Top Curr Chem (2012) 308: 365–404
DOI: 10.1007/128_2011_270

Published online: 28 September 2011

Biology of Fluoro-Organic Compounds

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Abstract Investigations on diverse aspects of fluoro-organic compounds have rapidly increased during the past decades. Because natural sources of fluoro-organic compounds are extremely rare, the industrial synthesis of fluorinated organic compounds and production of fluorinated natural product derivatives have greatly expanded in recent years because of their increasing importance in the agrochemical and pharmaceutical industries. Due to structural complexity or instability, synthetic modification is often not possible, and various biofluorination strategies have been developed in recent years for applications in the anti-cancer, anti-viral and anti-infection fields. Despite the industrial importance of fluorinated compounds, there have been serious concerns worldwide over the levels and synthetic routes of certain fluorinated organic compounds, in particular perfluorinated chemicals (PFCs). PFCs are emerging and recalcitrant pollutants which are widely distributed in the environment and have been detected in humans and wildlife globally. PFCs have been demonstrated to be potentially carcinogenic, adversely affect the neuroendocrine and immune systems, and produce neurotoxicity, heptatotoxicity and endocrine disrupting effects in vertebrate animals. Here, we provide an overview of recent advances in our understanding of the biology of various fluoro-organic compounds and perspectives for new enzymes and metabolic pathways for bioremediation of these chemicals.

Keywords Biodegradation · Defluorogenase · Environmental toxicity · Fluorinase · Human health · Perfluorinated compounds · Polyfluorinated compounds

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Contents

1 Introduction

1.1 Natural Sources of Fluorinated Compounds

Fluorine exists naturally in the Earth's crust and is the most abundant halogen and the 13th most abundant element. Compared with other halogens, fluorine shows very low levels in surface water and exists mainly in an insoluble form (CaF_2) in nature, and thus has very little effects on the environment and biota. More than 4,000 natural products that contain chlorine, bromine, and even iodine have been reported in living organism, whereas only about a dozen fluorinated natural products have been isolated to date [1]. Fluoroacetate was the first natural organofluorinated compound to be identified in 1943 as a metabolite from *Dichapetalum cymosum* [2]. The low bioavailability of natural fluorinated compounds and fluorine's very low concentration in surface water may be due to its largely insoluble form (CaF_2). The fluoride ion has a high heat of hydration in aqueous solution, which thus limits its participation in displacement reactions. Fluorine cannot be transformed into organic substrates by haloperoxidases (which is a family of peroxidase enzymes that mediate the oxidation of halides by hydrogen peroxide) [3].

1.2 Biofluorination and Fluorinase

Fluorine substitution is widely used in pharmaceutical and agricultural applications because of the effects of fluorine on membrane permeability, metabolic stability, and receptor-binding properties [4, 5]. Because fluorinated products are extremely rare in nature, a number of methods have been developed for synthesis of fluorinated

compounds [6, 7]. However, the greatest progress has been in the generation of nonselective fluorinated products, which often cause toxicity and are difficult to handle. Selective incorporation of fluorine is challenging; therefore, development of biologically-based methods for fluorochemical production is needed.

Some enzymatic systems have been reported to utilize fluoride ions. For example, pyruvate kinase is known to catalyze the generation of fluorophosphate from ATP fluoride [8], and more recently, mutant glycosyl transferases were reported to fluorinate 2,4-dinitrophenyl-activated sugars to form α-fluoroglycosides [9, 10]. However, these reactions are adventitious or the intermediates are unstable. In 2002, the first fluorinase was reported in *Streptomyces cattleya* (O'Hagan et al. 2002), which uses *S*-adenosyl-L-methionine (SAM) and a fluoride ion as substrates to catalyze the formation of 5-fluoro-5-deoxyadenosine (5-FDA) and L-methionine (L-Met), which is the first step in the biosynthetic pathway of the fluorometabolites, fluoroacetate and 4-fluorothreonine (Fig. 1; Hagan et al. 2002). As the only native fluorination enzyme that has been identified so far, fluorinase was used to explore the syntheses of diverse fluorinated derivatives. For example, an engineered organofluorine biosynthetic metabolite that is a potent anticancer agent, fluorosalinosporamide, was produced by introducing a fluorinase gene (*flA*) into *Salinispora tropica* using recombinant DNA technology [12]. This study showed that selective fluorination of drugs and drug candidates could be expanded by inserting the *flA* gene into a variety of microorganisms to initiate the biosynthesis of novel organofluorine compounds.

In 2009, a chemo-enzymatic approach for selective fluorination was established whereby fluorine substitutions were used to produce a set of organic molecules including some prodrugs via a two-step regio- or stereo-selective procedure. The initial reaction is catalyzed by cytochrome P450 monooxygenases to insert oxygen selectively into non-reactive C–H bonds with deoxofluorination. The generated hydroxyl group was substituted by a nucleophilic fluorinating reagent, leading to selective fluorine substitution [13].

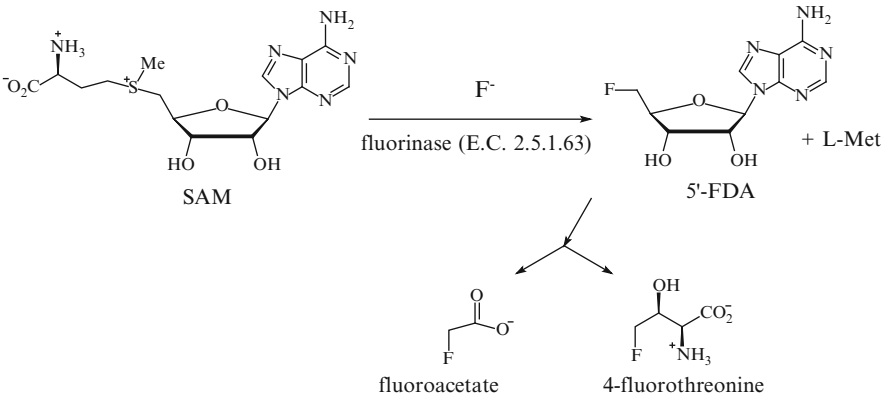

Fig. 1 The fluorinase enzyme of *S. cattleya* is the first committed step in the biosynthetic pathway to produce fluoroacetate and 4-fluorothreonine [11]

1.3 (Bio) Synthesis and Pharmaceutical Applications of Fluorinated Compounds

Burgeoning after the 1970s, the industrial synthesis of fluorinated organic compounds expanded because of their applications in pharmaceutical, agricultural, and other industrial areas. In medical applications, fluorine substitution often increases the hydrophobicity, metabolic stability, bioactivity, and bioavailability of molecules, thus improving their therapeutic indices. Medicinal production has focused on fluorinated drugs and drug candidates based on natural product analogs. While fluorinated natural products are very rare, the production of fluorinated natural product derivatives is increasingly common. Due to structural complexity or instability, synthetic modification is often not possible, and alternative strategies have been sought. In the past 20 years, synthetic methodologies in organic fluorine chemistry have focused on the biosynthesis of fluorinated analogs of natural products. Precursor-directed biosynthesis and mutasynthesis are two of the main industrial approaches for biosynthesis of fluorinated natural products. For example, fluorinated diazepinomicin analogs with modest anti-bacterial activity against *Staphylococcus aureus* have been generated through precursor-directed biosynthesis by supplementing *Micromonospora* cultures with various indole-related derivatives [14]. Using the mutasynthesis approach, auxotrophic strains of bacteria (which are unable to produce specific amino acids) have been successfully exploited to produce a number of fluorinated natural products [15]. For example, several new calcium-dependent antibiotics were produced by feeding 5-fluorotryptophan to a *Streptomyces coelicolor* tryptophan-auxotrophic strain [16].

Derivatives of anti-cancer drugs and other compounds such as the anti-inflammatory drugs fluorouracil and fluorocorticoids have been successfully biosynthesized. Other recent efforts have led to the development of fluorinated natural product derivatives, such as fluorine-substituted nucleosides, alkaloids, macrolides, steroids, amino acids, and prostaglandins, for applications in the anti-cancer, anti-viral, and anti-infection fields [15, 17]. Almost 20% of all pharmaceutical drugs on the market contain at least one fluorine atom, including the two best selling compounds, Lipitor (Atorvastatin; Fig. 2a), an inhibitor of cholesterol biosynthesis, and Advair Discus (a mixture of fluticasone (Fig. 2b) and salmeterol), a steroidal anti-inflammatory [18].

1.4 Perfluorinated Compounds

In industrial applications, fluorinated compounds, especially perfluorinated compounds perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA), play important roles in material science, including fluoropolymers, liquid crystals, and fire extinguishing products, due to their thermal and oxidative stability [19]. The phase-partitioning behavior of perfluoroalkanes makes them a prominent class of surfactants widely used in fire-fighting applications, herbicide and insecticide

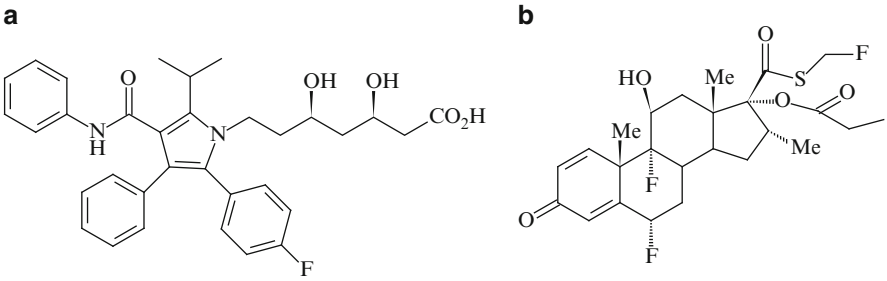

Fig. 2 Structures of the fluorine containing market leading pharmaceuticals. (**a**) Lipitor (Atorvastatin, (3*R*,5*R*)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid). (**b**) Advair Discus (a combination of fluticasone [*S*-(fluoromethyl) (6 *S*,8 *S*,9*R*,10 *S*,11 *S*,13 *S*,14 *S*,16*R*,17*R*)-6,9-difluoro-11,17-dihydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta [*a*] phenan-threne-17-carbothioate] and salmeterol-2-(hydroxymethyl)-4-{1-hydroxy-2-[6-(4-phenylbutoxy) hexylamino] ethyl} phenol) (O'Hagan 2010).

formulations, cosmetics, greases and lubricants, paints, polishes, and adhesives. In addition, poly/perfluorine derivatives are applied as oxygen carriers in blood substitutes [20]. Although production of many perfluorinated compounds such as PFOA and PFOS has ended in the USA and EU, these compounds are still produced in China and other developing countries.

1.4.1 Environmental Fate and Toxicity

Thousands of tons of fluorinated organic compounds have been emitted into the environment [19]. In recent years, concerns over the levels and synthetic routes of fluorinated organic compounds, especially perfluorinated compounds, have increased. Perfluorinated compounds show thermal, chemical, and biological stability, lipophilicity, worldwide distribution and accumulation in the atmosphere [21], river water [22], wildlife [22, 23], and in humans [24], which may lead to serious problems. The detection of organofluorines in wildlife and humans has been increasingly reported since 1968 [25, 26]. In 2003–2004, >99% of individuals sampled in one study in the US showed detectable PFOA in their serum [27]. In 2009, PFOS was included in Annex B of the Stockholm Convention on Persistent Organic Pollutants.

1.4.2 Fluorinated Compounds and Human Health

While fluorine is regarded as an essential element and is beneficial to human health at low concentrations, the environmental distribution of fluorinated organic compounds is dangerous to humans due to their bioaccumulation and potential impacts on metabolism. During the last two decades, concerns about the toxicity of fluorinated organic compounds, especially perfluorinated compounds, have increased.

Most toxicological studies on PFCs have been conducted on rats or monkeys. In animal research, common PFCs such as PFOA and PFOS have been demonstrated to be potentially carcinogenic, to affect the neuroendocrine and immune systems, to cause neurotoxicity and hepatotoxicity, and to reduce serum cholesterol and triglycerides [28–30]. Effects on gestational and developmental toxicity were also confirmed [31]. In vitro studies on human cells also demonstrated the toxicity of PFCs on DNA integrity, intracellular organelles, and hormones ([32]; Vanden Heuvel et al. 2006; [33]). In population studies, some PFCs were reported to act as hormone disruptors and thus to affect human fecundity [34]. Human fetal birth weight was also reported to be impaired by background exposure to PFOA [35]. Additionally, exposure to PFCs causes altered hepatic function, immune function, thyroid function, and cholesterol metabolism, and has carcinogenic potential in humans [36].

2 Biodegradation of Organofluorinated Compounds

Biodegradation is the chemical dissolution of materials by bacteria or through other biological means. Over the years, scientists and engineers have developed a number of bioremediation and biotransformation methods to degrade, transform, or accumulate a huge range of man-made contaminants. A great variety of microbes such as *Burkholderia*, *Rhodococcus*, *Pseudomonads*, *Aspergillus*, and *Beauveria* have shown an extraordinary capability to degrade artificial pollutants such as hydrocarbons (e.g., oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), heterocyclic compounds (such as pyridine or quinoline), and pharmaceutical substances [37]. Biodegradation by microorganisms is perhaps one of the most effective methods to remove organic pollutants from the environment and has attracted considerable interest in bioremediation of organofluorinated compounds.

Although fluoroorganic compounds are well known for their inertness and contain the strong C–F bond, some organisms such as bacteria, fungi, algae, and even vertebrates can still biotransform and biodegrade fluoroorganic compounds because of the steric size similarity between fluorine, hydrogen, and hydroxyl groups. To date, little is known about the bacterial metabolism of fluoroorganic compounds, even though several reports have been published on the degradation of monofluorinated compounds. In 1954, the first report on biological defluorination described fluoride elimination of *p*-fluoroaniline using a horseradish peroxidase. Fluoroaliphatics such as fluoroacetate can be degraded with monofluoroacetate dehalogenase (*Pseudomonas indoloxidans*, *Pseudomonas cepacia, Moraxella* sp., *Burkholderia* sp., etc.) and biodegradation of trifluoroacetic acid has also been reported [38, 39]. Fluoroaromatic compounds can be biodegraded aerobically and anaerobically. However, the biodegradation pathways of perfluorinated chemicals are still not known.

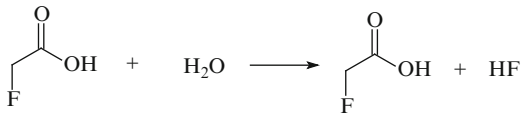

Fig. 3 Hydrolytic defluorination of fluoroacetate [40]

2.1 Fluoroaliphatics

2.1.1 Fluoroacetate

Fluoroacetate is one of the most highly toxic compounds for mammals [40]. The dissociation energy of its C–F bond is among the highest found in natural products [41]. The presence of fluoroacetate in the environment and biota results from its industrial use as a vertebrate pest control agent as well as from metabolites of other compounds such as fluoroacetamide, which is used to control rodents, the anticancer drugs 5-fluorouracil and fluoroethyl nitrosourea, and the industrial chemical fluoroethanol [42].

Microbial defluorination of fluoroacetate was first reported in 1961 [43], followed by reports of the first enzymatic release of fluoride ion from fluoroacetate in both vertebrates and bacteria [44]. A wide variety of microorganisms such as *Moraxella*, *Pseudomonas*, and *Burkholderia* were isolated and shown to be capable of defluorinating fluoroacetate [39, 45]. Fluoroacetate dehalogenases have been characterized in *Pseudomonas* strains as well as other bacteria for decades (Fig. 3) [46–48]. Microbial degradation of fluoroacetate is now well understood at the mechanistic level. Two possible mechanisms were delineated from the enzyme reaction [49]. The ester intermediate pathway has been examined for fluoroacetate dehalogenases and other enzymes such as rat liver microsomal epoxide hydrolase [45, 50–52]. The carboxylate group of the aspartate residue at the active site acts as a nucleophile and first attacks the α-carbon atom of fluoroacetate to displace the fluorine atom, leading to the release of a fluoride ion. An ester intermediate is formed, which is subsequently hydrolyzed by a water molecule activated by a histidine residue, thereby regenerating the carboxylate group of the aspartate molecule [53].

2.1.2 Fluoropyruvate

Fluoropyruvate is often used in the laboratory as an inhibitor to inactivate pyruvate carboxylase, lactate dehydrogenase, and the pyruvate dehydrogenase complex [54]. In recent years, there has been increasing focus on the use of 3-halopyruvate as an anti-cancer agent because it acts as an irreversible inhibitor of metabolic enzyme(s) associated with glycolysis. For example, it has been demonstrated that 3-bromo pyruvate shows high in vivo toxicity on tumors but has no adverse effect on healthy tissue [55]. In 1978, a pyruvate dehydrogenase component of *Escherichia coli* that catalyzes the conversion of 3-fluoropyruvate to acetate and fluoride ions was reported [56]. Fluoride is eliminated by β-elimination, which is the classical

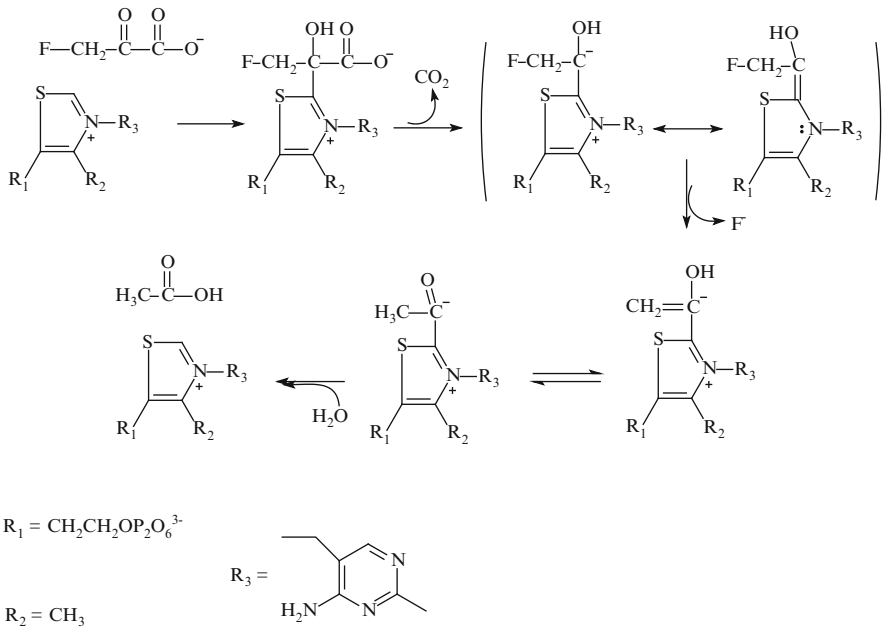

Fig. 4 Proposed enzymatic defluorination of 3-fluoropyruvate [38]

mechanism for dehydrogenases (Fig. 4). Recently, ^{19}F NMR spectroscopy studies demonstrated the conversion of fluoropyruvate to fluoroacetate by *D. cymosum*, where fluoroacetate is mineralized followed by the release of fluoride [57].

2.1.3 Maleylacetate

Fluorinated maleylacetates have been investigated as substrates of maleylacetate reductase for a number of years [58–61]. A maleylacetate reductase enzyme was first isolated in 1995 from *Pseudomonas* sp. strain B13 that catalyzes the halo-elimination of 2-fluoromaleylacetate as well as other halomaleylacetates (Fig. 5). This enzyme consumes two moles of NADH per mole of maleylacetate that contains a fluorine substituent in the 2-position, while only one mole of NADH is required for halide elimination in substrates without a fluorine substituent in the 2-position [58].

2.1.4 Fluorinated Cycloalkyl *N*-Phenylcarbamates

Fluorine substitution of a hydrocarbon position in fluorinated cycloalkyl *N*-phenylcarbamates occurs in hydroxylation reactions by *Beauveria bassiana*, a soil-borne filamentous fungus. The hydroxylation of 4-*cis*-fluorocycloalkyl

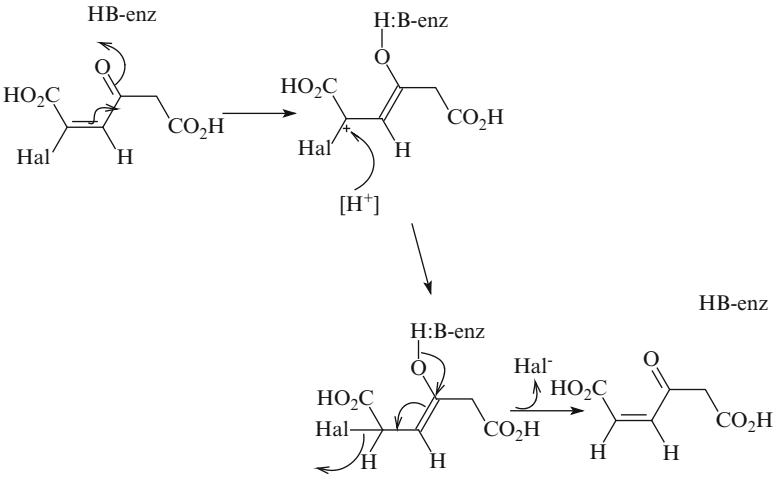

Fig. 5 Proposed mechanism for the elimination of halogen substituents from the 2-position of maleylacetate [58]

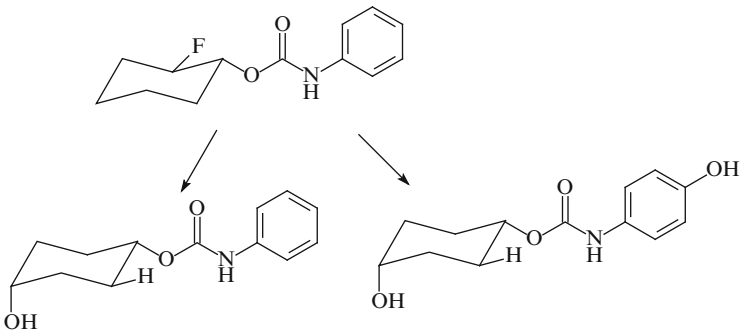

Fig. 6 Defluorination of trans-2-fluorocycloalkyl *N*-phenylcarbamate by *Beauveria bassiana* [63]

N-phenylcarbamates probably produces terminal fluorohydrins, which are not stable and thus are subsequently dehydrofluorinated to give the corresponding ketones [62]. Recently, the defluorination of *trans*-2-fluorocycloalkyl *N*-phenylcarbamate by *B. bassiana* was also reported, in which fluorine elimination could occur either via hydroxylation of the six member ring at C-4 or *p*-hydroxylation of the aromatic ring (Fig. 6).

2.1.5 Fluorinated Carbohydrates

Fluorinated carbohydrates have a broad range of pharmaceutical and biomedical applications ranging from metabolic and biochemical studies to disease diagnoses. Replacement of a hydroxyl group with a fluorine atom in carbohydrates can affect

their metabolic and biochemical behavior, including enzyme-carbohydrate interactions, lectin-carbohydrate affinities, antibody-carbohydrate binding [64, 65], and application in positron emission tomography for cancer diagnosis [66]. Therefore, fluorinated compounds are important reagents in metabolic studies and for disease diagnoses. The microbial catalytic defluorination of fluoromonosaccharides has been reported [67, 68]. Expression of a 65.5 kDa membrane protein is induced by 4-deoxy-4-fluoro-D-glucose (4-FG) or glucose and is associated with the active D-glucose transporter system in *Pseudomonas putida* [68]. *P. putida* defluorination of fluoro-D-glucose is stereospecific. In addition, 4-FG is converted to 2,3-dideoxy-D-glycero-pentonic acid with fluoride elimination while 3-deoxy-3-fluoro-D-glucose (3-FG) is metabolized without defluorination. Electron donors such as L-malate are required in these defluorination metabolic pathways [69].

2.2 *Fluoroaromatics*

Fluoroaromatics are widely used in industry as intermediates or end-products in the synthesis of pharmaceuticals, insecticides, plastics, and molecules related to liquid crystal technology [15, 70]. The broad applications of fluoroaromatics have led to their accumulation in the environment. Their widespread occurrence and potential toxicity have led to increasing interest in biodegradation and treatment processes for fluoroaromatics.

2.2.1 Fluorobenzoates

As model compounds of other fluoro-substituted aromatic compounds, fluorobenzoates have been widely used to study bacterial metabolism of fluorinated aromatics. For example, bacteria such as *Pseudomonas* [71, 72], *Xanthobacter* [73], and *Sphingomonas* [74] have been reported to exhibit fluorobenzoate degradation. In addition, the metabolism of 2-, 3-, and 4-fluorobenzoic acid has been well studied [71, 74, 75]. Using $^{18}O_2$, *Pseudomonas* sp. was shown to form catechol from 2-fluorobenzoic acid by incorporation of two oxygen atoms from a single dioxygen molecule. This defluorination proceeds through a cyclic peroxide intermediate. In the major pathway, 1,2-dioxygenation of 2-fluorobenzoic acid leads to an unstable fluorohydrin, which is then defluorinated to catechol. Muconate is finally formed, which subsequently goes in the TCA cycle to produce energy (Fig. 7, pathway a). The minor pathway, 1,6-dioxygenation, also takes place, leading to the formation of 3-fluorocatechol and then 2-fluoro-*cis*-*cis*-muconate (Fig. 7, pathway b) [75]. 3-Fluorobenzoate is degraded by 1,2-dioxygenation to yield fluorocatechol, which is metabolized to 2-fluorobenzoic acid in the minor pathway (Fig. 7, pathway c) [74, 75]. The predominant pathway of 3-fluorobenzoate includes a 1,6-dioxygenation reaction to yield fluoromuconic acids. Defluorination then occurs to yield muconate [74] (Fig. 7, pathway d).

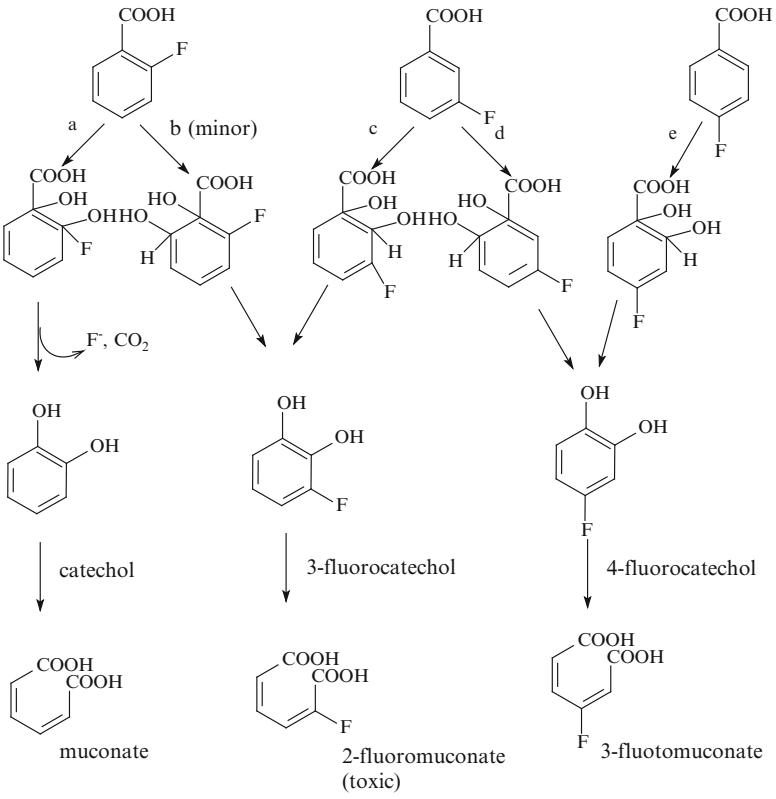

Fig. 7 Metabolism of 2-, 3-, and 4-fluorobenzoic acid [38]

4-Fluorobenzoate is degraded by *Pseudomonas* sp. in similar pathways to 3-fluorobenzoate (Fig. 7, pathway e) [75, 76].

The anaerobic degradation of monofluorobenzoates under various electron-accepting conditions including denitrifying, sulfate-reducing, iron-reducing, and methanogenic conditions has also been studied [77–80]. After long-term incubation, 2-fluoro- and 4-fluorobenzoates are degraded by *Pseudomonas* with fluoride elimination [79]. Recently, dehalogenated 3-fluorobenzoate was investigated in *Syntrophus aciditrophicus* culture. Two hydrogen atoms are added to 3-fluorobenzoate to form a 3-fluorocyclohexadiene metabolite, leading to stoichiometric accumulation of benzoate and fluorine [80].

2.2.2 Fluorophenols

Fluorophenolic compounds are widely used in agricultural industries as herbicides, insecticides, and fungicides [81]. Fluorophenols are transferred to fluorocatechols and fluoromuconates via microbial degradation [82]. The fluorophenol

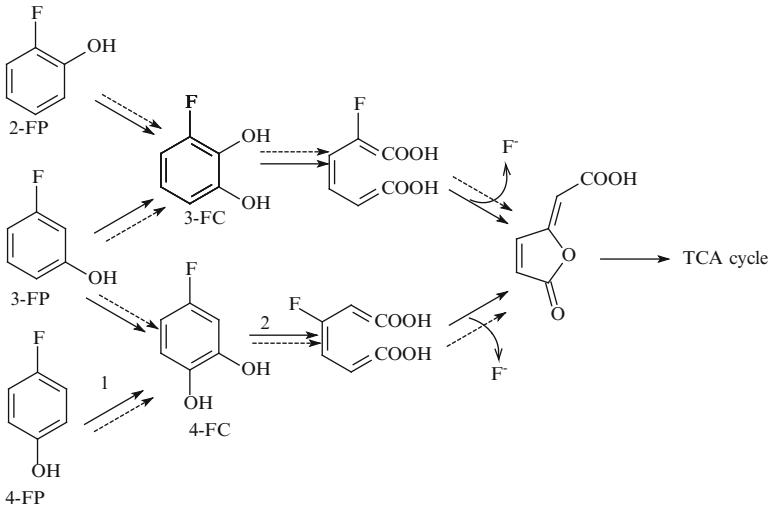

Fig. 8 Pathways for defluorination of fluorinated phenols by *P. benzenivorans*. *Dashed arrows* show the known monofluorophenols pathway for comparison. *1*: phenol hydroxylase, *2*: catechol 1,2-dioxygenase [83]

metabolites of *Exophiala jeanselmei*, a yeast-like fungus, which are converted by the phenol hydroxylase and catechol 1,2-dioxygenase enzymes, have been characterized by ^{19}F NMR spectroscopy. The conversion of fluorophenols (i.e., 3-fluoro-, 4-fluoro-, and 3,4-difluorophenol) via catechol 1,2-dioxygenase involves two common steps [81, 83]: (1) the introduction of *ortho*-hydroxyl groups and (2) ring cleavage by catechol dioxygenase. The resulting muconates and accumulation of stoichiometric amounts of fluoride anions have been detected (Fig. 8).

2.2.3 Fluorotoluene

3-Fluorotoluene was reported to be accumulated and co-metabolized by *Cladosporium sphaerospermum*, a fungi culture grown on toluene [84]. ^{19}F NMR was used to determine the catabolic pathway. A methyl group is first oxidized by the toluene monooxygenase enzyme followed by ring hydroxylation to form fluoroprotocatechuate. The remaining steps include decarboxylation of the fluoroprotocatechuate followed by *ortho*-cleavage (Fig. 9).

2.2.4 Fluorobiphenyls

Fluorobiphenyls can be co-metabolized via the classical aromatic degradation pathways by fungi and bacteria [85–87]. Recently, the degradation pathway of 4,4-difluorobiphenyl was proposed. The hydrolase BphD catalyzes the transformation from 3-fluoro-2-hydroxy-6-oxo-6-(4-fluorophenyl)-hexa-2,4-dienoate to 3-fluoro-2-hydroxypenta-2,4-dienoate. Then, (*Z*)-3-fluoro-2-oxo-pent-3-enoate is

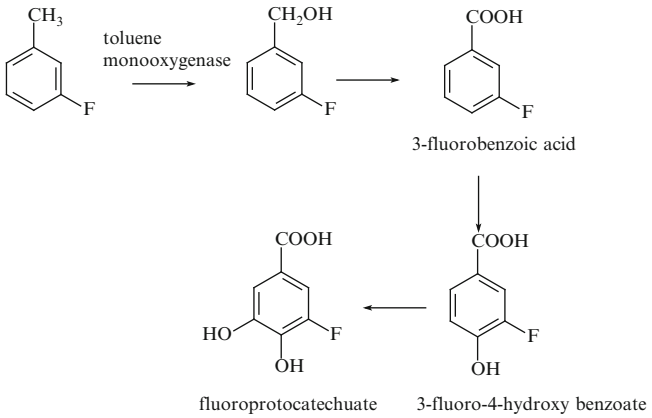

Fig. 9 Proposed fungal catabolism of fluorotoluene [38]

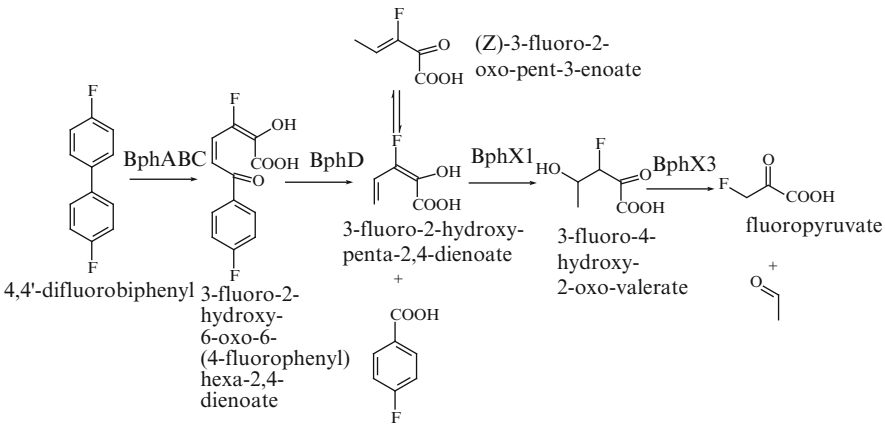

Fig. 10 Proposed catabolism of 4,4-difluorobiphenyl along the upper and lower pathways. *BphA* biphenyl 2,3-dioxygenase; *BphB* dehydrogenase; *BphC* 2,3-dihydroxybiphenyl 1,2-dioxygenase; *BphD* 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrolase; *BphX1* 2-hydroxypenta-2,4-dienoate hydratase; *BphX3* 4-hydroxy-2-oxovalerate hydrolase [88]

formed and further catabolized, eventually yielding acetaldehyde and fluoropyruvate (Fig. 10) [88].

2.2.5 Fluorophenylacetic Acid

The defluorination of *p*-fluorophenylacetic acid by *Pseudomonas* has been studied [76]. First, the aromatic ring is cleaved between C-1 and C-2. Then, C-2 is further modified by two alternative pathways. Hydrolyzation occurs to give 3-hydroxy-3-fluoroadipic acid. Fluorine elimination occurs and yields β-ketoadipic acid (Fig. 11, pathway a). Alternatively, after lactonization and formation of 4-carboxymethyl-

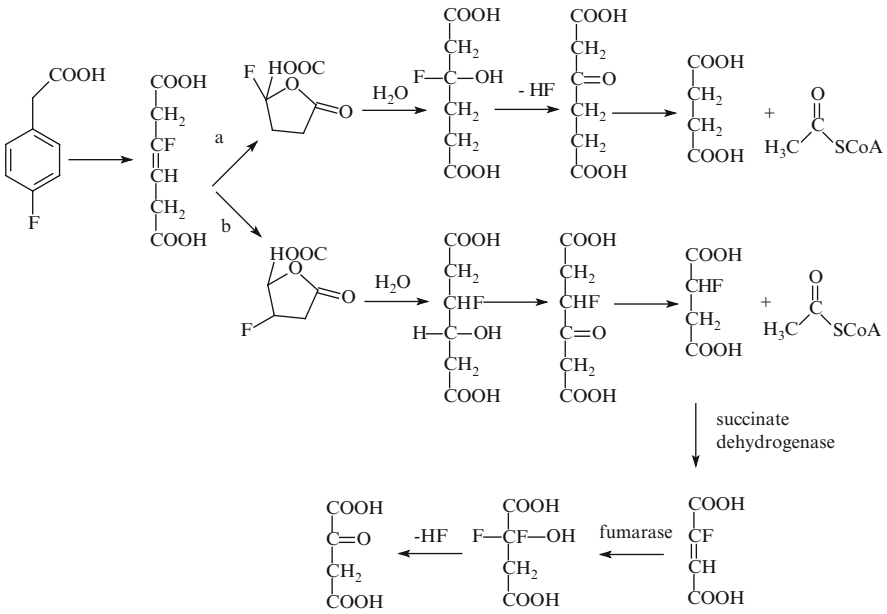

Fig. 11 Degradative pathways for 3-fluoro-3-hexenedioic acid [76]

3-fluoro-butanolide, hydrolyzation and cleavage of C–C bonds yield acetate and monofluorosuccinic acid (Fig. 11, pathway b). The latter compound is converted to oxaloacetate and hydrogen fluoride.

2.2.6 Fluorobenzene

A microbial consortium containing *Sphingobacterium*, *Flavobacterium*, and *β-Proteobacterium* was shown by Carvalho et al. in 2002 [89] to be capable of defluorinating fluorobenzene. In addition, a bacterial strain from the *Labrys portucalensis* group that uses fluorobenzene as a sole carbon and energy source has been purified [90]. The degradation of fluorobenzene via *ortho* cleavage of 4-fluorocatechol and catechol by *Rhizobiales* strain F11 has been investigated by Carvalho et al. in 2006 [91]. It was found that the initial attack on fluorobenzene by a dioxygenase enzyme could lead to two different pathways. In one pathway, a dihydrodiol dehydrogenase enzyme (step 1) transforms 4-fluoro- *cis*-benzene-1, 2-dihydrodiol to 4-fluorocatechol. In the second pathway (step 10), 1-fluoro-*cis*-benzene-1,2-dihydrodiol is converted to catechol (Fig. 12).

2.2.7 Fluoroquinolones

Fluoroquinolones are some of the most widely used antimicrobial agents for treating both Gram-negative and Gram-positive infections. Their widespread

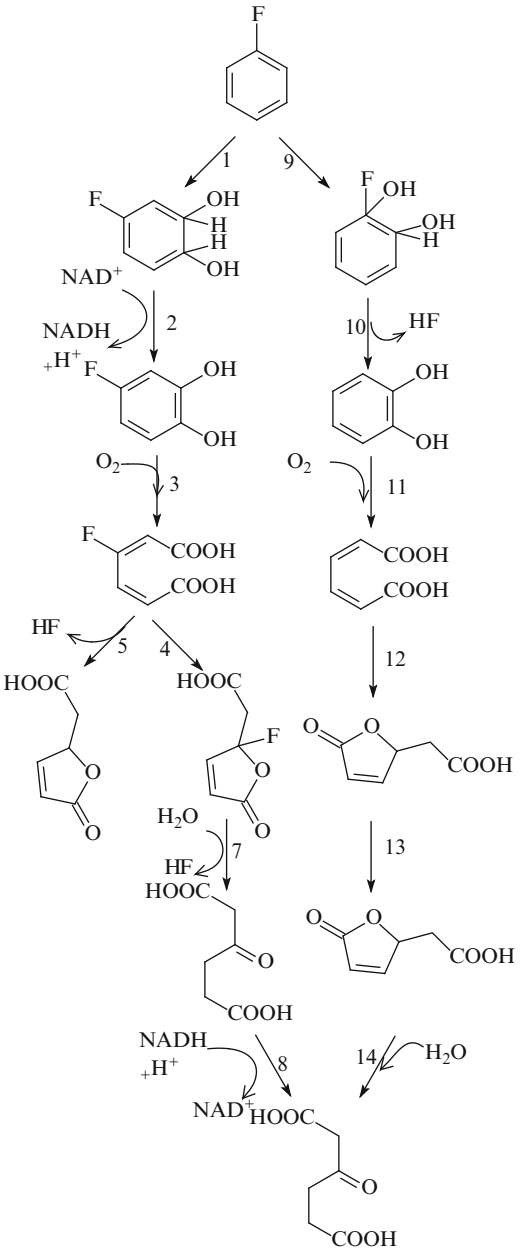

Fig. 12 Proposed pathway for fluorobenzene metabolism. The enzyme activities are denoted as follows: *1*: fluorobenzene dioxygenase; *2*: fluorobenzene dihydrodiol dehydrogenase; *3*: fluorocatechol 1,2-dioxygenase; *4*: fluoromuconate cycloisomerase; *5* and *6*: possible side reactions to *cis*-dienelactone by fluoromuconate cycloisomerase (activity 5) or by slow spontaneous conversion (activity 6); *7*: *trans*-dienelactone hydrolase; *8*: maleylacetate reductase; *9*: fluorobenzene dioxygenase; *10*: nonenzymatic defluorination; *11*: catechol-1,2-dioxygenase; *12*: muconate cycloisomerase; *13*: muconolactone isomerase; *14*: 3-oxoadipate enol-lactone hydrolase [91]

presence has been detected at multiple locations around the world [92]. Other reports have suggested their potential toxicity to plants and aquatic organisms [93, 94]. Many clinically relevant bacterial species including *S. aureus* and *Pseudomonas aeruginosa* are capable of developing resistance to quinolones [95].

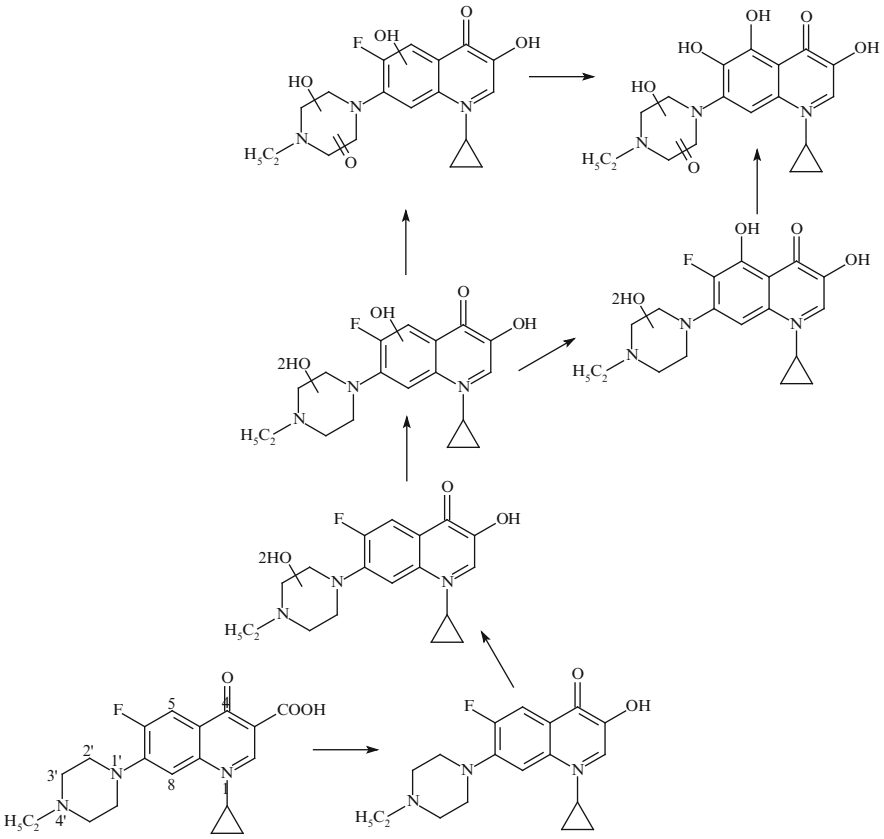

Fig. 13 Degradation of enrofloxacin by basidiomycetous fungi [97]

Degradation of the fluoroquinolone, enrofloxacin, was observed in *Gloeophyllum striatum*, a brown rot fungus where a hydroxyl radical attacks fluorine at the C-6 position to form 6-hydroxyen-rofloxacin which is further hydroxylated to 5,6- and 6,8-dihydrox-yenrofloxacin [96]. The metabolism of enrofloxacin by seven basidiomycetous fungi from agricultural sites was recently reported by Wetzstein et al. [97]. Oxidative decarboxylation of enrofloxacin first occurs, then defluorination takes place in multiply hydroxylation and acetylation steps (Fig 13) [97].

2.2.8 Fluorinated Anilines

Microsomal NADPH-dependent reaction pathways for biodehalogenation of fluorinated anilines have been investigated [98]. Three possible pathways for dehalogenation of fluorinated anilines, such as 2-fluoro-4-hydroxyaniline and pentafluoroaniline, in the presence of xanthine glutathione and NADPH were

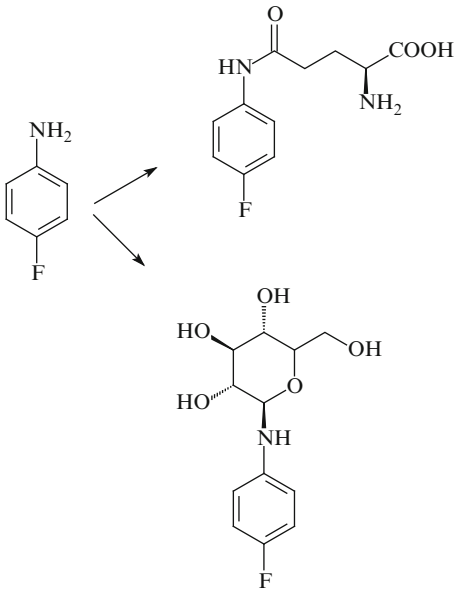

Fig. 14 Metabolism of 4-fluoroaniline in *Eisenia veneta* [100]

proposed. A study of the metabolism of 3,4-difluoroaniline with *Pseudomonas fluorescens* 26-K showed the formation of 3-fluoro-4-hydroxyaniline and the release of a fluoride ion [99]. Recently, biotransformation of 4-fluoroaniline was observed in the earthworm *Eisenia veneta*. The catabolic products were analyzed using 19-F NMR, but no fluoride ion was detected (Fig. 14) [100, 101].

2.3 Biodegradation of Polyfluorinated Compounds

The degradation of polyfluorinated compounds, such as fluorotelomer alcohols (FTOHs), fluorotelomer ethoxylates, and polyfluoroalkyl phosphates, in atmospheric and aqueous systems has been established and has been reported to be a source of perfluorinated carboxylic acids (PFCAs). However, published information on the biodegradation of PFCAs is very limited. The aerobic and anaerobic biodegradability of three fluorinated surfactants have been described [102]. However, no release of fluoride has been found.

2.3.1 Fluorotelomer Alcohols

FTOH is the generic name of fluorinated compounds that contain even-numbered fluorocarbon chains and an ethanol moiety [103]. FTOHs are used in fire-fighting foams, grease-resistant food packaging, leather protectants, and stain-resistant carpeting and textiles. In addition, FTOHs are used industrially to generate acrylate

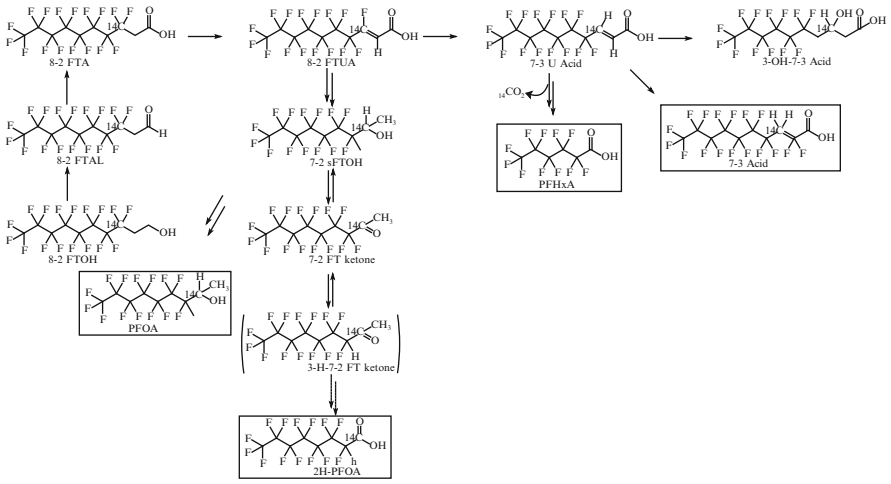

Fig. 15 Proposed biodegradation pathways of 8–2 FTOH [109]

polymers and as intermediates in the production of fluorinated surfactants. Consequently, FTOHs are widely detected in air. Furthermore, estrogen-like properties have been reported for these compounds [104].

8–2 FTOH degradation was first reported in detail in reactions catalyzed by a mixed microbial consortium [105–109]. Based on ^{14}C analysis, 8–2 FTOH biodegradation in aerobic soils was proposed (Fig. 15). 8–2 FTOH is converted rapidly to 8–2 fluorotelomer aldehyde (FTAL) by an alcohol dehydrogenase and to 8–2 fluorotelomer acid (8–2 FTA) by an aldehyde dehydrogenase. The conversion of 8–2 FTA to 8–2 fluorotelomer unsaturated acid (8–2 FTUA) in soils is so rapid that no 8–2 FTA above the limit of quantification was observed.

Recently, the first study to investigate aerobic biodegradation of 6–2 FTOH [$F(CF_2)_6CH_2CH_2OH$] was described by Liu et al. [110]. Based on this investigation and previous studies on the mechanism of 8–2 FTOH biodegradation [107–109, 111, 112], several pathways for 6–2 FTOH degradation have been proposed. 6–2 FTOH is first converted to 6–2 FTAL through oxidation by alcohol dehydrogenase or cytochrome P450, and then to 6–2 FTA by aldehyde dehydrogenase. Using the 2,4-dinitrophenylhydrazine (DNPH) derivatization method previously described for the detection of 8–2 FTAL from 8–2 FTOH degradation in soil and mammals [108, 109], 6–2 FTAL was not detected in the soil extracts. Hydrogen fluoride (HF) is removed from 6–2 FTA to form 6–2 FTUA either because α-oxidation is not operable or because rapid HF elimination to 6–2 FTUA supersedes 6–2 FTA α-hydroxylation, which is necessary for α-oxidation (Martin et al. 2005). 6–2 FTUA degradation proceeds by two pathways (Fig. 16).

Biodegradation of a novel fluorotelomer alcohol, 1*H*,1*H*,2*H*,2*H*,8*H*,8*H*-perfluorododecanol (degradable telomer fluoroalcohol, DTFA), was investigated in a mixed bacterial culture obtained from activated sludge and the pathway was also proposed (Fig. 17) [103], First, through the catalytic activity of alcohol dehydrogenase and

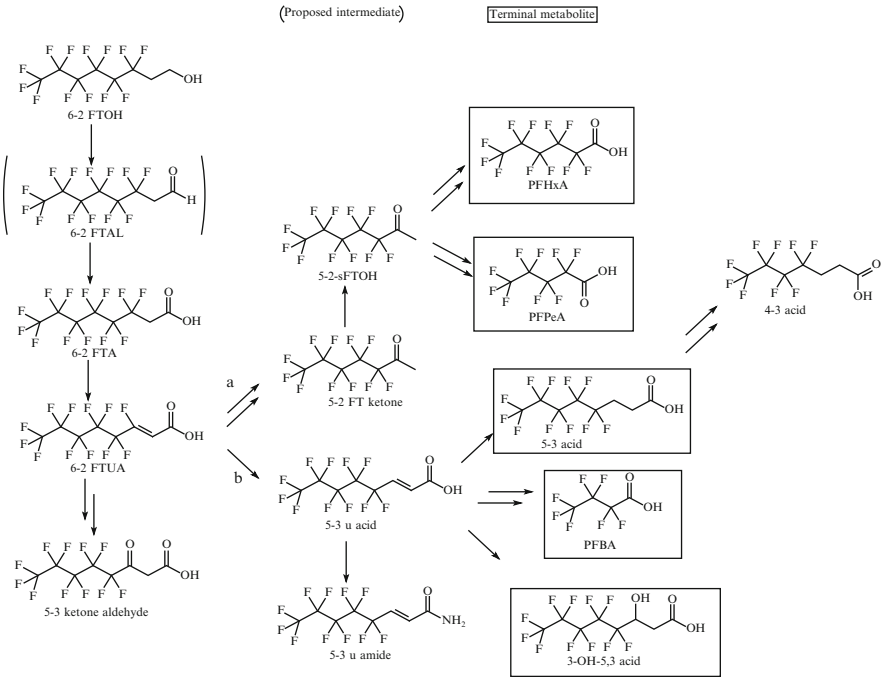

Fig. 16 Proposed 6–2 FTOH aerobic biodegradation pathways. The *double arrows* indicate multiple transformation steps [110]

aldehyde dehydrogenase, DTFA is oxidized to 2*H*,2*H*,8*H*,8*H*-perfluorododecanoic acid (2*H*,2*H*,8*H*,8*H*-PFDoA) which is then defluorinated to 2*H*,8*H*,8*H*-2-perfluorododecenoic acid (2*H*,8*H*,8*H*-2-PFUDoA). Double bonds are formed between the internal –CH2– and –CF2– groups in 2*H*,8*H*,8*H*-2-PFUDoA which is then further degraded via two different β-oxidation pathways. In pathway a, through the removal of –CF2–, 2*H*,8*H*-2,8-PFUDoA is transformed into three different long-chain carboxylic acids which are further degraded into perfluorobutanoic acid (PFBA) with dicarboxylic acids containing different fluorocarbon lengths (C4–C6 compounds), whereas in pathway b, 2*H*,8*H*-2,8-PFUDoA is transformed into perfluoropentanoic acid (PFPeA) with three different fluorinated dicarboxylic acids (Fig. 17).

2.3.2 Fluorotelomer Ethoxylates

Fluorotelomer ethoxylates [F–$(CF_2–CF_2–)_x$–$(CH_2–CH_2–O)_y$–H] are an important class of non-ionic fluorinated surfactants and are regarded as a potential source of per- and polyfluorinated organic pollutants. Aerobic biotransformation of FTEOs was recently demonstrated by Frömel and Knepper [113]. Distinct from the biodegradation of FTOHs, ω-oxidation occurs and is responsible for the transformation of FTEOs to FTEO carboxylates (FTEOCs). After oxidation of the terminal

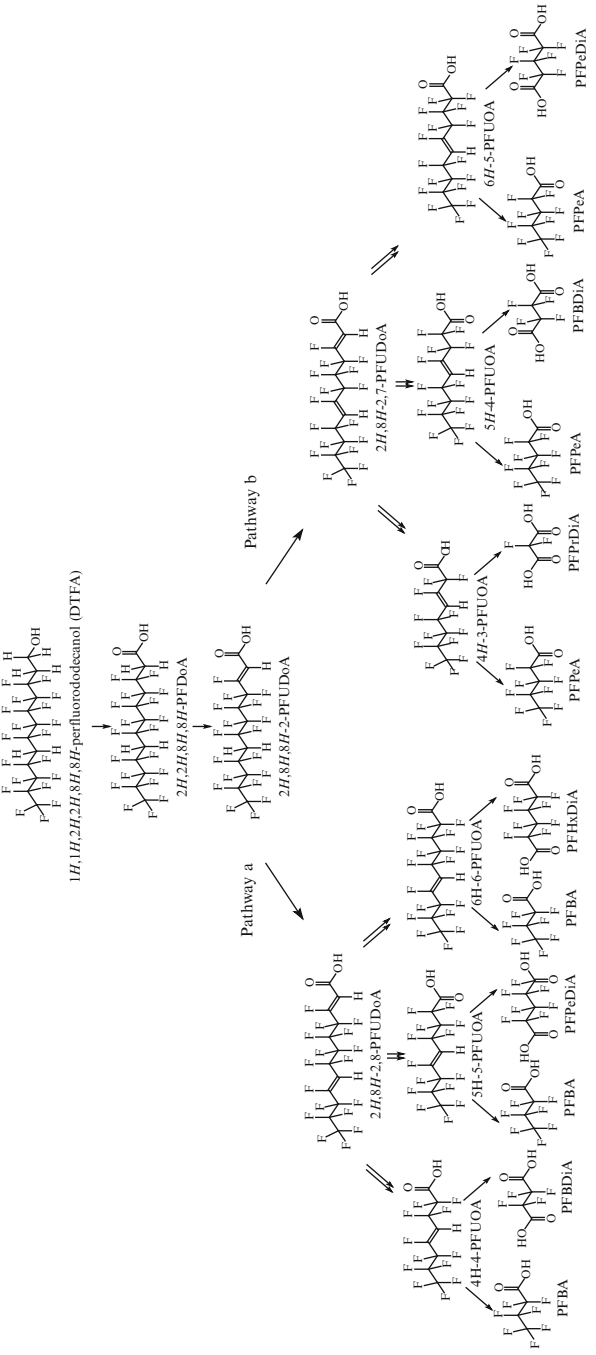

Fig. 17 Proposed biodegradation pathway for DTFA. The *double arrows* indicate multiple transformation steps [103]

hydroxyl group to a carboxylic acid, the carbon chain is subsequently shortened whereby the short-chained FTEOCs are not further degraded. In this chapter, no PFCA formation attributable to FTEO degradation was observed.

2.3.3 Fluorotelomer-Based Urethanes

Fluorotelomer-based urethanes are urethane polymers consisting of a series of fluorotelomer side-chains attached to a hydrocarbon backbone and are commercially used as stains and soil repellents for textiles. Russell et al. [114] tested the potential for microbial activities in four different soil samples to degrade a fluorotelomer-based urethane polymer under aerobic conditions over a 24-month period and demonstrated that fluorotelomer side-chains were released and transformed to perfluorocarboxylic acids including PFOA.

2.3.4 Polyfluoroalkyl Phosphates

Polyfluoroalkyl phosphates (PAPs) are used as commercial surfactants for oil repelling applications, and have been shown to be degraded to PFCAs in a rat model and waste water treatment plant system [115]. The pathway in Fig. 18

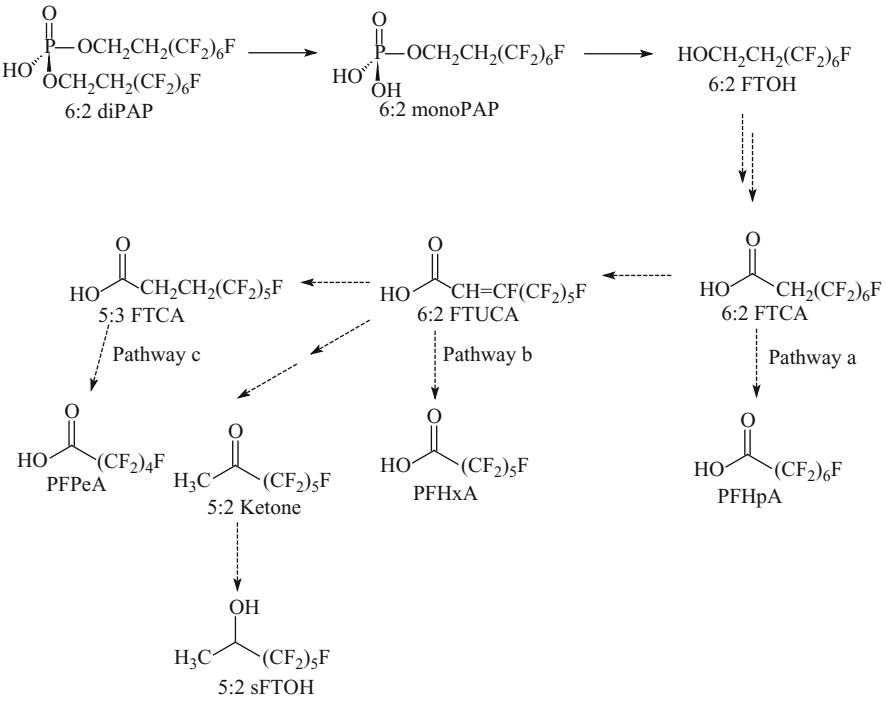

Fig. 18 Proposed degradation pathway of 6:2 diPAP and 6:2 monoPAP [115]

describes the aerobic degradation routes of 4:2, 6:2, 8:2, and 10:2 monosubstituted PAPs (monoPAPs) and 6:2 disubstituted PAP (diPAP) by a microbial mixture collected from sewage of a wastewater treatment plant. In the microbial system, 6:2 FTOH was initially oxidized into a series of acid metabolites. The intermediate metabolite, 6:2 saturated fluorotelomer carboxylic acid (6:2 FTCA), is converted via β-oxidation to 6:2 unsaturated fluorotelomer carboxylic acid (6:2 FTUCA) and perfluorohexanoic acid (PFHxA) (Fig. 18, pathway b), while 5:3 FTCA is transformed to perfluoropentanoic acid (PFPeA) (Fig. 18, pathway c). However, the production of PFPeA may also be attributed to other precursors. For example, 6:2 FTUCA may degrade into 5:2 fluorotelomer ketone ($F(CF_2)_5CH(OH)CH_3$) which is further reduced to 5:2 secondary fluorotelomer alcohol (sFTOH, $F(CF_2)_5CH(OH)CH_3$), and subsequently transformed to PFPeA (Fig. 18, pathway d). 6:2 FTCA and PFHpA production have been observed, supporting the possibility of oxidation of the α-carbon in FTCA to form odd-chain PFCAs (Fig. 18, pathway a).

2.3.5 ω-(Bis(trifluoromethyl)amino)alkane-1-Sulfonates

Biodegradation of ω-(bis(trifluoromethyl)amino)alkane-1-sulfonates was detected in a fixed-bed bioreactor. Its incomplete mineralization revealed that degradation mostly takes place via desulfonation, oxidation, and further β-oxidation [116]. The C–F and C–N bonds in the bis(tri-fluoromethyl)amino (BTFMA) group cannot be accessed by microbes for biodegradation; therefore no defluorination was observed (Fig. 19).

2.3.6 *N*-Ethyl Perfluorooctane Sulfonamide Ethanol

N-Ethyl perfluorooctane sulfonamide ethanol (*N*-EtFOSE) is present in protective paper coatings. Although the only producer in the USA, 3M, has stopped production since 2002, *N*-EtFOSE can still be detected in the North American atmosphere [117]. Aerobic biotransformation of *N*-EtFOSE in activated sludge has been studied [118]. Fast oxidation of *N*-EtFOSE forms *N*-ethyl perfluorooctane sulfonamido acetic acid (*N*-EtFOSAA) through an aldehyde intermediate. *N*-Ethyl perfluorooctane sulfonamide (*N*-EtFOSA) undergoes direct dealkylation to perfluorooctane sulfonamide (FOSA), while perfluorooctane sulfonamido acetic acid (FOSAA) production proceeds at a slower rate. The extremely stable compound PFOS was observed as the final product (Fig. 20) [118].

2.3.7 10-(Trifluoromethoxy) Decane-1-Sulfonate

10-(Trifluoromethoxy) decane-1-sulfonate is a fluorinated surfactant that has been globally distributed, thus leading to increasing concern on its environmental fate and toxicity. Biomineralization of 10-(trifluoromethoxy) decane-1-sulfonate was reported by Peschka et al. in 2008. Two proposed pathways, major and minor, have been

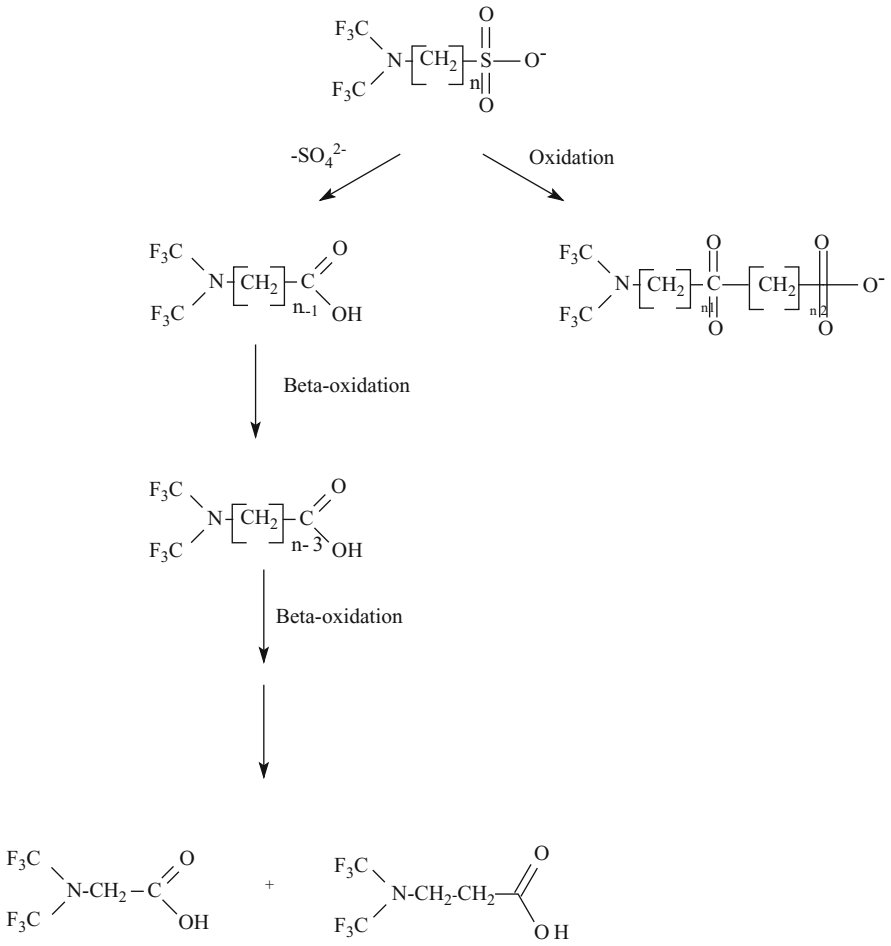

Fig. 19 Biotransformation pathways of ω-(bis(trifluoromethyl)amino)alkane-1-sulfonates [116]

described (Fig. 21). In the major degradation pathway, the carbon chain of the fluorinated alkylsulfonate derivative is shortened by β-oxidation after desulfonation and oxidation. The formed trifluoromethanol is unstable and mineralizes immediately (Fig. 21, pathway a). In the minor degradation pathway, insertion of oxygen occurs, and then, the molecule is subsequently cleaved and degraded (Fig. 21, pathway b).

2.4 Perspectives for the Biodegradation of Perfluorinated Compounds

As previously mentioned, biodegradation and biotransformation of several polyfluorinated compounds such as FTOHs, FTEOs, and PAPs have been reported.

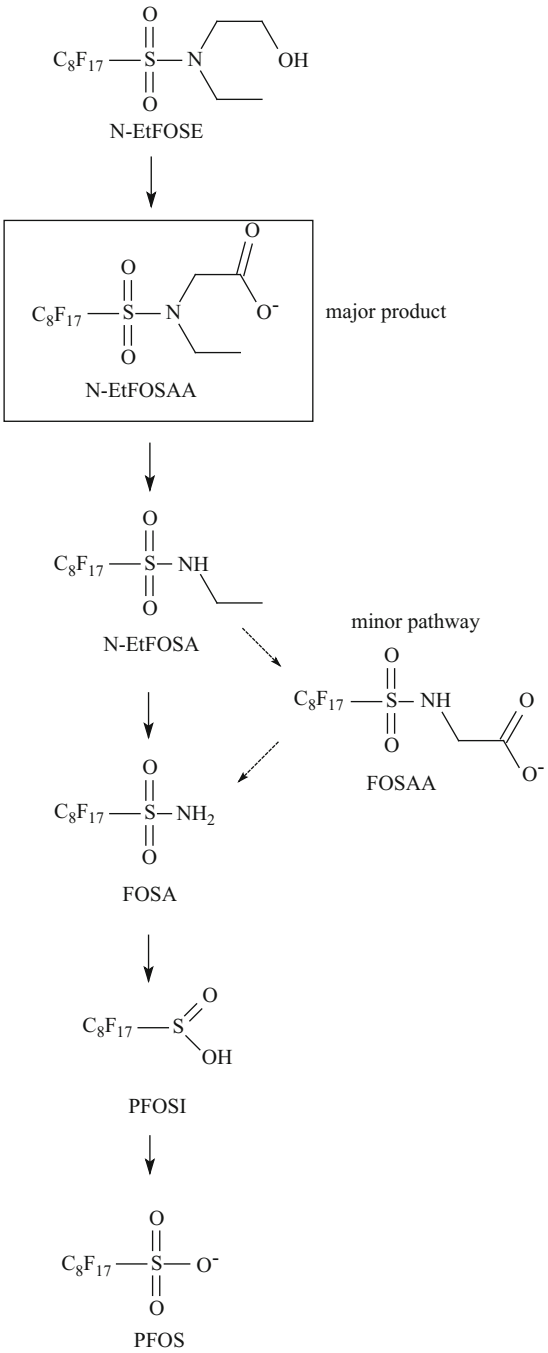

Fig. 20 Proposed transformation pathway of N-EtFOSE in activated sludge [118]

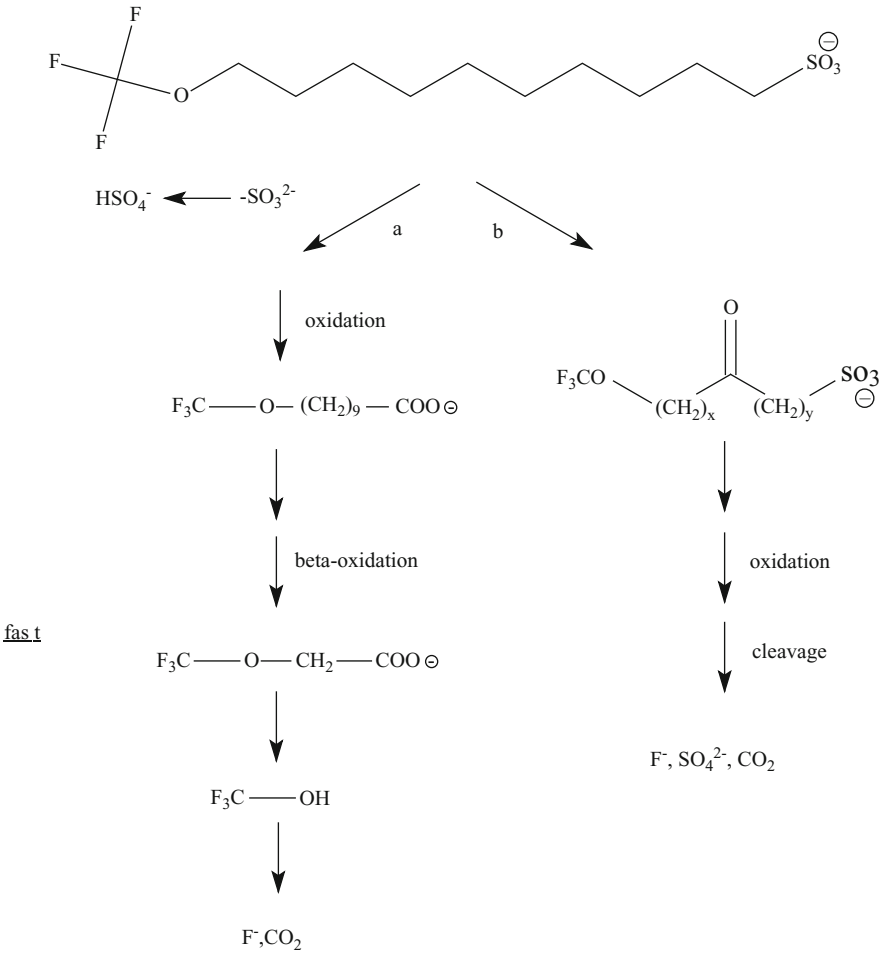

Fig. 21 Degradation pathways of 10-(trifluoromethoxy)decane-1-sulfonate [119]

But to date there are still no reliable reports on the biodegradation or biotransformation of perfluoroalkyl compounds such as PFOS and PFOA. To date, the studies examining biodegradation and transformation of PFCs is very limited. PFCAs, including PFOA, are common transformation products from fluorotelomer chemicals [105, 106, 109, 120, 121]. No evidence about the biodegradation and biotransformation of PFCAs has been found. A recent study about the biodegradability of PFOA using five different microbial communities incubated for up to 259 days showed that PFOA is still microbiologically inert and thus is environmentally persistent [122]. Because of the high stability of the strong C–F covalent bonds, the rigidity of the perfluoroalkyl chain, and the lack of reactive substituent, PFOS is highly recalcitrant to biodegradation or chemical degradation under

ambient conditions. Only two reports about the chemical degradation of PFOS have been published [123, 124]. No studies on biodegradation or biotransformation have been reported. Recently, the first report of reductive dehalogenation of PFOS catalyzed by vitamin B12 was published, in which PFOA was reduced and dehalogenated by Ti(III)-citrate [125]. These results suggested the potential for reductive dehalogenation of PFCs.

2.4.1 Thermodynamics of Organofluorine Biodegradation

Thermodynamics can be used to evaluate whether organisms can obtain energy for growth by catalyzing certain reactions. This approach has been applied to the study of reductive biodechlorination of chlorinated compounds such as 3-chlorobenzoate and chloromethanes. The amount of energy available from reductive dechlorination was reported to be between 100 and 180 kJ/mol [126], which is enough to support microorganism growth by using halogenated compounds as electron acceptors. The Gibbs free energy values of fluorinated compounds showed that the amount of energy obtained from defluorination is similar to the amount available from dechlorination and could support microorganism growth. Therefore, organisms may be able to obtain energy by catalyzing certain defluorination reactions for growth.

Although the thermodynamic properties of perfluoroalkylated compounds are not available, the Gibbs free energy values for the reductive removal of one fluorine atom from fluoropropane molecules (Table 1) showed that the energy yields from the hydrogenolysis of perfluorinated compounds and from less fluorinated compounds are similar. These results reveal the thermodynamic basis for reductive biodefluorination of perfluoroalkylated compounds, especially under anaerobic conditions.

Table 1 Gibbs free energy values for reductive dehalogenation (hydrogenolysis) of selected fluorinated aromatic and aliphatic compounds and their chlorinated analogs [127]

Substrate	Product	ΔG° (kJ/mol)
$CF_3CF_2CH_3$	$CHF_2CF_2CH_3$	−100.0
$CF_3CF_2CH_3$	CF_3CHFCH_3	−117.4
CF_3CHFCH_3	CHF_2CHFCH_3	−88.2
$CHF_2CF_2CH_3$	CHF_2CHFCH_3	−105.5
CF_3CHFCH_3	CF_3CH2CH_3	−163.2
CF_3CH2CH_3	CHF_2CH2CH_3	−78.8
CHF_2CHFCH_3	$CH_2FCHFCH_3$	−96.0
CHF_2CHFCH_3	$CHF_2CH_2CH_3$	−153.9
$CHF_2CH_2CH_3$	$CFH_2CH_2CH_3$	−88.4
$CH_2FCHFCH_3$	$CFH_2CH_2CH_3$	−146.3
$CFH_2CH_2CH_3$	$CH_3CH_2CH_3$	−140.0

All calculations used the following standard conditions: T=298.15K, pH=7, methanes and H_2 in the gas phase at 1 atm, and benzoates and halides in the aqueous phase at 1 M

2.4.2 Perspectives for the Biodegradation of Perfluorinated Compounds

Thermodynamically, perfluorinated compounds should be potentially biodegradable, especially under anaerobic conditions [127]. To date, the reductive biodefluorination of perfluorinated compounds has not yet been observed. Moreover, the rate at which microorganisms can evolve the capability to grow on this potential source of energy and the function of the enzymatic machinery that catalyzes this reaction are largely unknown. However, co-metabolic degradation of several polyfluorinated compounds under aerobic conditions and without thermodynamic facilitation has been studied in detail [109, 110, 115]. This information formed the basis for technology that has been applied in the field for the degradation of other polyhalogenated compounds such as trichloroethene. The search for co-metabolic degradation of poly- and perfluorinated compounds, and studies to understand its mechanisms better will continue.

3 Defluorination Pathways and Defluorogenases

3.1 Enzymatic Metabolic Pathways

3.1.1 Aerobic Metabolism

Limited reports are available about the biodegradation of fluorinated organic compounds, and therefore little is known about the enzyme-catalyzed defluorination pathway. Under aerobic conditions, fluorinated organic compounds are usually degraded via the electron donor or co-metabolic pathways. It has been reported that 4-fluorophenol can be utilized as the sole source of carbon and energy for *Arthrobacter* sp. strain IF1, and that two gene clusters are involved [128]. Cluster A harbors *fpd*A1DE and includes an FADH2-dependent monooxygenase, a putative maleylacetate reductase, and a hydrolase gene. In Cluster B, *fpd*A2 encodes a 4-FP monooxygenase, *fpdB* encodes a flavin reductase, and *fpdC* encodes a putative hydroxyquinol dioxygenase (Fig. 22). The proposed catabolic pathway is shown in Fig. 23.

To date, the well-known enzymes involved in fluoro-degradation are normally responsible for the catabolism of non-fluorinated compounds. Evidence suggests that enzymes are specifically employed for the catabolism of these substrates. Enzymes for degrading aromatic compounds such as monooxygenases, cleavage dioxygenases, and maleylacetate reductase have exhibited biodefluorination activity. As shown in Fig. 24, a number of enzymes that do not show specific activity for fluoroaromatic compounds are involved in the catabolism of 3-fluorobenzoic acid [86].

Because of the similar steric sizes of hydrogen and fluorine, substitution of hydrogen for fluorine is considered to have an important role in defluorination. Many oxygenases and (de)hydroxylases make up a group of defluorinating enzymes

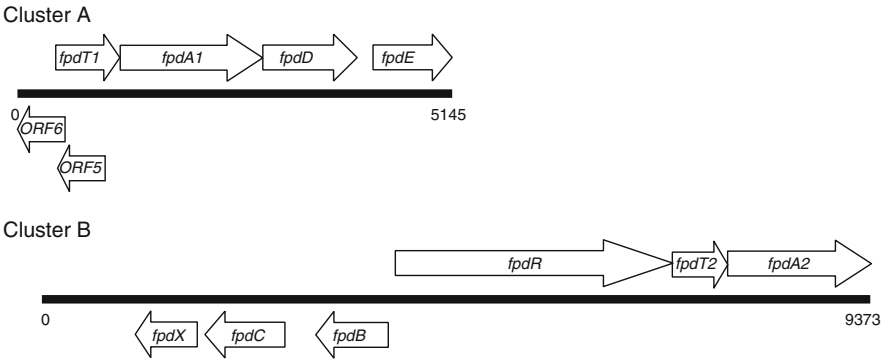

Fig. 22 Organization of the open reading frames (ORFs) in the *fpd* gene regions of *Arthrobacter* sp. strain IF1. *Open arrows* indicate the size and direction of each ORF [128]

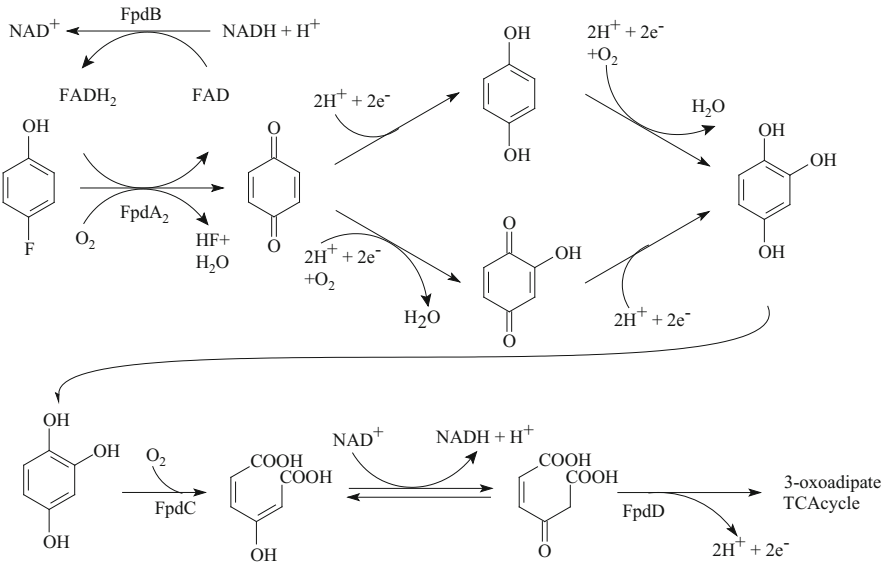

Fig. 23 Proposed catabolic pathway for 4-FP degradation [128]

that act on both fluorinated aromatics and fluoroaliphatics. In 1978, a pyruvate dehydrogenase component of *E. coli* was found to catalyze the conversion of 3-fluoropyruvate to acetate and fluoride ions and to eliminate the fluorine [56]. Later, the proposed mechanism of fluorine elimination by dehydrogenases was proposed. *p*-Hydroxybenzoate hydroxylase, a NADPH-dependent flavin-containing monooxygenase from *P. fluorescens* and *Candida parapsilosis*, was reported to

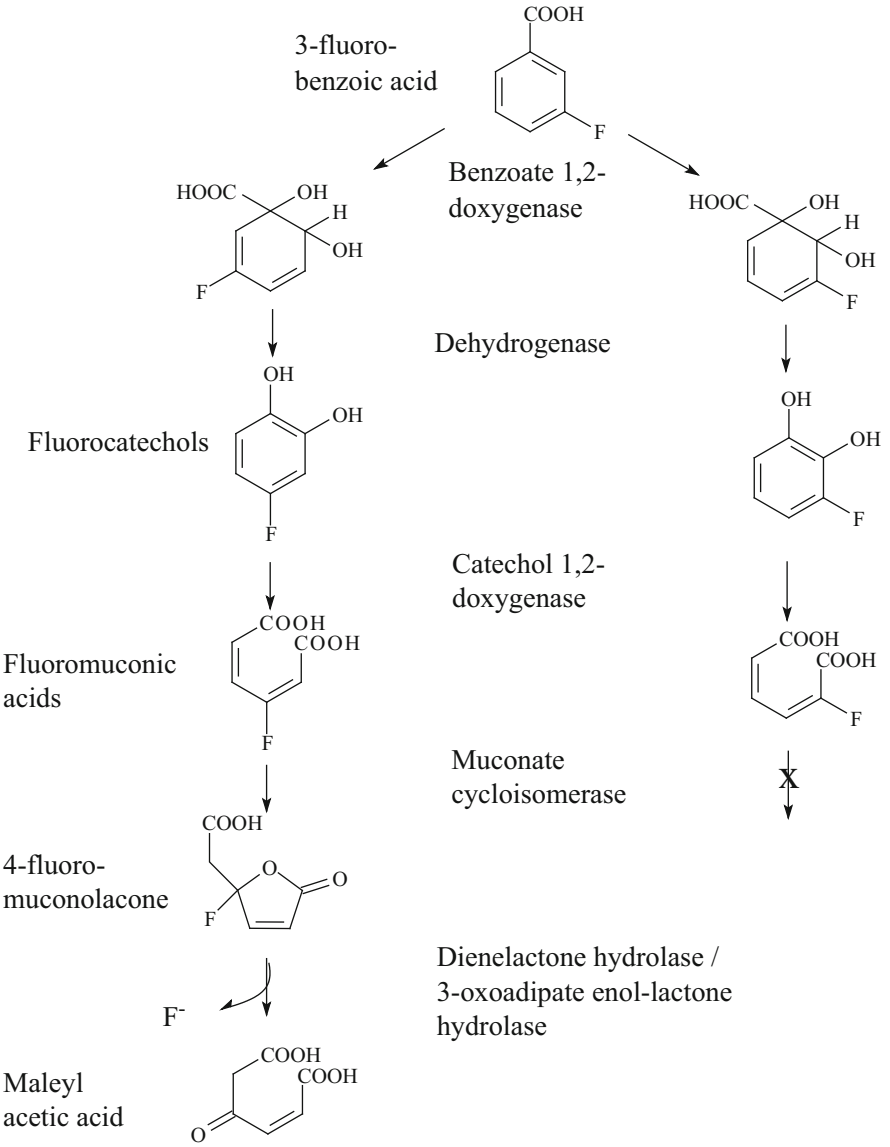

Fig. 24 Catabolism of 3-fluorobenzoic acid in aerobic bacteria [40]

degrade several fluorine-substituted *p*-hydroxybenzoates such as fluorohydroxybenzoate [129, 130]. Fluorobiphenyl metabolism is catalyzed by a series of dioxygenases dehydrogenases and hydrolases to yield fluoropyruvate and 4-fluorobenzoate [86, 87].

3.1.2 Anaerobic Metabolism

Less is known about the degradation of fluoroaromatic compounds under anaerobic conditions. Defluorination of 2-hydroxybenzoate and 3-fluorobenzoate was observed in *S. aciditrophicus* cultures under anaerobic conditions (Mouttaki et al. 2009). Recently, co-metabolism in a bacterial culture was found to catalyze 4-fluorobiphenyl to a carboxylic acid derivative [131]. Both methanogenic and sulfate-reducing defluorination generated trifluoroacetic acid (TFA) via a co-metabolism pathway [132, 133]. Denitrifying bacteria have also been reported to mineralize 2- and 4-fluorobenzoate [79].

3.2 Defluorinases

Among all the dehalogenations, defluorination is most difficult because the C–F bond is one of the most stable bonds in nature. Partly because of limited studies on defluorinases, most man-made organofluorine compounds are degraded via co-metabolism pathways. Enzymes with alterable substrates play an important role, although few fluorine-specific enzymes have been identified.

3.2.1 Fluoroacetate Dehalogenase

As the most common fluorinated natural product, fluoroacetate was reported in 1965 to be degraded by *Pseudomonas* fluoroacetate dehalogenase, which catalyzes the hydrolytic cleavage of the C–F bond to yield glycolate and a fluoride ion [46]. Other fluoroacetate dehalogenases have been isolated from microorganisms such as *Moraxella*, *Delftia*, and *Burkholderia* [45, 48, 134]. Fluoroacetate dehalogenase belongs to the α/β hydrolase superfamily protein. The mechanism of C–F bond cleavage by fluoroacetate dehalogenase has been extensively investigated (Keuning et al. 1985; [45]). The three-dimensional structure of FAc-DEX FA1, a fluoroacetate dehalogenase from *Burkholderia sp.* strain FA1 [53], suggested a mechanism whereby fluoroacetate is degraded by an initial nucleophilic attack on the α-carbon atom by the carboxylate group of Asp104 which displaces the fluoride ion to form an ester intermediate. The ester intermediate is then hydrolyzed by a His271-activated water molecule, which yields glycolate and regenerates the carboxylate group of Asp104 (Fig. 25).

3.2.2 Fluoroacetate-Specific Defluorinases

Detoxification of fluoroacetate in mammals is catalyzed by fluoroacetate-specific defluorinases (FSDs) such as the glutathione-*S*-transferase isozyme GSTZ which is distinct from bacterial fluoroacetate dehalogenases (Fig 26) [135]. Two distinct

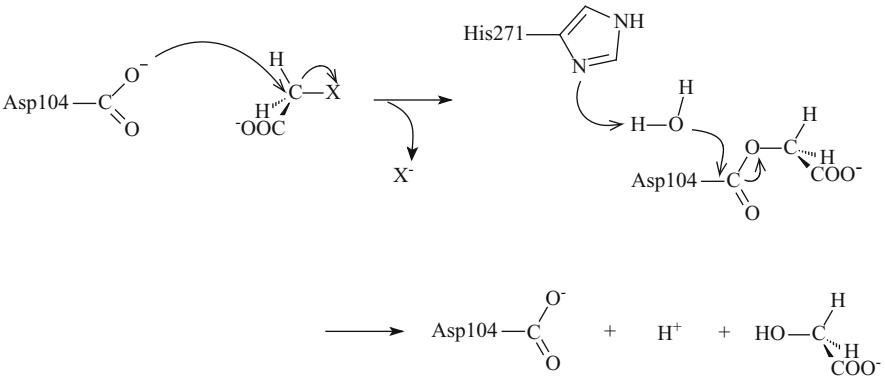

Fig. 25 Proposed reaction mechanism of FAc-DEX FA1 [53]

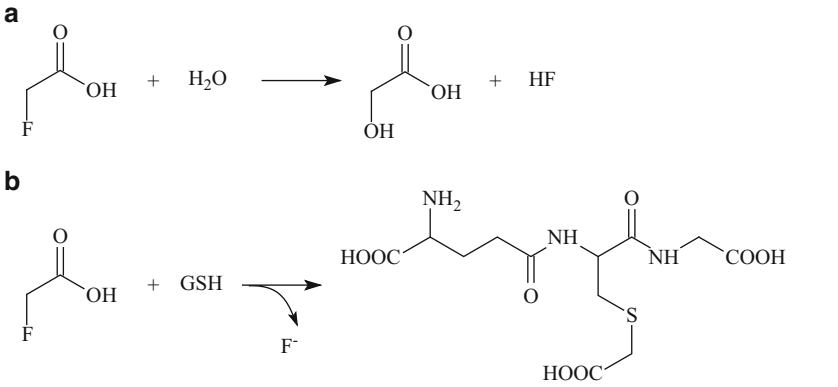

Fig. 26 Enzymatic C–F bond cleavage. (**a**) Fluoroacetate dehalogenase. (**b**) Fluoroacetate-specific defluorinase [40]

FSD activities have been identified in rat liver: one is glutathione-*S*-transferase-like and the other more predominant activity is apparently a new type of dehalogenase, which is considered to be an FSD. Interestingly, the amino acid sequence of the latter FSD is similar to the sorbitol dehydrogenase protein, which does not show defluorination activity on fluoroacetate.

3.2.3 4-Fluorobenzoate Dehalogenase

4-Fluorophenol (4-FP) monooxygenase (FpdA2) was first cloned and purified from *Arthrobacter* sp. strain IF1. In combination with FpdB, which uses NADH to reduce either flavine-adenine dinucleotide (FAD) or flavin Mononucleotide (FMN), FpdA2 transforms various halogenated phenols via *para*-substitution, leading to halide release and hydroquinone formation (Fig. 27) [128].

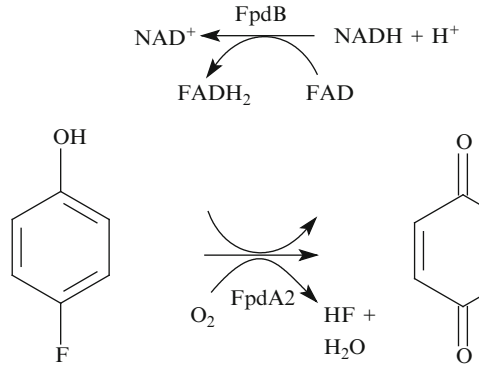

Fig. 27 4-FP monooxygenase [128]

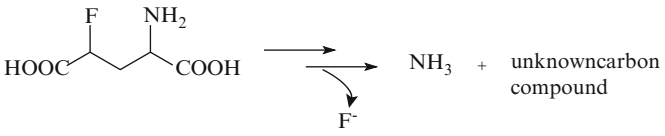

Fig. 28 4-Fluoroglutamate dehalogenase/deaminase. *Arrows* indicate that more than one reaction might be occurring. GSH=glutathione [40]

3.3 Perspectives for New Enzymes and Metabolic Pathways

As mentioned above, co-metabolism is the main degradation pathway for mono- and polyfluorinated organic compounds. However, due to very limited research, only a few co-metabolism pathways have been found in the laboratory. Further studies in this area will help to investigate the pathways and enzymes involved in defluorination. Compared with a series of dechlorinases that catalyze various kinds of reactions, only three kinds of defluorinases have been identified to date. In 2007, biodegradation of 4-fluoroglutamate was reported via an unusual pathway, yielding equimolar concentrations of fluoride ions and ammonia, indicating that an enzyme such as glutamate dehydrogenase is not responsible for the biotransformation (Fig. 28) [136]. In addition, the defluorinating/deaminating activity was found in the soluble fraction of the cell and was not related to the dechlorinating/deaminating activity, which was located in the cell membrane. These results suggest the existence of a potential new fluoroglutamate dehalogenase. Under anaerobic conditions, defluorination was detected in methanogenic, sulfate-reducing, and denitrifying bacteria, indicating that extensive defluorination occurs under anaerobic conditions [79, 132]. Reductive defluorination is a thermodynamically feasible mechanism to derive cellular energy under anaerobic conditions. However, microbes that are able to obtain energy for growth by reductive defluorination have yet to be isolated [127]. And there is much be done to elucidate the defluorination mechanisms and properties of the enzymes involved.

4 Summary and Perspectives

Brominated and chlorinated compounds have been investigated in previous research on the biodegradation of halogenated compounds. However, fluorinated chemicals have thus far received much less attention [127]. The inertness of fluorine results in persistence and leads to accumulation in the environment, making it necessary to explore microbial degradation of fluoroorganic compounds. Until recently, only a few microbes including bacteria, fungi, and algae have been found to be capable of fluoro-degradation. For most fluorinated substrates, the mechanism of fluoro-degradation is still not clear. Several monofluorinated compounds, including fluoroaliphatics [57, 136, 137], fluoroaromatics [71, 74, 81, 88], and a few other polyfluorinated compounds [105, 106, 109, 110], can be degraded. However, the mechanisms of these degradation reactions are largely unknown. No biodegradation of perfluorinated compounds has been observed [25, 122]. Perfluorinated and polyfluorinated compounds are widely used as surfactants, catalysts, and insecticides [18, 19]. These compounds are highly recalcitrant and have been detected throughout the global environment [26, 27]. Biodegradation of perfluorinated compounds is thermodynamically possible under reductive conditions, but has not been measured [127]. Despite a great increase in knowledge over the last few decades, we are still far from being able to predict the biodegradation of fluorinated organic compounds as well as the mechanism of defluorination. Although the dehalogenation of both fluorinated and chlorinated organic compounds is largely mediated by soil microflora, limited knowledge of the factors influencing these microorganisms is available. Development of systematic biological and molecular genetics studies will help in the study of soil microbial species and communities, thus facilitating the discovery of new microbes capable of defluorination.

New technologies for chemical analysis have made highly sophisticated studies practical in the laboratory. Fluorine-19 nuclear magnetic resonance spectroscopy (^{19}F NMR) and isotopic labeling techniques have helped to contribute to a deeper understanding of several key processes in the catalyzed reactions of fluorinated substances [74, 109, 110]. The rapid growth of bioinformatics has led to the development of databases that search for organic persistence information. Furthermore, scientists have created computer programs such as MultiCASE based on general quantitative structure-degradation relationships (QSDRs) to predict the degradation/persistence of organic chemicals in the environment that have not been characterized [138]. One of these popular computer programs, BIOWIN, contains a series of models collectively referred to as biodegradability probability. Based on QSDR models as well as six aerobic biodegradation models and one anaerobic model, BIOWIN can predict the biodegradability probability under aerobic and anaerobic conditions. If a metabolic pathway is available for a chemical, it is assumed to be biodegradable [139]. Similar programs, including the UM-BBD Pathway Prediction and System CATABOL program, have also been developed for determining the biodegradability probability [140, 141]. These tools provide unique approaches to studying biodifluorination.

In general, biodegradation studies need interdisciplinary collaborations between microbiology, ecology, genetics, biochemistry, and analytical chemistry to resolve complex problems. As more research attention is given to this field and more technologies are developed and applied, further mechanisms of the biodegradation of fluorine-containing organic compounds will be elucidated.

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