial mineralization of ring-substituted anilines through an ortho-cleavage pathway," *Applied and Environmental Microbiology*, vol. 50, no. 2, pp. 447–453, 1985.

[25] E.-R. Leadbetter and J.-W. Foster, "Bacterial oxidation of gaseous alkanes," *Archiv für Mikrobiologie*, vol. 35, no. 1, pp. 92–104, 1960.

[26] J.-T. Wilson and B.-H. Wilson, "Biotranformation of TCE in soil," *Applied and Environmental Microbiology*, vol. 29, pp. 242–243, 1985.

[27] V. Elango, H. D. Kurtz, and D. L. Freedman, "Aerobic cometabolism of trichloroethene and cis-dichloroethene with benzene and chlorinated benzenes as growth substrates," *Chemosphere*, vol. 84, no. 2, pp. 247–253, 2011.

[28] R.-S. Horvath, "Enhancement of Co metabolism of chlorobenzoates by the Co substrate enrichment technique," *Journal of Applied Microbiology*, vol. 25, no. 6, pp. 961–963, 1973.

[29] J. G. Bundy, E. M. Lenz, N. J. Bailey et al., "Metabonomic assessment of toxicity of 4-fluoroaniline, 3,5-difluoroaniline and 2-fluoro-4-methylaniline to the earthworm *Eisenia veneta* (rosa): identification of new endogenous biomarkers," *Environmental Toxicology and Chemistry*, vol. 21, no. 9, pp. 1966–1972, 2002.

[30] S.-K. Hong, D.-K. Anestis, T. T. Henderson, and G. O. Rankin, "Haloaniline-induced in vitro nephrotoxicity: effects of 4-haloanilines and 3,5-dihaloanilines," *Toxicology Letters*, vol. 114, no. 1-3, pp. 125–133, 2000.

[31] P. Hongsawat and A. S. Vangnai, "Biodegradation pathways of chloroanilines by *Acinetobacter baylyi* strain GFJ2," *Journal of Hazardous Materials*, vol. 186, no. 2-3, pp. 1300–1307, 2011.

[32] S. A. Ensign, M. R. Hyman, and D. J. Arp, "Cometabolic degradation of chlorinated alkenes by alkene monooxygenase in a propylene-grown *Xanthobacter* strain," *Applied and Environmental Microbiology*, vol. 58, no. 9, pp. 3038–3046, 1992.

[33] M. J. Nelson, S. O. Montgomery, and P. H. Pritchard, "Trichloroethylene metabolism by microorganisms that degrade aromatic compounds," *Applied and Environmental Microbiology*, vol. 54, no. 2, pp. 604–606, 1988.

[34] S.-A. Hasan, M. I.-M. Ferreira, M.-J. Koetsier, M.-I. Arif, and D.-B. Janssen, "Complete biodegradation of 4-Fluorocinnamic acid by a consortium comprising *Arthrobacter* sp. strain G1 and *Ralstonia* sp. Strain H1," *Applied and Environmental Microbiology*, vol. 77, no. 2, pp. 572–579, 2011.

[35] D. L.-N. Cardy, V. Laidler, G. P.-C. Salmond, and J. C. Murrell, "Molecular analysis of the methane monooxygenase (MMO) gene cluster of Methylosinus trichosporium OB3b," *Molecular Microbiology*, vol. 5, no. 2, pp. 335–342, 1991.