REMOVAL OF PERFLUOROOCTANE SULFONATE (PFOS) AND RELATED COMPOUNDS FROM INDUSTRIAL EFFLUENTS

by

Valeria Lourdes Ochoa Herrera

Copyright © Valeria Lourdes Ochoa Herrera 2008

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF CHEMICAL AND ENVIRONMENTAL ENGINEERING

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY
WITH A MAJOR IN ENVIRONMENTAL ENGINEERING

In the Graduate College

THE UNIVERSITY OF ARIZONA

2008

THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Valeria Lourdes Ochoa Herrera, entitled Removal of Perfluorooctane Sulfonate (PFOS) and Related Compounds from Industrial Effluents, and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy

____ Date: 11/17/08
Maria R. Sierra Alvarez

____ Date: 11/17/08
James A. Field

____ Date: 11/17/08
James Farrell

____ Date: 11/17/08
Craig A. Aspinwall

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copies of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

____ Date: 11/17/08
Dissertation Director: Maria R. Sierra Alvarez

STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at the University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this dissertation are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the copyright holder.

SIGNED: Valeria Lourdes Ochoa Herrera

AKNOWLEDGEMENTS

I would like to express my deepest gratitude to my advisors Dr. Reyes Sierra-Alvarez and Dr. Jim Field for their guidance, support and most of all for their friendship. Their motivation and encouragement has greatly contributed to my academic formation but even more to my personal development. It has been a great pleasure to work for them.

Much of the work presented in this dissertation would not have been possible without the help and positive input of several people. I'm thankful to Dr. Jeanne Pemberton, Dr. Craig Aspinwall and Dr. Jim Farrell for their scientific advices and support. I gratefully acknowledge Dr. Javier Torres for his aids with ab initio calculations and MS Omar Gonzalez for his contribution to analytical purifications. I also want to recognize our collaborators and friends, Dr. Arpad Somogyi and Dr Penn (Stephanie) Shu (Mass Spectrometry Facility), Dr. Neil Jacobsen and Dr. Guangxin Lee (NMR Facility) from the Chemistry Department and Dr. Leif Abrell (Arizona Laboratory for Emerging Contaminants) from the Soil, Water and Environmental Science Department. I would like to give them special thanks for running my samples, for training me to operate the instruments and for helping me to analyze data. I am also very grateful to my undergraduate's students Chandra Katri and Beshoy Latiff for their invaluable contribution. I give my sincere thanks to former and current members of the Field-Sierra group for all the shared experiences and the long working hours spent in the labs during these four years.

I am grateful to my family in Tucson, Nana and Papa, for their love, help and support and to my friends for all the fun times.

I want to honor my mother and my father for their unconditional love, guidance and encouragement. They have played a transcendental role in my professional and personal success. I am truly thankful to my dear husband Javi and to my lovely Valerie. I would not have made it without their love and support; this dissertation is mine as much as it is theirs.

Finally, I would like to thank the Semiconductor Research Center/Sematech Engineering Research Center for Environmentally Benign Semiconductor Manufacturing for financial support.

DEDICATION

A mis queridos padres Irma y Marcelo,

a mi amado esposo Javi,

y a mi adorada hija Valerie.

TABLE OF CONTENTS

LIST OF TABLES…………………………………………………………………….. 11

LIST OF FIGURES………………………………………………………………… 13

ABSTRACT……………………………………………………………….…... 21

OBJECTIVES…………………………………………………………………. 25
Aim……………………………………………………………………….…... 25
Specific objectives…...………..…………………………………………….... 25

CHAPTER 1……………………………………………………………………. 27

Introduction……………………………………………………….……….. 27

1.1 Chemistry………………………………………………………………. 27
1.2 Synthesis……………………………………………………………….. 32
1.3 Production and Uses…..……………………………………………….... 36
1.4 Environmental Regulations…………………………………………….. 41
1.5 Environmental Persistence, Fate and Bioaccumulation…………………... 42
1.6 Toxicity………………………………………………………………… 49
1.7 Analytical Techniques………………………………………………….. 52
1.8 Treatment Methods…………………………………………………….. 54
1.8.1 Biological Methods…………………………………………………. 54
1.8.2 Physico-chemical Methods…………………………………………. 56
1.9 Conclusions……………………………………………………………. 57
1.10 References……………………………………………………………… 58

CHAPTER 2……………………………………………………………………. 75

Development of Analytical Methods to Quantify Perfluorooctane Sulfonate (PFOS) and Related Compounds in the Environment………………………….. 75

2.1 Abstract………………………………………………………………… 75
2.2 Introduction…………………………………………………………….. 77
2.3 Materials and Methods…………………………………………………. 79
2.3.1 Chemicals…………………………………………………………… 79
2.3.2 ^{19}F NMR quantification…………………………………………….. 80
2.3.3 HPLC-suppressed conductivity detection…………………………... 81
2.3.4 TOC Analysis……………………………………………………….. 82
2.4 Results and Discussion………………………………………………… 82
2.4.1 ^{19}F NMR assignments and quantification of PFOS………………… 82

TABLE OF CONTENTS – *Continued*

2.4.2 HPLC-suppressed conductivity detection………………………….… 91
2.4.3 Quantification by TOC and COD analysis……………...………. 98
2.5 Conclusions………………………………………………………….... 101
2.6 Acknowledgments…………………………………………………… 101
2.7 References……………………………………………………………. 102

CHAPTER 3………..……………………………………………………… 110

Application of LC-MS/MS Methods to Determine the Fate of Perfluorinated Chemicals in Municipal Wastewater Treatment Plants in Arizona…………….. 110

3.1 Abstract……………………………………………………………… 110
3.2 Introduction………………………………………………………….... 112
3.3 Materials and Methods……………………………………………….. 116
3.3.1 Chemicals……………………………………………………..... 116
3.3.2 Sludge extraction……………………………………………… 116
3.3.3 Sample clean-up……………………………………………..… 117
3.3.4 LC-MS/MS…..………………………………………………… 118
3.3.5 Quantification………………………………………………….. 119
3.4 Results and Discussion……………………………………………….. 121
3.4.1 Application of LC-MS/MS for the detection and quantification of PFOS and related compounds……………………………………… 121
3.4.2 Assessment of PFC levels in wastewater treatment sludge……….... 125
3.5 Conclusions………………………………………………………….... 130
3.6 Acknowledgements………………………………………………….... 130
3.7 References……………………………………………………………. 131

CHAPTER 4…………………..…………………………………………… 138

Microbial Toxicity and Biodegradability of Perfluorooctane Sulfonate (PFOS) and Related Compounds…………………………………………………….... 138

4.1 Abstract……………………………………………………………..... 138
4.2 Introduction………………………………………………………….. 140
4.3 Materials and Methods……………………………………………….. 144
4.3.1 Chemicals……………………………………………………… 144
4.3.2 Sludge and sediment samples……………………………………… 145
4.3.3 Electrolyzed PFOS and PFBS samples……………………………... 146
4.3.4 Culture media……………………………………………………….. 146
4.3.5 Microbial toxicity assay…………………………………………….. 147
4.3.6 Microbial degradation………………………………………………. 148

TABLE OF CONTENTS – *Continued*

4.3.7 GC-FID……………………………………………………………….. 150
4.3.8 HPLC-suppressed conductivity detection………………………….. 150
4.3.9 ^{19}F NMR………………………………………………………….. 151
4.3.10 Other analytical determinations………………………………….. 152
4.4 Results and Discussion…………………………………………………. 153
Microbial toxicity…………………………………………………… 153
4.4.1 Microbial toxicity of PFOS and related compounds…..…………… 153
4.4.2 Microbial toxicity of short-chain (per)fluorinated compounds…….. 157
4.4.3 Microbial toxicity of electrolyzed PFOS and PFBS samples………. 160
Microbial degradation………………………………………………….. 171
4.4.4 Anaerobic microbial degradation of PFOS and related compounds... 171
4.4.5 Microbial degradation of short-chain perfluorocarboxylates……….. 174
4.4.6 Anaerobic microbial degradation of electrolyzed PFOS and PFBS samples………………………………………………………… 175
4.5 Conclusions……………………………………………………………... 177
4.6 Acknowledgments………………………………………………………. 178
4.7 References……………………………………………………………… 179

CHAPTER 5……………..……………………………………………………. 186

Biomimetic Reductive Defluorination of Perfluorooctane Sulfonate (PFOS) with Vitamin B_{12}………………………………..…………………………… 186

5.1 Abstract…………………………………………………………………… 186
5.2 Introduction……………………………………………………………….. 188
5.3 Materials and Methods…………………………………………………. 192
5.3.1 Chemicals……………………………………………………………… 192
5.3.2 Identification and quantification of structural PFOS isomers………. 193
5.3.3 Purification of PFOS isomers and linear PFOS…………………….… 194
5.3.4 Reductive defluorination……………………………………………. 194
5.3.5 Clean-up and concentration of samples…………………………….. 195
5.3.6 ^{19}F NMR…………………………………………………………….. 196
5.3.7 LC-MS/MS…………………………………………………………….. 196
5.3.8 HPLC-suppressed conductivity detection…………..………………. 197
5.3.9 EPR spectroscopy…………………………………………………... 198
5.3.10 Ab initio calculations……………………………………………….. 198
5.4 Results and Discussion...………………………………………………….. 201
5.5 Conclusions……………………………………………………………….. 227
5.6 Acknowledgments………………………………………………………… 227
5.7 References………………………………………………………………… 228

TABLE OF CONTENTS – *Continued*

CHAPTER 6……………………………………..…………………………… 238

Characterization of Degradation Products from the Reductive Dehalogenation of Perfluorooctane Sulfonate (PFOS)…...……………………………………… 238

6.1 Abstract……………………………………………………………… 238
6.2 Introduction………………………………………………………….. 240
6.3 Materials and Methods………………………………………………. 243
6.3.1 Chemicals………………………………………………………… 243
6.3.2 Purification of PFOS isomers and linear PFOS…………………….. 244
6.3.3 Clean-up and concentration of samples…………………………….. 244
6.3.4 Purification of PFOS isomers by preparative HPLC……………….. 245
6.3.5 Reductive defluorination……………………………………………. 245
6.3.6 ^{19}F NMR……………………………………………………….. 246
6.3.7 HPLC-suppressed conductivity detection…………………………... 247
6.3.8 LC-MS/MS……...………………………………………………… 247
6.3.9 GC-MS/MS………………………………………………………… 248
6.4 Results and Discussion……………………………………………… 250
6.4.1 Purification of branched PFOS isomers…………………………….. 250
6.4.2 Characterization of degradation products from the biomimetic reductive defluorination of branched PFOS isomers……………….. 260
6.5 Conclusions………………………………………………………….. 274
6.6 Acknowledgments…………………………………………………… 274
6.7 References…………………………………………………………… 275

CHAPTER 7……………………………..……………………………………… 281

Removal of Perfluorinated Surfactants by Sorption onto Granular Activated Carbon, Zeolite and Sludge…………………………………………………... 281

7.1 Abstract.……………………………………………………………... 281
7.2 Introduction….………………………………………………………. 283
7.3 Materials and Methods………………………………………………. 286
7.3.1 Chemicals………………………………………………………… 286
7.3.2 Sorbents……………………………………………………………... 286
7.3.3 Adsorption of perfluorinated surfactants…………………………… 287
7.3.4 Solid phase extraction………………………………………………. 289
7.3.5 Analytical methods…………………………………………………. 289
7.4 Results and Discussion……………………………………………… 291
7.4.1 PFOS adsorption onto activated carbon…………………………….. 291
7.4.2 PFOA and PFBS adsorption onto activated carbon………………… 298

TABLE OF CONTENTS – *Continued*

7.4.3 Adsorption of PFOS on faujasite zeolites………………………….. 301
7.4.4 Adsorption of PFOS onto wastewater treatment sludge……………. 304
7.5 Conclusions……………………………………………………………….. 307
7.6 Acknowledgments………………………………………………………… 308
7.7 References………………………………………………………………… 308

CHAPTER 8……………………..……………………………………………… 315

Toxicity of Fluoride to Microorganisms in Biological Wastewater Treatment Systems……………………………………………………………………… 315

8.1 Abstract…………………………………………………………………… 315
8.2 Introduction……………………………………………………………….. 317
8.3 Materials and Methods……………………………………………………. 320
8.3.1 Chemicals………………………………………………………… 320
8.3.2 Sludge sources……………………………………………………. 320
8.3.3 Culture media…………………………………………………….. 322
8.3.4 Microbial toxicity assays………………………………………… 323
Nitrification toxicity assays……………………………………… 323
Denitrification toxicity assays…………………………………… 324
Toxicity to aerobic heterotrophs………………………………… 324
Inhibition of glucose fermentation……………………………….. 325
Methanogenic toxicity assays…………………………………… 326
Toxicity to anaerobic propionate and butyrate-degrading bacteria… 327
8.3.5 Aquatic toxicity…………………………………………………... 328
Microtox® assay………………………………………………….. 328
Toxicity to biomonitoring species………………………………... 329
8.3.6 Analytical methods……………………………………………… 329
8.4 Results and Discussion……………………………………………………. 331
Microbial toxicity assays………………………………………………… 331
8.4.1 Nitrification and denitrification toxicity assays…………………….. 331
8.4.2 Inhibition of aerobic heterotrophs and glucose fermenters by fluoride…………………………………………………………… 338
8.4.3 Toxicity to anaerobic microorganisms: methanogens and microorganisms involved in propionate- and butyrate degradation… 340
8.4.4 Inhibition of aquatic organisms by fluoride....……………………… 348
8.5 Conclusions……………………………………………………………….. 350
8.6 Acknowledgments………………………………………………………… 350
8.7 References………………………………………………………………… 351

CONCLUSIONS AND FUTURE DIRECTIONS………………………………… 359

LIST OF TABLES

Table 1.1 Physical and chemical properties of PFOS as the potassium salt…… 29

Table 1.2 Physical and chemical properties of PFOA…………………………. 31

Table 1.3 Physical and chemical properties of PFBS………………………….. 32

Table 1.4 EU consumption of PFOS related substances in semiconductor manufacturing and concentrations utilized………………………….. 41

Table 1.5 Toxicity levels of PFOS in different organisms…………………….. 51

Table 2.1 ^{19}F NMR assignments of the PFOS molecule……………………… 87

Table 4.1 Microbial toxicity of PFOS and related perfluoroalkyl sulfonates to hydrogenotrophic methanogens in anaerobic sludge. IC_{20}, IC_{50} and IC_{80} are the concentrations of the perfluoroalkyl surfactants causing 20, 50 and 80% decrease in the activity of the methanogenic microorganisms, respectively…………………………………….. 156

Table 4.2 Fluoride release during the electrochemical treatment of PFOS and PFBS after different periods of time……………………………… 162

Table 4.3 Fluoride release corrected with control samples of PFOS, PFBS and TH-PFOS (200 mg L^{-1}) in biodegradability assays inoculated with different municipal and industrial sludge and sediments………..…. 173

Table 4.4 Fluoride release corrected with control samples during the microbial degradation of electrolyzed PFOS and PFBS samples in bioassays inoculated with anaerobic wastewater treatment sludge after 1.5 and 1.1 years of incubation, respectively……………………………... 176

Table 5.1 Percent concentration of technical PFOS based on LC-MS/MS studies………………………………………………………………….. 199

Table 5.2 Optimal treatment conditions of those tested for the reductive dehalogenation of PFOS by Ti(III) citrate and vitamin B_{12}………… 209

Table 5.3 Ab initio quantum mechanical calculations of the Gibbs free energy for the linear and branched isomers structures of PFOS. The differences in energy are referred to the most stable structure (*i.e.,* linear molecule). Values were obtained at standard conditions of temperature and pressure (298.15 K and 1 atm)…………………….. 217

Table 6.1 Tentative assignments of branched PFOS isomers separated by means of HPLC-suppressed conductivity detection………………... 255

Table 6.2 Organic solvents employed in the solid-liquid extraction of the precipitate grouped into polar protic, polar aprotic and non-polar solvents. The polarity is expressed as dielectric constant…………... 261

Table 7.1 Physical properties of GAC Calgon F300, Calgon F400 and Calgon URV-MOD1……………………………………………………… 293

Table 7.2 Langmuir isotherm constants a (mg PFC g sorbent^{-1}) and b (L mg PFC^{-1}) and Freundlich isotherm constants K_F [(mg PFC g sorbent)$^{-1}$ (mg PFC L^{-1})$^{-n}$] and n for the adsorption of PFCs onto various sorbent materials at 30ºC and solution pH 7.2………………………. 297

LIST OF TABLES – *Continued*

Table 7.3 Physical properties of faujasite zeolites evaluated in this study…….. 301

Table 8.1 Inhibitory effect of sodium fluoride on the key microbial populations in biological wastewater treatment systems. IC_{20}, IC_{50} and IC_{80} are the concentrations of fluoride causing 20, 50 and 80% decrease in the activity of the target microorganisms, respectively…. 333

Table 8.2 96-h lethal concentrations of fluoride towards two organisms commonly used in effluent biomonitoring, water fleas (*Ceriodaphnia dubia*) and fathead minnow (*Pimephales promelas*). LC_{20}, LC_{50}, LC_{80} are the concentrations of the toxic lethal to 20, 50 and 80% of the test organisms, respectively………………………… 349

LIST OF FIGURES

Figure 1.1 Chemical structures of perfluorinated compounds of environmental importance. Legend: A) perfluorooctane sulfonate, PFOS ($C_8F_{17}SO_3^-$), B) perfluorooctanoic acid, PFOA ($C_7F_{15}COOH$), and C) perfluorobutane sulfonate, PFBS ($C_4F_9SO_3^-$)…………………… 30

Figure 1.2 Chemical structure of linear PFOS and branched isomers, perfluoroisopropyl (6-CF_3-PFOS), 5-perfluoromethyl (5-CF_3-PFOS), 4-perfluoromethyl (4-CF_3-PFOS), 3-perfluoromethyl (3-CF_3-PFOS) and 1-perfluoromethyl (1-CF_3-PFOS)………………….. 35

Figure 1.3 Production of PFOS and related compound by 3M Company in 2,000………………………………………………………… 38

Figure 2.1 Molecular structure of perfluoroalkyl surfactants. A) PFOS and B) TH-PFOS……………………………………………………….. 84

Figure 2.2 ^{19}F NMR spectrum of an aqueous PFOS sample (31.5 mg L^{-1}) relative to the internal standard, 4'-(trifluoromethoxy)acetanilide, 4-TFMeAc, (-58.08 ppm)…………………………………………… 85

Figure 2.3 ^{19}F NMR spectra of aqueous solutions containing a mixture of PFOS and TH-PFOS in a 1:1 molar fluoride ratio (100 mg PFOS L^{-1}: 112 mg TH-PFOS L^{-1}) (upper panel) and PFOS only solution (100 mg L^{-1}) (lower panel)………………………………………………… 88

Figure 2.4 Calibration curve of aqueous PFOS based on the terminal CF_3 peak by ^{19}F NMR ($r^2 > 0.98$)…………………………………………… 90

Figure 2.5 HPLC-suppressed conductivity detection chromatogram of aqueous PFOS (25 mg L^{-1})…………………………………………………… 93

Figure 2.6 Calibration curve of aqueous PFOS by HPLC suppressed conductivity detection ($r^2 > 0.99$)…………………………………… 96

Figure 2.7 HPLC-suppressed conductivity detection chromatograph of an aqueous solution containing PFBS, PFOA, and PFOS (21 mg L^{-1} each)………………………………………………………………… 97

Figure 2.8 Calibration curve of aqueous PFOS by TOC analysis ($r^2 > 0.99$)…... 100

Figure 3.1 Chemical structures of perfluorinated compounds evaluated in this study. A) Perfluorosulfonates: perfluorohexane sulfonate (PFHXs), perfluorooctane sulfonate (PFOS) and perfluorodecano sulfonate (PFDS). B) Perfluorosulfonamides: 2-(N-methylperfluorooctanesulfonamido) acetate (*N*-MeFOSAA) and 2-(N-ethylperfluorooctanesulfonamido) acetate (*N*-EtFOSAA). C) Perfluorocarboxylates: perfluorooctanoic acid (PFOA) and perfluorodecanoic acid (PFDA)…………………………………... 115

Figure 3.2 LC-MS chromatograms of 5 mg L^{-1} of PFHXs, PFOA, PFOS, PFDA and PFDS in a mixed standard solution (top) and individual standard solutions…………………………………………………… 122

LIST OF FIGURES – *Continued*

Figure 3.3 Negative ESI/MS spectra of a mixed standard solution containing 5 mg L^{-1} of PFHXs, PFOA, PFOS, PFDA and PFDS obtained under SIM analysis………………………………………………………… 123

Figure 3.4 SIM analysis of a sewage sludge sample in Ina Road WWTP from Tucson, Arizona. A) Typical LC-MS chromatogram and, B) MS/MS spectrum of extract sludge sample spiked with 0.5 mg PFDS L^{-1}………………………………………………………….. 129

Figure 4.1 Sequential reduction of PCE to ethane by anaerobic reductive dechlorination……………………………………………………….. 143

Figure 4.2 Time course of the methane formation by a methanogenic microbial consortium in anaerobic granular sludge (1.5 g VSS L^{-1}) in the presence of increasing concentrations of PFOS (in mg L^{-1}): (■) 0; (○) 50; (▲) 125; (□) 250, and (●) 500………………………………. 154

Figure 4.3 Chemical structures of short-chain perfluorocarboxylates. I) C_2: A) trifluoroacetic acid. II) C_3: A) pentafluoropropionic acid, PFPA, B) 3H-tetrafluo-ropropionic acid, 3H-PFPA, C) 3,3,3-trifluoropropionic acid, 2H,2H-PFPA and D) 2,2-bis(trifluoromethyl)propionic acid, $2CH_3,2CF_3$-PFPA. II) C_5: A) perfluoropentanoic acid, PFPeA, B) 5H-perfluoropentanoic acid, 5H-PFPeA and C) 1H,1H,2H,2H,3H,3H-perfluoropentanoic acid, 1H,1H,2H,2H,3H,3H-PFPeA……………………………………….. 159

Figure 4.4 Concentrations of total organic carbon (TOC) and PFOS as a function of time during the electrochemical of PFOS in a BDD batch reactor operated at a current of 10 mA. Legend: (□) TOC and (●) PFOS…………………………………………………………….. 161

Figure 4.5 Concentrations of total organic carbon (TOC) and PFBS as a function of time during the electrochemical of PFBS in BDD flow-through reactor operated with a current density of 2.5 mA cm^{-2}. Legend: (□) TOC and (▲) PFBS…………………………………… 163

Figure 4.6 ^{19}F-NMR spectra of technical PFOS (214 mg L^{-1}) (upper panel) and PFOS subjected to electrochemical treatment for 18 h (lower panel) in a BDD batch reactor operated at a current of 10 mA…………….. 165

Figure 4.7 ^{19}F-NMR spectra of PFBS (120 mg L^{-1}) and PFBS subjected to electrochemical attack for 24 h in a BDD flow-through reactor operated with a current density of 2.5 mA cm^{-2}…………………… 166

Figure 4.8 Normalized hydrogenotrophic methanogenic activities of PFOS samples electrolyzed for 6, 12, 18 and 24 h. Error bars (shown if larger than the symbols) represent standard deviations of duplicate assays…………………………………………………………….... 169

LIST OF FIGURES – *Continued*

Figure 4.9 Normalized hydrogenotrophic methanogenic activities of PFBS samples subjected to electrochemical attack for different time periods. Error bars (shown if larger than the symbols) represent standard deviations of duplicate assays………………………….. 170

Figure 5.1 Proposed mechanism of the biomimetic reductive dehalogenation of PFOS with vitamin B_{12} and Ti(III) citrate………………………….. 191

Figure 5.2 Chemical structure of linear PFOS and branched isomers, perfluoroisopropyl (6-CF_3-PFOS), 5-perfluoromethyl (5-CF_3-PFOS), 4-perfluoromethyl (4-CF_3-PFOS), 3-perfluoromethyl (3-CF_3-PFOS) and 1-perfluoromethyl (1-CF_3-PFOS)………………….. 200

Figure 5.3 Effect of temperature on the biomimetic reductive dehalogenation of technical PFOS (332 μM) with vitamin B_{12} (260 μM) and Ti(III) citrate (36 mM) and pH 9.0 on day 36, 12 and 7 at 30, 50 and 70°C, respectively. Legend: control samples (PFOS + Ti(III) citrate) (white bars) and treatment samples (PFOS + Ti(III) citrate + vitamin B_{12}) (black bars). Error bars (shown if larger that the symbols) represent standard deviations of triplicate assays…………………… 202

Figure 5.4 Arrhenius plot of the rate constant of fluoride release during the biomimetic reductive dehalogenation of technical PFOS (332 μM) with vitamin B_{12} and Ti(III) citrate (36 mM) at pH 9.0 at different temperatures……………………………………………………… 205

Figure 5.5 Effect of vitamin B_{12} concentration on the biomimetic reductive dehalogenation of technical PFOS (332 μM) with vitamin B_{12} and Ti(III) citrate (36 mM) at pH 9.0 in samples treated for 36 days at 30ºC (▲) and 7 days at 70ºC (■). Error bars (shown if larger that the symbols) represent standard deviations of triplicate assays…….. 206

Figure 5.6 Effect of Ti(III) citrate dosages on the biomimetic reductive dehalogenation of technical PFOS (332 μM) with vitamin B_{12} (260 μM) and Ti(III) citrate at pH 9.0 in samples treated for 36 days at 30ºC (▲) and 7 days at 70ºC (■). Error bars (shown if larger that the symbols) represent standard deviations of triplicate assays…….. 207

Figure 5.7 Effect of solution pH on the biomimetic reductive dehalogenation of technical PFOS (332 μM) with vitamin B_{12} (260 μM) and Ti(III) citrate (36 mM) in samples treated for 36 days at 30ºC (▲) and 7 days at 70ºC (■). Error bars (shown if larger that the symbols) represent standard deviations of triplicate assays…………………… 208

LIST OF FIGURES – *Continued*

Figure 5.8 Effect of buffers on the biomimetic reductive dehalogenation of technical PFOS (332 μM) with vitamin B_{12} (260 μM) and Ti(III) citrate (36 mM) in samples treated for 7 days at 70ºC. Legend: control samples (PFOS + Ti(III) citrate) (white bars) and treatment samples (PFOS + Ti(III) citrate + vitamin B_{12}) (black bars). Error bars (shown if larger that the symbols) represent standard deviations of triplicate assays…………………………………………………… 210

Figure 5.9 Effect of catalyst on the biomimetic reductive dehalogenation of technical PFOS (332 μM) with vitamin B_{12} (260 μM) and Ti(III) citrate (36 mM) at 70°C and pH 9.0 on day 7. The concentrations of cobalt (II) in treatment samples were 260 uM (PFOS-Ti-Co) and 5,236 uM (PFOS-Ti-20xCo). Legend: control samples (PFOS + Ti(III) citrate) (white bars) and treatment samples (PFOS + Ti(III) citrate + catalyst) (black bars). Error bars (shown if larger that the symbols) represent standard deviations of triplicate assays……….. 212

Figure 5.10 Time course of fluoride release of technical PFOS (332 μM) during the biomimetic reductive dehalogenation with vitamin B_{12} (260 μM) and Ti(III) citrate (36 mM) at 70ºC and solution pH 9.0 monitored by fluoride-selective electrode. Legend: control samples (PFOS + Ti(III) citrate) (○) and treatment samples (PFOS + Ti(III) citrate + vitamin B_{12}) (●). Error bars (shown if larger that the symbols) represent standard deviations of triplicate assays…………………… 214

Figure 5.11 ^{19}F NMR of spectrum of technical PFOS (332 μM) during the biomimetic reductive dehalogenation with vitamin B_{12} (260 μM) and Ti(III) citrate (36 mM) at 70ºC and pH 9.0 in samples treated for 7 days. Chemical shifts are referenced to the internal standard, 4-TFMeAc, at -58.08 ppm…………………………………………….. 215

Figure 5.12 Time course disappearance of branched PFOS isomers (54 μm) in the chemical reductive dehalogenation with vitamin B_{12} (260 μM) and Ti(III) citrate (36 mM) at 70ºC and pH 9.0 based on HPLC-suppressed conductivity detection analysis. Control samples (PFOS + Ti(III) citrate) (○) and treatment samples (PFOS + Ti(III) citrate + vitamin B_{12}) (●). Error bars (shown if larger that the symbols) represent standard deviations of triplicate assays…………………… 220

Figure 5.13 Pseudo-first order plot for the degradation of branched PFOS isomers (54 μM) with reaction time. Samples were incubated vitamin B_{12} (260 μM) and Ti(III) citrate (36 mM) at 70ºC and pH 9.0 for 7 days. Error bars (shown if larger that the symbols) represent standard deviations of triplicate assays…………………… 221

LIST OF FIGURES – *Continued*

Figure 5.14 LC-MS/MS chromatograms of branched PFOS isomers in control samples (PFOS + Ti(III) citrate) (thick line) and treatment samples (PFOS + Ti(III) citrate + vitamin B_{12}) (thin line) during the biomimetic reductive dehalogenation of branched PFOS isomers (54 μM) with vitamin B_{12} (260 μM) and Ti(III) citrate (36 mM) at 70ºC and pH 9.0 on day 7…………………………………………………. 222

Figure 5.15 ^{19}F NMR spectra of branched PFOS isomers in control (upper panel) and treatment samples (lower panel) during the biomimetic reductive dehalogenation of branched PFOS isomers (54 μM) with vitamin B_{12} (260 μM) and Ti(III) citrate (36 mM) at 70ºC and pH 9.0 on day 7. Chemical shifts are referenced to the internal standard, 4-TFMeAc, at -58.08 ppm………………………………………... 223

Figure 5.16 EPR spectra of the chemical reductive dehalogenation of technical PFOS (332 μM) with vitamin B_{12} (260 μM) and Ti(III) citrate (36 mM) at 70ºC and pH 9.0 on day 7 in the presence of the spin trap DMPO (27 mM). Legend: (A) PFOS + Ti(III) citrate + B_{12} + DMPO, (B) Ti(III) citrate + B_{12} + DMPO, and (C) PFOS + Ti(III) citrate + DMPO... 226

Figure 6.1 Chemical structure of linear PFOS and branched isomers, perfluoroisopropyl (6-CF_3-PFOS), 5-perfluoromethyl (5-CF_3-PFOS), 4-perfluoromethyl (4-CF_3-PFOS), 3-perfluoromethyl (3-CF_3-PFOS) and 1-perfluoromethyl (1-CF_3-PFOS)………………….. 251

Figure 6.2 HPLC-suppressed conductivity chromatogram of PFOS isomers concentrated by SPE during the preparative HPLC fractionation. Legend: Branched *f* (blue), branched *s* (black), branched *0* (orange), branched *1* (green), branched *2* (navy blue), branched *3* (brown) and *linear PFOS* (red). The methanol concentration is displayed in black in the secondary Y-axis…………………………………………... 253

Figure 6.3 LC-MS/MS chromatograms of technical PFOS and branched PFOS isomers fractions purified by preparative HPLC. Legend: Technical PFOS (black), branched *A* (red) and fraction *B* (blue)……………… 256

Figure 6.4 HPLC-suppressed conductivity detection chromatogram of purified branched PFOS isomers (343 μM) employed in the biomimetic reductive dehalogenation experiments………………………………. 258

Figure 6.5 ^{19}F NMR spectra of technical PFOS (332 μM) (upper panel) and branched PFOS isomers (343 μM) concentrated and purified by SPE followed by preparative HPLC (lower panel)……………………….. 259

LIST OF FIGURES – *Continued*

Figure 6.6 ^{19}F NMR spectra of the precipitate in treatment samples (PFOS + Ti(III) citrate + vitamin B_{12}) during the chemical reductive dehalogenation of branched PFOS isomers (343 µM) with vitamin B_{12} (260 µM) and Ti(III) citrate (36 mM) at 70ºC and pH 9.0 on day 7. Precipitate extracted with methanol (upper panel) and diethyl ether (lower panel)………………………………………………….. 262

Figure 6.7 Time course of fluoride release of purified branched PFOS isomers (343 µM) with vitamin B_{12} (260 µM) and Ti(III) citrate and Ti(III) ($TiCl_3$) (36 mM) at 70ºC and pH on day 7 in treated samples by fluoride-selective electrode. Legend: bicarbonate buffer: control samples (PFOS + Ti(III) citrate) (○) and treatment samples (PFOS + Ti(III) citrate + vitamin B_{12}) (●) and phosphate buffer: control samples (PFOS + Ti(III)) (∆) and treatment samples (PFOS + Ti(III) + vitamin B_{12}) (▲). Error bars (shown if larger that the symbols) represent standard deviations of triplicate assays…………………… 265

Figure 6.8 SIM (m/z 44) GC-MS chromatograms of the headspace of the biomimetic reduction of branched PFOS isomers (343 µM) with vitamin B_{12} (260 µM) and Ti(III) ($TiCl_3$) (36 mM) at 70ºC and pH 9.0 on day 7. Legend: control samples (PFOS + Ti(III)) (thin line) and treatment samples (PFOS + Ti(III) + vitamin B_{12}) (thick line)… 267

Figure 6.9 SIM (m/z 69) GC-MS chromatograms of the headspace of the biomimetic reduction of branched PFOS isomers (343 µM) with vitamin B_{12} (260 µM) and Ti(III) ($TiCl_3$) (36 mM) at 70ºC and pH 9.0 on day 7. Legend: control samples (PFOS + Ti(III)) (thin line) and treatment samples (PFOS + Ti(III) + vitamin B_{12}) (thick line)… 269

Figure 6.10 GC/MS spectrum of the treatment sample (PFOS + Ti(III) + vitamin B_{12}) of the reductive dehalogenation of branched PFOS isomers (343 µM) with vitamin B_{12} (260 µM) and Ti(III) (36 mM) at 70ºC and pH 9 on day 7…………………………………………………… 270

Figure 6.11 GC/MS spectrum of the headspace of aqueous 1H-perfluorohexane standard (76 mg L^{-1})……………………………………………… 271

Figure 6.12 Tentative chemical structure of the volatile fluorinated compounds detected in the headspace of reaction flasks following reductive dehalogenation of branched PFOS isomers (343 µM) with vitamin B_{12} (260 µM) and Ti(III) (36 mM) at 70ºC and pH 9.0 on day 7. Legend: A) 2H-perfluorohexane, B) 3H-perfluorohexane, and C) 1H-perfluoroethane………………………………………………….. 272

LIST OF FIGURES – *Continued*

Figure 7.1 Adsorption isotherms of PFOS onto granular activated carbon at intermediate-high equilibrium concentrations. Legend: Calgon F400 (■), Calgon F300 (●), and Calgon URV-MOD 1 (▲). Experimental data fit to Langmuir model (dashed line); experimental data fit to Freundlich model (solid line). Error bars (shown if larger that the symbols) represent standard deviations of duplicate assays………… 294

Figure 7.2 Adsorption isotherm of PFOS onto granular activated carbon Calgon F400 at low equilibrium concentrations. Experimental data fit to Freundlich model. Error bars (shown if larger that the symbols) represent standard deviations of duplicate assays………… 295

Figure 7.3 Adsorption isotherms of PFOS (■), PFOA (▲), and PFBS (●) PFBS onto activated carbon Calgon F400. Experimental data fit to Langmuir model (dashed line); experimental data fit to Freundlich model (solid line). Error bars (shown if larger that the symbols) represent standard deviations of duplicate assays…………………... 300

Figure 7.4 Adsorption isotherms of PFOS onto various zeolite types: NaY80 (Si/Al 80) (■); NaY (Si/Al 5.5) (●), and 13X (Si/Al 2.8). Experimental data fit to Langmuir model (dashed line); experimental data fit to Freundlich model (solid line). Error bars (shown if larger that the symbols) represent standard deviations of duplicate assays…………………………………………………... 303

Figure 7.5 Adsorption isotherms of PFOS onto different wastewater treatment sludges: anaerobic granular sludge (■), anaerobic digested sludge or ADS (▲), aerobic activated sludge or RAS (●). Experimental data fit to Langmuir model (dashed line); experimental data fit to Freundlich model (solid line). Error bars (shown if larger that the symbols) represent standard deviations of duplicate assays………… 306

Figure 8.1 Time course of ammonium consumption by a mixed microbial culture obtained form the nitrification stage of a full-scale municipal wastewater treatment plant (0.5 g VSS L^{-1}) in the presence of increasing F^- concentrations (in mg L^{-1}): (●) 0, (○) 50, (ж) 90, (■) 130, (□) 180, (Δ) 230, and (▲) 300………………………………… 332

Figure 8.2 (A) Inhibitory effect of F^- on the nitrifying specific activity of a mixed microbial culture obtained from the nitrification stage of a full-scale municipal wastewater treatment plant (0.5 g VSS L^{-1}) as measured by ammonia depletion. (B) Inhibitory effect of F^- on the denitrifying specific activity of an industrial anaerobic granular sludge (1.0 g VSS L^{-1}) as determined by nitrate depletion. The nitrifying and denitrifying specific activities were normalized with respect to the control. Error bars (shown if larger than the symbols) represent standard deviations of duplicate assays………………….. 337

LIST OF FIGURES – *Continued*

Figure 8.3 (A) Inhibitory effect of F^- on the glucose-degrading specific activity of an enrichment culture obtained from aerobic wastewater treatment sludge. (B) Inhibitory effect of F^- on the glucose fermentation by a mixed anaerobic culture inoculated with 1.5 g VSS L^{-1} anaerobic granular sludge (■), and an anaerobic glucose-degrading enrichment culture (●). The glucose-degrading specific activities were normalized with respect to the control in which no contaminant was added. Error bars (shown if larger than the symbols) represent standard deviations of triplicate assays………… 339

Figure 8.4 Inhibitory effect of F^- on the activity of mesophilic and thermophilic acetoclastic (A and B) and hydrogenotrophic (C and D) methanogens, respectively. Bioassays were conducted with Ina Road anaerobically digested sludge (A and C) and Hyperion anaerobically-digested thermophilic sludge (B and D), respectively. Legend: (●) First substrate feeding and (○) second substrate feeding. The methanogenic activities were normalized with respect to the control. Error bars (shown if larger than the symbols) represent standard deviations of triplicate assays…………………… 343

Figure 8.5 Inhibitory effect of F^- on the maximum specific activity of propionate (A) and butyrate (B) utilization by mesophilic anaerobic consortium (Aviko sludge). The activities were normalized with respect to the control. Error bars (shown if larger than the symbols) represent standard deviations of triplicate assays…………………… 347

ABSTRACT

Perfluorooctane sulfonate (PFOS) and related perfluoroalkyl surfactants (PFAS) are ubiquitous contaminants of increasing public concern due to their environmental persistence, toxicity, and bioaccumulation. These perfluorinated compounds have been used for more than half a century in a wide variety of industrial and consumer products ranging from stain repellents such as Teflon® to aqueous fire-fighting foams and to grease-proof food packing. The public health and environmental risks posed by PFAS have driven environmental agencies and the industry to restrict their use to specific applications where they cannot be replaced by other chemicals.

The sources and pathways of PFOS and its derivatives in the environment are not well understood. Analysis of environmental samples is critical to understand the fate, transport and persistence of these emerging contaminants. Techniques based on fluorine nuclear magnetic resonance (^{19}F NMR) spectroscopy and high performance liquid chromatography (HPLC) with suppressed conductivity detection were successfully developed to monitor the presence of PFAS in water samples. Chromatographic separation of C_4 to C_8 PFAS surfactants was achieved using a C_{18} reversed-phase column and a mobile phase consisting of a mixture of boric acid and acetonitrile at mixing ratios ranging from 75:25 to 45:55 (v/v). The combination of these two techniques was very effective for characterization and routine quantification of PFOS and related chemicals. Analytical methods based on ^{19}F NMR, HPLC-suppressed conductivity detection, and liquid chromatography with tandem mass spectrometry (LC-MS/MS) were employed to

characterize commercial PFOS samples. Linear and branched PFOS isomers in a percentage ratio of 75:25 were identified.

Municipal wastewater treatment systems are one of the major sources of PFAS emissions into the environment. The presence of PFAS in sewage sludge from two wastewater treatment plants in Tucson, Arizona, was investigated. Sludge samples were washed with acetic acid and extracted with a mixture of acetic acid and methanol. The extract was cleaned and concentrated by means of solid phase extraction. LC-MS/MS operating in the selective ion monitoring (SIM) mode was employed to assess the presence of perfluorosulfonates, perfluorosulfonamides, and perfluorocarboxylates in sewage sludge samples. PFOS was the only perfluoroalkyl chemical detected in municipal sludge samples at a concentration of 77 ± 5 μg kg^{-1} sludge dry weight.

Cost-effective treatment techniques for removing PFAS from industrial effluents are needed to minimize discharges of these pollutants. Reductive dehalogenation is widely applied to the degradation of highly chlorinated compounds. Hence, the susceptibility of PFOS and related compounds to biological and chemical reductive dehalogenation was evaluated in batch assays. PFAS were not reductively dehalogenated by different microbial consortia even after periods of incubation exceeding 2 y, confirming the high resistance of these compounds to microbial degradation. The anaerobic biodegradability of PFOS and perfluorobutane sulfonate (PFBS) samples exposed to electrochemical pretreatment with boron-doped diamond film electrodes was also investigated. The oxidation decreased the concentration of PFAS and dissolved organic carbon in solution, confirming the destruction of these compounds. However, the

oxidative treatment did not enhance the susceptibility of PFAS to microbial degradation even after extended periods of incubation (> 1 y).

In contrast, PFOS was reductively dehalogenated with a biomimetic system based on vitamin B_{12} as the catalyst and Ti(III) citrate as the reducing agent. The optimal treatments conditions of the reaction were 260 µM vitamin B_{12}, 36 mM Ti(III) citrate, 70°C and solution pH 9.0. Interestingly, branched PFOS isomers were more prone to degradation by vitamin B_{12} catalysis compared to the linear isomer. Removal of 3 mol F^- per mol of technical PFOS and 12 mol F^- per mol of branched PFOS isomers was achieved. Defluorination of PFOS was also observed at environmental relevant conditions of 30°C and pH 7.0, albeit at lower degradation rates. Fluoride and carbon dioxide were identified as the major products of the chemical defluorination. Traces of partially fluorinated volatile compounds were also detected in the headspace.

The feasibility of removing PFAS compounds from aqueous streams by sorption onto granular activated carbon (GAC), zeolite, and wastewater treatment sludge was examined in batch isotherm experiments. The fluorocarbon chain and the functional group influenced sorption of the anionic surfactants, PFOS adsorbed more strongly to GAC than perfluorooctanoic acid (PFOA) and PFBS. Activated carbon showed the highest affinity for PFOS (Freundlich K_F values of 36.7 to 60.9) followed by the hydrophobic, high-silica zeolite NaY (Si/Al 80, K_F of 31.8) and lastly anaerobic sludge (K_F of 0.95 to 1.85). GAC sorption is a suitable treatment for the removal of anionic perfluoroalkyl surfactants when present at low concentrations.

Fluoride has been identified as the major product of the reductive dehalogenation of PFOS and derivatives. Thus, the toxicity of inorganic fluoride towards the main microbial populations responsible for the removal of organic constituents and nutrients in wastewater treatment processes was also studied. Fluoride concentrations ranging from 18 to 43 mg L^{-1} caused 50% inhibition (IC_{50}) of the activity of propionate- and butyrate-degrading microorganisms and of acetate-utilization by methanogens evaluated under mesophilic and thermophilic conditions. All other microbial populations evaluated in this study, *i.e.*, glucose fermenters, aerobic glucose-degrading heterotrophs, denitrifying bacteria, and H_2-utilizing methanogens tolerated fluoride at very high concentrations (> 500 mg L^{-1}). In the same manner, H_2-utilizing methanogens also tolerated PFOS and PFBS at concentrations as high as 200 and 500 mg L^{-1}, respectively.

OBJECTIVES

Aim

The aim of this research was to develop physico-chemical and biological methods to remove perfluorooctane sulfonate and related perfluorinated surfactants from industrial effluents.

Specific Objectives

The specific objectives of this PhD dissertation are as follows:

I. Develop analytical techniques to monitor PFOS and related perfluoroalkyl surfactants (PFAS) in liquid and solid environmental samples.

II. Apply analytical techniques based on LC-MS/MS to determine the significance of PFAS in discharges from selected municipal wastewater treatment plants in Tucson, Arizona.

III. Identify and characterize the various structural PFOS isomers present in commercial samples.

IV. Investigate the susceptibility of technical PFOS and branched PFOS isomers to chemical reductive dehalogenation with vitamin B_{12} and Ti(III) citrate.

V. Evaluate the anaerobic biodegradability and the methanogenic toxicity of PFOS and related PFAS compounds.

VI. Assess the impact of electrochemical pretreatment on the susceptibility of PFOS and perfluorobutane sulfonate (PFBS) to microbial degradation.

VII. Study the feasibility of removing PFOS and related PFAS surfactants, *i.e.*, perfluorooctanoic acid (PFOA) and PFBS from aqueous solutions by adsorption onto various types of granular activated carbon, zeolite and wastewater treatment sludge.

VIII. Assess the inhibitory effect of inorganic fluoride, a degradation product of the reductive dehalogenation of PFOS and related chemicals, towards the main microbial populations involved in biological wastewater treatment processes

CHAPTER 1

Introduction

1.1 Chemistry

Perfluorinated alkyl surfactants (PFAS) are fully fluorinated compounds in which all the hydrogen atoms have been replaced by fluorine atoms. They have a molecular formula of $CF_3(CF_2)_nX$ where n usually varies from 3 to 11 and X could be a hydroxyl group, carboxylic acid, sulfonic acid, quaternary ammonium group, or other substituents [1]. Among these chemicals, perflurooctane sulfonate (PFOS) and perflurooctanoic acid (PFOA), are the most studied fluorinated alkyl surfactants due to their global distribution, environmental persistence and bioaccumulative potential.

Per fluorinated alkyl surfactants such as PFOS and PFOA are a unique class of chemicals which exhibit hydrophobic and oleophobic (oil-repellent) characteristics [1]. The non-polar perfluorocarbon tail of PFAS is more effective in reducing surface tension compared to hydrocarbon chains, improving the wetting properties and stability of these surfactants under harsh conditions [1, 2].

PFAS are the most chemically and thermally stable fluorinated alkyl substances. The unusual properties of these compound arise from the nature of the fluorine element

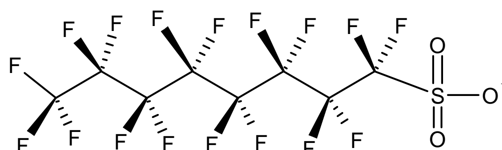
[1]. The strength of the carbon-fluorine bond (485 KJ mol^{-1}), the presence of three non-bonding electron pairs in the outer shell of the fluorine atom, the effective shielding of carbon by the fluorine atom, the rigidity of perfluorinated chain and the absence of structures susceptible to electrophilic or nucleophilic attack make fluorinated surfactants inert to acids, bases, oxidizing and reducing agents, and microbial attack [1, 3, 4].

The structural formula of PFOS is illustrated in Figure 1.1. The physical and chemical properties of PFOS as the potassium salt are listed in Table 1.1. PFOS, a key ingredient in 3M's popular Scotchgard ™ fabric and surface protector, has been detected in blood from general populations, wildlife biota, and wastewaters samples throughout the world [5-8]. PFOS is the final degradation product of the perfluoro alkyl sulfonate family [9-11].

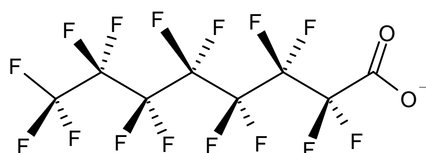
Table 1.1 Physical and chemical properties of PFOS as the potassium salt [8].

Properties	PFOS potassium salt
CAS number	275-39-3
Molecular weight	538.22 g mol^{-1}
Solubility	570 mg L^{-1} in pure water, 370 mg L^{-1} in fresh water and 12.4 mg L^{-1} in natural sea water.
Appearance	White powder
Melting point	≥ 400ºC
Vapor pressure	3.31 x 10^{-4} Pa at 20ºC
Henry's law constant	3.19 10^{-4} Pa m^3 mol^{-1}
Log Kow	Not possible to determine at neutral pH
pKa	-3.27 (calculated)

A)



B)



C)

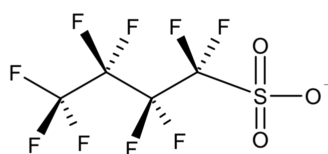


Figure 1.1 Chemical structures of perfluorinated compounds of environmental importance. Legend: A) perfluorooctane sulfonate, PFOS ($C_8F_{17}SO_3^-$), B) perfluorooctanoic acid, PFOA ($C_7F_{15}COOH$), and C) perfluorobutane sulfonate, PFBS ($C_4F_9SO_3^-$).

The structural formula PFOA is illustrated in Figure 1.1. The main physical and chemical properties of the compound are listed in Table 1.2. PFOA is an anthropogenic perfluorinated compound commonly used in the manufacturing of fluoroelastomers and fluoropolymers including polytetrafluoroethylene (Teflon®). PFOA is the ultimate product of the degradation of telomers and other perfluorinated chemicals [12, 13].

Table 1.2. Physical and chemical properties of PFOA [12].

Properties	PFOA
CAS number	335-67-1
Molecular weight	430.06 g mol^{-1}
Solubility	3.4 g L^{-1}
Melting point	40 - 50ºC
Boiling point	189 - 192ºC
Vapor pressure	1.33 x 10^{-5} Pa at 25ºC
Henry's law constant	Not available
Log Kow	Not possible to determine at neutral pH
pKa	2.5

The chemical structure of PFBS is illustrated in Figure 1.1. The physical and chemical properties of PFBS are listed in Table 1.3. PFBS, a four-carbon chain fluorinated alkyl surfactant, is considered one of the alternatives to PFOS and related compounds [14]. In fact, PFOS has been replaced by PFBS in Scotchgard ™ products of 3M [15].

Table 1.3 Physical and chemical properties of PFBS [16].

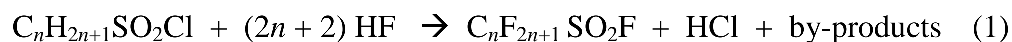
Properties	PFBS potassium salt
CAS number	29420-49-3
Molecular weight	329.21 g mol^{-1}
Solubility	46.2 g L^{-1} at 20ºC
Appearance	White powder
Density	Not available
Melting point	270.4ºC
Boiling point	200ºC
Vapor pressure	< 1.22 X 10^{-5} Pa at 20ºC
Henry's law constant	9.06 X 10^{-8} Pa m^3 mol^{-1}
Log Kow	Not possible to determine at neutral pH
pKa	Fully dissociated in water over pH 4 - 9

1.2 Synthesis

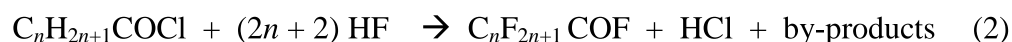
All perfluorinated compounds found in the environment are anthropogenic. PFOS and derivatives have been in the market for half a century in well-known consumer products such as Teflon®, Scotchgard®, Gore-Tex®, Silverstone®, and Stanimaster® [12].

The majority of perfluorinated compounds are commercially synthesized by two methods electrochemical fluorination process (EFC) which uses anhydrous hydrofluoric acid as the fluorine source and telomerization where tetrafluoroethylene and perfluoroalkyl iodide are the starting materials [1].

In the electrochemical fluorination process (EFC), an electric current is passed through the organic substance previously dissolved in anhydrous hydrogen fluoride. All C-H bonds are replaced by C-F bonds but some functional groups are retained. The EFC process primarily yields sulfonyl (reaction 1) and carbonyl (reaction 2) halides [1].



$$C_nH_{2n+1}SO_2Cl + (2n+2)\,HF \rightarrow C_nF_{2n+1}SO_2F + HCl + \text{by-products} \quad (1)$$



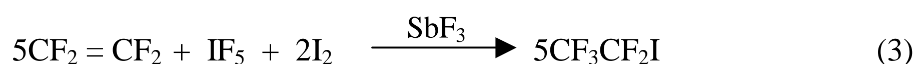
$$C_nH_{2n+1}COCl + (2n+2)\,HF \rightarrow C_nF_{2n+1}COF + HCl + \text{by-products} \quad (2)$$

Because the EFC process is not efficient or selective, a complex chemical mixture varying in molecular weight and chain length is obtained. Perfluoroalkyl isomers with linear and branched fluorochemical tails, homologues series of even- and odd- carbons numbered, cyclic perfluoroalkanes, ethers and other byproducts of unknown composition are formed [12]. In fact, the EFC process yields only 35 to 40% linear perflurooctanesulfonyl fluoride [2]. The generated perfluorosulfonyl and perfluorocarbonyl chemicals are intermediates for the manufacture of commercial and industrial perfluorinated surfactants. Perfluorooctanesulfonyl fluoride ($C_8F_{17}SO_2F$) is enzymatic or chemically hydrolyzed to produce PFOS in the acid ($C_8F_{17}SO_3H$) and salt form ($C_8F_{17}SO_3M$, where *M* is a cation) [1].

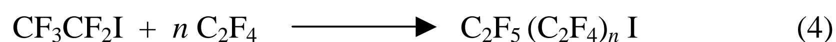
Commercial PFOS produced by the ECF process is a mixture of linear and branched isomers as described above. The content of branched isomers varies with each batch production between 20 to 30% of the total mass [17-19]. The isomer distribution of PFOS compounds in commercial samples has been studied by LC-MS/MS

chromatography [20-22] and ^{19}F NMR spectroscopy [17, 18, 23]. The structures of the perfluoromonomethyl and perfluoroisopropyl PFOS isomers are depicted in Figure 1.2.

In the telomerization process, iodine pentafluoride and iodine react with tetrafluoroethylene to generate pentafluoroethyl iodide, known as telogen (reaction 3). Telogen then reacts with tetrafluoroethylene to produce a mixture of perfluoroalkyl iodides (reaction 4) [1],



$$5CF_2 = CF_2 + IF_5 + 2I_2 \xrightarrow{SbF_3} 5CF_3CF_2I \quad (3)$$



$$CF_3CF_2I + n\, C_2F_4 \longrightarrow C_2F_5\,(C_2F_4)_n\, I \quad (4)$$

In contrast to the EFC products, the perfluorinated compounds obtained during the telomerization process are mixtures of linear and even-carbon numbered telomers with different carbon chain lengths (*n*) that can be purified by distillation (98%) [1]. The perfluoroalklyl iodides obtained are reacted with ethylene to form perfluoroalkylethyl iodides ($C_2F_5(C_2F_4)_nCH_2CH_2I$) which can be converted to olefins, alcohols, thiocyanates, sulfonly chloride, and thiols [1, 3]. These fluorotelomers are used as intermediates in the manufacture of perfluorinated surfactants.

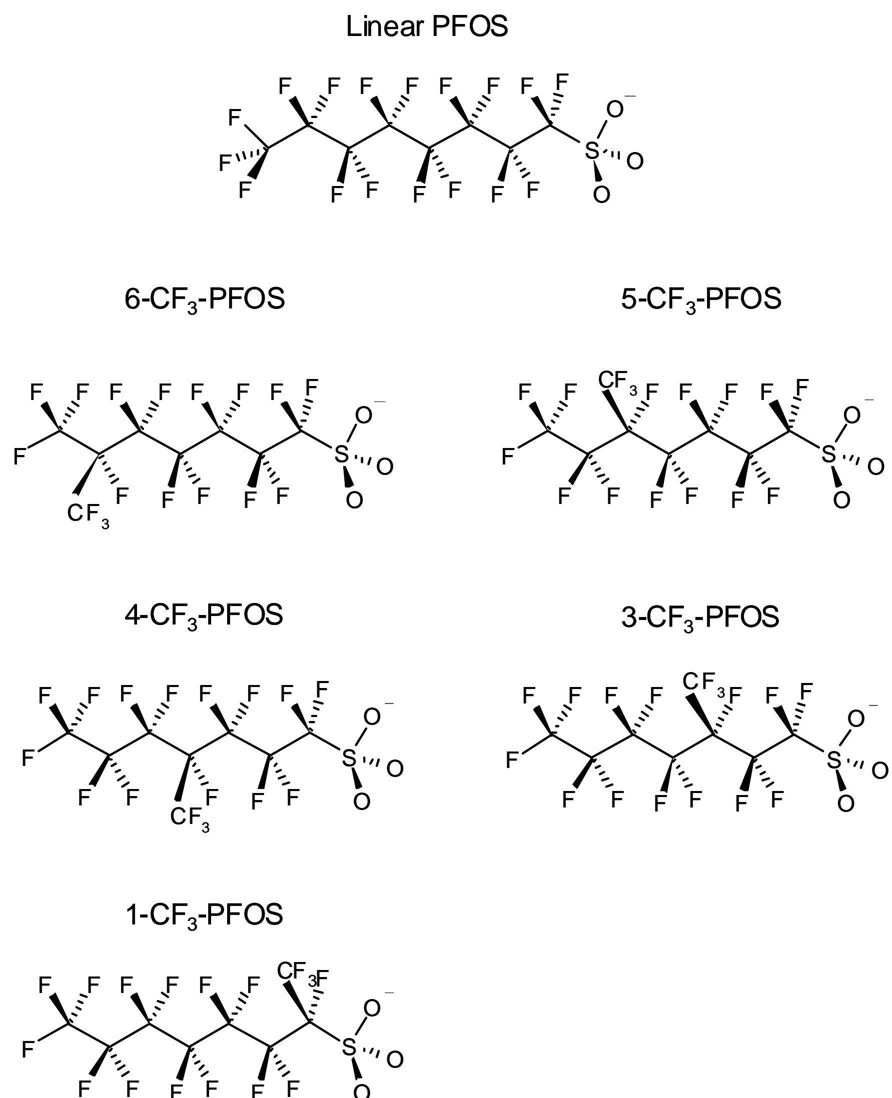


Figure 1.2 Chemical structure of linear PFOS and branched isomers, perfluoroisopropyl (6-CF_3-PFOS), 5-perfluoromethyl (5-CF_3-PFOS), 4-perfluoromethyl (4-CF_3-PFOS), 3-perfluoromethyl (3-CF_3-PFOS) and 1-perfluoromethyl (1-CF_3-PFOS).

1.3 Products and Uses

3M Company has been the only manufacturer of PFOS and related perfluorosulfonyl substances in the United States. PFOS compounds are important ingredients in many consumer and industrial products due to their unique physico-chemical properties. Their ability to repel grease, water and oil make them excellent surface-active agents in different industrial applications. Their chemical and thermal stability are responsible for the use in high temperatures and strong acid and base applications [1, 3, 4].

3M phased out PFOS and related chemicals from the production lines at the end of 2,002. Increasing reports confirming the environmental persistence of PFOS and its tendency to accumulate in human and animal tissue motivated 3M to voluntarily eliminate these surfactants from the market [24]. 3M also reduced the manufacture of PFOA. Nevertheless, PFOA and its salts are still being produced by other companies such as DuPont for the manufacturing of Teflon® and similar products [12]. The U.S. Environmental Protection Agency (EPA) has asked the manufacturers of fluoropolymers to eliminate the use of PFOA and precursors from emissions and consumer products no later than 2,015 [25].

In 2000, 3M produced approximately 6.5 million pounds of PFOS-containing products which can be divided into three main categories of use: paper protection, surface treatment and performance chemicals [26] (Figure 1.3). The production of PFOS in the U.S. decreased by 93% by the end of 2,002, and stopped completely by the beginning of 2,003 [27]. Although, the production of PFOS and derivatives has ceased in the U.S.,

PFOS-based products such as aqueous fire-fighting foams (AFFF) are still in used today from inventories previously in place [9].

Perfluorosulfonyl compounds were extensively utilized as surface protectors in leather, fabrics, upholstery, carpet, automobile interiors, among others. In 2,000, the production of these chemicals for surface treatment applications was approximately 2.4 million pounds [26]. The use of PFOS and related compounds in paper applications represented the largest volume of PFOS production (2.7 million pounds) in 2000. Plates, food containers, bags and wraps (food contact) as well as cartons, containers and masking papers (non-food contact) were some of the products that contained these surfactants [26]. PFOS substances in the performance chemicals group were divided into industrial, commercial and consumer applications. Specific areas included cleaning agents, waxes and floor polishes, paint and varnish, pesticides and insecticides, fire fighting foams, photographic industry, photolithography and manufacturing of semiconductors, hydraulic oils – airplane industry, metal surface treatment, plumbing and other uses. The production of PFOS compounds for performance chemicals was approximately 1.5 million pounds in 2,000 [15, 26].

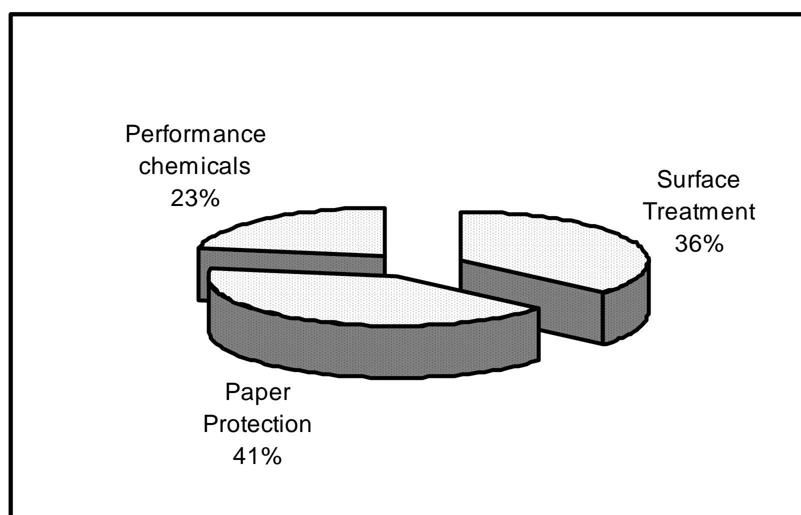


Figure 1.3 Production of PFOS and related compound by 3M Company in 2,000 [26].

Currently, the uses of PFOS and PFOS-based substances are limited to specific applications where they cannot be replaced by any other compound [26, 28]. Since the production of PFOS in the U.S. has ceased completely, these perfluorinated surfactants are imported as chemicals or in final products from Asia and Europe [9]. The exempted applications include the use as additives in aviation hydraulic fluids (aviation industry), as components of the photoresist solvent or as anti-reflective coating (photolithography process to produce semiconductors or electronic miniaturized devices), as coatings for surface tension, static discharge and adhesion control for analog and digital films (photography industry) and as intermediates to produce other chemical substances with restricted use [26, 27, 29].

PFOS is widely utilized in photolithography and semiconductor industry in photoresist formulations as photo-acid generators (PAGs) and surfactants, or as top and bottom anti-reflecting coatings, TARCs and BARCs, respectively. PFOS is currently the only PAG that can provide the necessary acid catalyst to create a desired pattern responding appropriately at a 248 nm and shorter wavelengths [28]. PFOS-based PAGs are very stable compounds that act as light activated charges, the specific wavelength charges the sulfonate group without breaking the PFOS molecule due to the properties of the perfluorinated chain [30]. Then, the acid produced catalyzes another reactions and the photoresist is chemically transformed in the areas where it was exposed. Besides, PFOS PAGs dissolve in the photoresist without phase separation and are non-volatile at room temperature. PFOS based PAG are used predominantly for 193 nm and for photoresists designed for 157 nm wavelengths [28]. PFOS is primarily utilized in the ARCS as a

surfactant. PFOS is very inert and aligns with the reflective index of the resistant being used. PFOS based surfactants are effective in lowering the overall surface tension, reducing thickness variation and producing more uniform coatings [28]. PFOS makes a unique contribution to the semiconductor manufacturing processes due to its optical characteristics and acid-generating efficiency [8]. Currently there are no commercial surfactants that provide the same chemical properties [28]. PFOS concentrations employed in this industral operations range from 0.02 to 1% [31]. The uses of PFOS-related compounds in the semiconductor industry in the European Union (EU) are summarized in Table 1.4. The consumption of PFOS-based chemicals in semiconductor manufacturing process in the U.S. is estimated to be in the same range [32]. Although the use of PFOS and related compounds has decreased considerably in commercial and industrial applications in the U.S., other perfluorinated compounds are being employed. PFBS, a 4-carbon chain compound and flurotelomers-based substances with C_6 or lower chain lengths are utilized as alternative to PFOS in non-critical uses in the semiconductor industry [30].

The bioaccumulation potential of PFAS decreases with decreasing perfluoroalkyl chain length [33]. Martin *et al.* [34] reported that the bioconcentration factors (BCF) in rainbow trout of PFOS (C_8), PFHS (C_6) and PFBS (C_4) were 3,100, 59 and less than 1, respectively. Perfluorinated chemicals with seven fluorinated carbons and less are not considering bioaccumulative according to the regulatory criteria [33]. Unfortunately, these PFOS-alternative compounds are still recalcitrant and will persist in the environment [14, 16].

Table 1.4 EU consumption of PFOS related substances in semiconductor manufacturing and concentrations utilized [28].

Applications	Concentration (%)	EU Consumption (kg $year^{-1}$)
Photoresists	0.02 - 0.10	46
TARCs	0.10	135
BARCs	----	8
Surfactant	0.01	195

1.4 Environmental Regulations

After 3M announced in the year 2,000 the withdraw of PFOS and related compounds from its production lines, U.S. EPA issued a proposed Toxic Substances Control Act (TSCA) and Significant New User Rule (SNUR) in later 2,002 [26]. The use of 90 pefluorooctyl sulfonate compounds in commercial and industrial applications was prohibited with limited exemptions for the aviation, photolithography, and semiconductor industries. In January 2,006, EPA asked eight companies including DuPont and 3M to reduce the use of PFOA emissions from manufacturing processes and consumer products by 95% by 2,010 and completely eliminated by 2,015 [25]. In 2,006, U.S. EPA issued a

Significant New Use Rule for perfluoroalkyl sulfonates [27]. The use of 183 PFAS is restricted because they could pose a risk to human health and the environment.

U.S. EPA and other environmental agencies in industrialized countries such as Canada and the EU have initiated studies to quantify the use of perfluorinated compounds, assess their potential risks, and consider regulations restricting or banning their use [35].

1.5 Environmental Persistence, Fate and Bioaccumulation

PFOS is highly persistent in the environment. PFOS is not known to hydrolyze, photolyze, or biodegrade under environmental conditions [8, 36, 37]. Studies conducted by 3M demonstrated that the fluorinated surfactant does not undergo photolytic or hydrolytic degradation at temperatures of 25ºC and 50ºC, and pH values ranging from 1.1 to 11 [37]. Similarly, no microbial degradation of PFOS has been observed under aerobic or anaerobic conditions [8, 38, 39].

Perfluorinated chemicals show potential for bioaccumulation in human and animals. PFOS is the predominant chemical detected in biotic samples and the concentrations are higher in samples collected in urbanized regions closed to emission sources. Biomonitoring studies suggested that PFOS and related chemicals can bioaccumulate and biomagnify through food webs, animals feeding higher up in the food chain had higher PFOS concentrations [7, 33].

The distribution of perfluoroalkyl compounds in human blood, wildlife and aqueous and solid matrices is still not well characterized. Since PFOS is a xenobiotic compound its presence in the environment must be a result of human manufacture and use. The release of these contaminants can occur from production and processing plants, the use and disposal of consumer and industrial products, e.g., fire-fighting foams, and the transformation of PFOS precursors [9, 40]. PFOS is known to be the ultimate degradation product of a number of PFAS of commercial applications and will persist in that form [2, 7, 11].

Environmental sources and pathways of exposure to PFOS and related compounds are unclear. The presence of PFOS in remote areas can be attributed to atmospheric and ocean transport of their volatile precursors such as *n*-methyl perflurooctanesulfonamido-ethanol, *n*-MeFOSE [$C_8F_{17}SO_2N(CH_3)CH_2CH_2OH$], and *n*-ethyl perflurooctane-sulfonamidoethanol, *n*-EtFOSE [$C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$], where they ultimately degrade to stable PFOS [9, 10, 41, 42]. Dispersion in the environment is also thought to occur through ocean transport of PFAS themselves [9, 43]. Perfluoroalkyl surfactants have been detected in municipal effluents, drinking, surface and ground waters worldwide and will be discussed in more detail below.

Traces of PFOS have been found in animals throughout the world [7, 44]. In 2,001, Giesy and coworkers [5] reported for the first time the global distribution of PFOS in fish, bird, amphibians and marine mammals including seals, dolphin, polar bear, mink, among others. The liver, plasma or egg tissues of various samples were studied to monitor the presence of perfluorooctanesulfonylamide, PFOSA [$C_8F_{17}SO_2NH_2$],

perfluorohexanesulfonate, PFHxS [$C_6F_{13}SO_3^-$], PFOA and PFOS. However, only PFOS was detectable at concentrations > 1ng g^{-1}. PFOS was present in all species even in those from remote locations, such as polar bears in Artic (180 - 680 ng PFOS g^{-1}). Livers of mink from the Midwestern U.S. registered the highest PFOS concentration with a mean of 2,630 ng g^{-1} (970 – 3680 ng g^{-1}). PFOS was found in birds and fish eggs (max. 320 ng g^{-1}) suggesting possible maternal transfer during yolk formation [45]. Concentrations of PFOS in biota were several-fold greater in urbanized areas than those from remote locations. In another study, Martin e*t al.* [46] investigated the level of PFOS in the liver of Canadian Artic animals. PFOS was found to be the most predominant organohalogen contaminant. The presence of PFOS in animals far from anthropogenic sources demonstrates the long-transport potential through its volatile precursor. Smithwick *et al.* [47] reported temporal trends of PFAS in polar bears from the Artic. Liver samples collected between 1,972 and 2,002 were analyzed. Concentrations of PFOS and PFAS with carbon chain lengths from C_9 to C_{11} showed an exponential increase between 1,972 and 2,002. PFOS doubling times of 13.1 $\pm$ 4.0 years were reported. This value is similar to the doubling time of production of PFOS-based chemicals during 1,990. Recently, van de Vijver and colleagues [48] reported PFAS in harbor porpoises from the Black Sea. PFOS was the predominant compound detected accounting for 90% of the total PFAS measured which are comparable to the levels found in porpoises from the German Baltic Sea and the coastal areas near Denmark. The highest PFOS concentration registered was found in the liver (327 ng g^{-1} wet wt). Perfluorononanoic acid, perfluorodecanoic acid,

perfluoroundecanoic acid, and perfluorododecanoic acid were detected in liver tissue of approximately 25% of the individuals.

Perfluoroalkyl compounds are present in human blood from several countries [6, 49-52]. Kannan and coworkers [6] studied the concentrations of PFOS, PFHxS, PFOA, and PFOSA in blood/serum/plasma samples from United States, Colombia, Brazil, Belgium, Italy, Poland, India, Malaysia and Korea. PFOS was the most abundant perfluorochemical found in blood samples followed by PFOA. Age and gender had no influence in the concentrations of PFOS and PFOA in the serum samples investigated. Samples collected from various locations in the U.S. (Michigan, Kentucky, New York) and Poland registered the highest PFOS concentration (> 30 ng mL^{-1}). Yeung *et al.* [53] evaluated the presence of PFOS and related derivatives in 85 human samples from China. Among the PFAS tested, PFOS was the predominant compound detected followed by PFHXs. PFOS concentrations ranged from 3.7 to 79.2 ng mL^{-1}. Only gender related differences were found with males higher for PFOS and PFHXs. PFOS and PFOA concentration were also detected in human sera from Japan [54-56]. As an example, Harada and coworkers [54] reported that PFOS levels in samples collected in 2,003 ranged from 3.5 to 28.1 ng mL^{-1}. Perfluorinated chemicals (PFCs) concentrations in females in historical samples suggested that the levels of PFOS and PFOA have increased by a factor of 3 and 14, respectively, in the last 25 years. PFOS has also been detected in maternal and cord serum samples [57-59]. Apelberg and colleagues [59] measured ten PFAS in umbilical cord serum samples from 299 babies born between 2,004 and 2,005 in Baltimore, MD. Geometric mean concentrations of 4.9 and 1.6 ng mL^{-1} were found for

PFOS and PFOA, respectively. The other eight PFAS were detected less frequently and at lower concentrations than those reported for PFOS and PFOA. These chemicals have also been measured in human breast milk in the samples collected in the U.S. and other countries [57, 58, 60]. For instance, Volkel *et al.,* [60] measured PFOS in samples from Germany and Hungary. PFOS concentrations ranging from 0.03 to 0.31 ng mL^{-1} were detected in samples from Germany. PFOA was detected in 16% of the samples analyzed at lower concentrations than PFOS. The concentration of PFOS in samples from Hungary was about 3-fold higher than those reported in Germany. Maestri and coworkers [61] measured PFOS and PFOA in human liver, kidney, adipose tissue, brain, basal ganglia, hypophysis, thyroid, gonads, pancreas, lung, skeletal muscle and blood. PFOS concentrations ranged from 1.0 to 13.6 ng g^{-1}. PFOA concentrations varied from 0.3 to 3.8 ng g^{-1}.

The presence of branched PFOS in human blood samples was firstly reported by Hansen and coworkers [51]. However, no isomer identification was conducted. Recently, Karrman and coworkers [62] evaluated the isomer pattern of PFOS in human blood samples from Sweden, United Kingdom, and Australia. The linear isomer was the major compound detected (58 to 70%) followed by 1- and 6-CF_3-PFOS (18 to 22 %) and 3/4/5-CF_3-PFOS (13 to 18%). The fact that the linear isomer is the major compound detected in biological samples could be attributed to slower eliminations rates as well as higher exposure concentrations as a result of the production process [33, 63, 64].

PFOS and related perfluorosulfonyl compounds have also been detected in aqueous and solid environmental matrices. These chemicals are expected to partition into

water compartments on release into the environment due to their high solubility, low vapor pressure, and moderate sorption to soil and sediments [9, 33]. The presence of perfluorinated compounds in various aqueous streams has been reported around the globe. Simcik and coworkers [65] reported PFOS levels of 2.7 to 49.0 ng L^{-1} in remote and urban surface waters in the mid western U.S. including Lake Michigan. Hansen and colleagues [66] measured PFOS at levels ranging from 16.8 to 140.0 ng L^{-1} in the Tennessee River downstream from a fluorochemical manufacturing plant. Boulanger and coworkers [67] monitored the presence of PFOS and related compounds in 16 Great Lake water samples and reported PFOS concentrations ranging from 21 to 70 ng L^{-1}. Higher PFOS concentrations ranging from 0.8 to as much as 1,090 ng L^{-1} were detected in surface waters in the state of New York [68]. Saito and coworkers [69] reported PFOS values from 0.24 to 37.3 ng L^{-1} in surface water in Japan. PFOS concentrations as high as 3,160 ng L^{-1} were found in surface waters in an industrialized area in Germany [70]. PFOS concentrations in rainwater in Canada in the order 0.59 ng L^{-1} were reported by Loewen *et al.* [71]. Yamashita and coworkers [43] measured PFAS in the Pacific Ocean, mid-Atlantic Ocean, South China Sea, Sulu Sea and Labrador Sea. Tokyo Bay registered the highest concentrations of PFOS (57.7 ng L^{-1}) and PFOA (192 ng L^{-1}) while levels in the open oceans were in the range of pg L^{-1}. PFOS has also been detected in drinking water in Japan [69, 72], Germany [70] and Northern Italy [73] at concentrations ranging from 0.1 to 73 ng L^{-1}.

Relatively high PFAS concentrations have been measured in wastewaters from fluorochemical plants and other industrial applications that utilize PFAS such as

operations of semiconductor industry (1,650 mg PFOS L^{-1}) [31, 74]. PFOS and related perfluorinated compounds have also been detected in municipal wastewater, wastewater treatment sludge, and in sediments. In the U.S., PFAS concentrations in sewage sludge from wastewater treatment plants ranging from 5 to 3,370 ng g^{-1} have been detected in Alabama, California [75], Florida [76], Georgia [76], Iowa [77], Kentucky [78], New York [79] and Tennessee [76]. PFOS and PFOA were detected in sludge samples at levels ranging from 5 to 120 ng g^{-1} in Germany [80], 0.4 to 74.1 ng g^{-1} in Denmark [81] and 278 to 5,383 ng g^{-1} in China [82], among others. The concentrations of PFOS detected in the sewage sludge in the U.S. are in the range of those measured in other countries [83]. These results suggest that PFOS may be present as residual in consumer products as well as final product of the degradation of PFOS-related substances.

In addition to contaminated aqueous streams, contact with other matrices can contribute to human exposure to perfluoroalkyl compounds. PFOS precursors have been utilized as coatings for food paper packing. Tittlemier and coworkers [84] studied the potential migration of PFOS-precursors from packing as a route of exposure. Perfluorooctanesulfonamides were detected in baked goods, candy, dairy, eggs, fast food, fish and meat. PFOSA concentrations as high as 27.3 ng g^{-1} were found in fast foods. An exposure level of 73 ng day^{-1} per person was estimated with these data. Also, PFOS and PFOA have been detected in indoor dust samples in homes in Canada at average concentrations of 450 and 100 ng g^{-1}, respectively [85].

1.6 Toxicity

In higher organisms, PFOS and related compounds are mainly detected in the serum, kidney and liver [44, 52]. In contrast with many priority environmental pollutants, PFOS does not accumulate in fatty tissue, rather it binds to proteins. This unusual behavior is related to the simultaneous hydrophobic and lipophobic character of PFOS [32]. The chemical was shown to bind strongly to β-lipoproteins [86] and bovine serum albumin in a linear stoichiometric relationship, and interact with liver fatty acid-binding proteins (L-FABP) producing displacement of endogenous ligands and toxicity [87].

PFOS is not metabolized or excreted [52]. The elimination half-life ($T_{½}$) of PFAS varies considerably among species. Half life time of PFOS is 100 days in rats, 100-200 days in monkeys, and 5.4 years in humans [44, 52].

Several studies have been conducted to determine the toxicity of PFOS in animals and humans [44, 52]. The chemical shows moderate acute toxicity to aquatic vertebrates, invertebrates, and mammals as shown in Table 1.5 [88]. The toxicity effects of PFOS in monkey, rabbits and rats are similar. Exposure of animals to repeated PFOS doses lead to significant weight loss accompanied by hepatotoxicity and reduction of serum cholesterol and thyroid hormones [15, 52]. Exposure of rats during gestation to PFOS resulted in a decrease in fetal body weight, increase in external and visceral anomalies, delay in ossification, and variations in skeletal formations [52, 89, 90]. Reductions in fetal body weight and increases in delay ossification were also observed in maternal rabbits [89].

The mechanisms of PFOS toxicity are still not clearly understood and additional research need to be conducted to elucidate the modes of action [44, 52].

Numerous studies have been conducted on workers occupationally exposed to perfluorooctansulfonyl compounds in fluorochemical plants [44]. For instance, Olsen and coworkers [91] measured the concentration of PFOS and other PFAS in worker populations in a fluorochemical facility. The average PFOS concentrations for chemical operators were 1780 ng mL^{-1}. Although, these values are approximately 50-fold higher than those measured in U.S. general populations, no correlation between PFOS concentrations in serum plasma and adverse health effects has been observed [44, 52, 92]. Interestingly, an increase in mortality resulting from bladder cancer was found in three male workers who had worked for a year in high PFOS exposed jobs at a fluorochemical facility [93]. Follow-up studies confirmed the increased bladder cancer mortality ratio but no definitive trends were obtained [44, 52].

Table 1.5. Toxicity levels of PFOS in different organisms [88].

Organism	Toxicity level*	Concentration
Aquatic vertebrates		
Fish (*Fathead minnow*)	96-hour LC50	4.7 mg L^{-1}
Aquatic invertebrates		
Fresh water species (*Daphnia magna*)	48-hour EC50	27 mg L^{-1}
Saltwater species (*Mysid shrimp*)	96-hour LC50	3.6 mg L^{-1}
Mammals		
Rats	LD50	251 mg kg^{-1}
	NOAEL	1 mg kg^{-1} day^{-1}
	LOAEL	5 mg kg^{-1} day^{-1}

* 96-hour LC50: 50% lethal concentration after 96 h of exposure; 48-hour EC50: 50% effective response after 48 h of exposure; NOAEL: Non-observed adverse effect level; LOAEL: Low-observed adverse effect level

1.7 Analytical Techniques

Commonly available analytical techniques such as high performance liquid chromatography-ultraviolet detection (HPLC-UV) is not suitable for the analysis of perfluorinated sulfonates in the environment due to their absence of chromophoric groups [3]. Besides, the lack of authentic standards limits the accurate detection and quantification of these emerging pollutants [22, 35, 94].

Liquid chromatography coupled to mass spectrometry (LC-MS) and tandem mass spectrometry (LC-MS/MS) are the two most common analytical techniques employed for the measurement of PFAS in biological and environmental samples [94-96]. Hansen *et al.* [51] described the first method for the analysis of PFOS and related chemicals in 2,001. The procedure consisted of liquid solvent extraction, solid-phase clean-up, and analysis by HPLC with electrospray tandem mass spectrometry (LC/ESI-MS/MS). Since then, there has been a tremendous progress in the development of LC-MS methods for the analysis of PFAS. ESI operating in the negative ionization mode seems to be the dominant method for analysis of most perfluorinated surfactants [95, 96]. Selective ion monitoring (SIM) is commonly utilized for quantification aids allowing detection limits in the pg to low ng PFAS L^{-1} levels [95]. The use of solid phase extraction (SPE) procedures has become very popular for the extraction and concentration of PFOS from aqueous environmental matrices [94, 96, 97]. The main drawbacks of LC-MS/MS techniques are ion suppression due to matrix interferences, sample contamination, and the elevated cost of the analysis.

Quantitative analysis of perfluoroalkyl sulfonate anions in water has also been accomplished by attenuated total reflected Fourier transform infrared spectroscopy (ATR-FTIR) [98], by direct injection negative-ion electrospray ionization mass spectrometry ESI-MS [99], ion-exclusion chromatography (IEC) [100], gas chromatography mass spectrometry (GC/MS) [101, 102], fluorine nuclear magnetic resonance (^{19}F NMR) [103], and high-performance liquid chromatography with conductimetric detection [104]. Although, ATR-FTIR seems to be a suitable technique for the analysis of these contaminants, no environmental applicability has been demonstrated yet. Direct injection MS is less expensive and time consuming than LC-MS/MS and could offer an interesting alternative for samples that do not cause interfering matrix effects. Ion exclusion chromatography is an effective method for the analysis of short-chain (C_4 to C_6) perfluoroalkyl compounds; nevertheless, it is not useful for the analysis of perfluorooctane sulfonate. GC-MS analysis of perfluorosulfonyl compounds requires derivatization steps prior measurements limiting, therefore, the applicability of the technique to environmental monitoring. In contrast, the specificity of NMR spectroscopy towards fluorinated compounds together with the lack of matrix interferences makes this technique suitable for structural characterization of PFAS in environmental samples. However, the low sensitivity is a drawback of the technique. HPLC-suppressed conductivity detection has a great potential for the accurate quantification of PFAS in aqueous streams in a timely and efficient manner.

1.8 Treatment Methods

Although more environmentally friendly alternatives to PFOS-compounds are under study, PFC chemicals are still being utilized in photolithographic steps of semiconductor manufacturing [26, 29]. Therefore, the development of treatment techniques to remove PFOS from wastewater effluents is critical to reduce discharges to the environment.

The removal of perfluorochemical compounds from industrial effluents by biological and physico-chemical methods has been studied recently. High temperature incineration (~ 1200ºC) is known to degrade PFOS and related compounds [27]. Incineration seems to be an effective approach, but preconcentration procedures are required and the cost is relatively high $ 3 to 4 per gallon [30]. Published studies concerned with the application of biological and physico-chemical methods for the removal of perfluorochemicals are reviewed in the next section.

1.8.1 Biological methods

Few studies have evaluated the potential for degradation of perfluorinated or partially fluorinated surfactants by microbial processes [8, 37-39]. The resistance of PFOS to microbial degradations was reported for the first time by Remde *et al.* [38]. PFOS was not degraded under aerobic nor anaerobic conditions. In agreement with these results, PFOS was not degraded under aerobic sulfur limiting conditions by *Pseudomonas* sp

strain D2 nor in wastewater treatment sludge or pure cultures [8, 39]. Information on the fate of PFOS during biological wastewater treatment is very scarce. Schroder [105] evaluated the removal of PFOS in short-them lab-scale aerobic and anaerobic bioreactor experiments. The author claimed that the surfactant was microbially degraded. However, no evidence was presented (e.g. stoichiometric fluoride release) to support the claim. Adsorption to sludge may have been responsible for the elimination.

A fluorinated surfactant closely related to PFOS, 1H,1H,2H,2H-perfluoroctane sulfonate (TH-PFOS, $C_6F_{13}C_2H_4SO_3^-$), was partially defluorinated and supported growth under sulfur limiting and aerobic conditions by several bacterial strains [39]. The microbial degradation yielded unidentified volatile fluorinated chemicals. These results suggest that in the presence of hydrogen, the carbon-sulfur bond becomes more accessible, making the compound more susceptible to microbial attack than fully fluorinated chemicals.

Biosorption may be an effective approach for the removal of PFOS due to the tendency of this contaminant to accumulate in biological and solid environmental matrices [7, 44, 77, 106]. Additional research needs to be conducted to determine the removal of PFOS from industrial effluents by sorption to organic matter.

1.8.2 Physico-chemical methods

Several studies on the removal of PFOS and related compounds by physico-chemical treatment methods have been published in the last three years. Moriwaki and coworkers first reported the breakdown of PFOS and related chemicals by sonochemical means [107]. Ultrasonic irradiation of PFOS under an argon saturated atmosphere for 60 min resulted in degradation of 60% of the initial concentration. PFOA and other short-chain perfluorinated carboxylic compound were the products of the reaction. The sonochemical decomposition of PFOA was also observed and short-chain PFOA related compounds were the degradation products. The breakdown of PFOS and related chemicals using zero-valent iron in sub-critical water was examined by Hori *et al.* [108]. The reaction was conducted at 350ºC for 6 h and yielded about 50% of F^- ion and no degradation products were identified. Only CHF_3 was detected in a small amount in the gas phase. No PFOS was detected in solution after reaction completion. The authors suggested that PFOS was thermally decomposed following adsorption onto the iron surface, leading to the slow release of F^- ions into solution. Yamamoto and colleagues [109] evaluated the degradation of PFOS by UV-light (254 nm, 32W) in aqueous or alkaline 2-propanol solutions. Photodegradation of PFOS was faster and more efficient in alkaline 2-propanol solutions than that in water, and 92% PFOS removal was obtained after 10 days of irradiation using the alkaline solution. Lower molecular weight and C_6-C_7 fluorinated compounds were identified as degradation products. The oxidation of PFOS using boron-dopped diamond electrodes was reported recently [110]. Between 11 and 14 fluoride ions

were released per mol of PFOS oxidized, depending on the reactor system used. Fluoride, sulfate, and traces levels of trifluoroacetic acid were the main degradation products detected.

Membrane processes have also been considered for the treatment of PFOS from aqueous streams. Tang and coworkers [31] investigated the use of reverse osmosis membranes for the removal of PFOS from semiconductor effluents. PFOS was shown to be effectively rejected (99%) by commercial membranes. Activated carbon (AC) is a promising adsorbent for the removal of PFOS and related compounds from wastewater due to its versatility, efficiency and low cost. However, no studies have been published to date on the adsorption of perfluorinated chemicals to AC.

Further research is needed to develop feasible remedial treatments for the removal of PFOS and related compounds from wastewater and contaminated groundwater.

1.9 Conclusions

Perfluoroalkyl surfactants are emerging contaminants that have received a lot of attention in the last decade due to their environmental persistence, bioaccumulation potential, and toxicity. Their widespread in polluted as well as pristine areas is a major concern and a threat to human and environmental health. Hence, analysis of PFAS in biological and environmental matrices is critical to understand their fate, transport and persistence.

Moreover, the development of cost-effective treatment techniques for removing these contaminants from industrial effluents is needed to minimize discharges.

1.10 References

1. Kissa, E., *Fluorinated surfactants and repellants*. Marcel Dekker, Inc.: New York, 2001.
2. 3M. *The Science of Organic Fluorochemistry*; 3M: St Paul, MN, 1999.
3. Schultz, M. M.; Barofsky, D. F.; Field, J. A., Fluorinated alkyl surfactants. *Environmental Engineering Science* **2003,** *20*, (5), 487-501.
4. Moody, C. A.; Field, J. A., Perfluorinated surfactants and the environmental implications of their use in fire-fighting foams. *Environ. Sci. Technol.* **2000,** *34*, (18), 3864-3870.
5. Giesy, J. P.; Kannan, K., Global distribution of perfluorooctane sulfonate in wildlife. *Environ. Sci. Technol.* **2001,** *35*, (7), 1339-1342.
6. Kannan, K.; Corsolini, S.; Falandysz, J.; Fillmann, G.; Kumar, K. S.; Loganathan, B. G.; Mohd, M. A.; Olivero, J.; Van Wouwe, N.; Yang, J. H.; Aldous, K. M., Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. *Environ. Sci. Technol.* **2004,** *38*, (17), 4489-4495.

7. Houde, M.; Martin, J. W.; Letcher, R. J.; Solomon, K. R.; Muir, D. C. G., Biological monitoring of polyfluoroalkyl substances: A review. *Environ. Sci. Technol.* **2006,** *40*, (11), 3463-3473.

8. Brooke, D.; Footitt, A.; Nwaogu, T. A., Environmental risk evaluation report: Perfluorooctanesulphonate (PFOS). *Environment Agency, Chemicals Assessment Section, Wallingford, UK* **2004**.

9. Prevedouros, K.; Cousins, I. T.; Buck, R. C.; Korzeniowski, S. H., Sources, fate and transport of perfluorocarboxylates. *Environ. Sci. Technol.* **2006,** *40*, (1), 32-44.

10. Richardson, S. D., Water analysis: Emerging contaminants and current issues. *Analytical Chemistry* **2007,** *79*, (12), 4295-4323.

11. Dimitrov, S.; Kamenska, V.; Walker, J. D.; Windle, W.; Purdy, R.; Lewis, M.; Mekenyan, O., Predicting the biodegradation products of perfluorinated chemicals using CATABOL. *Sar and Qsar in Environmental Research* **2004,** *15*, (1), 69-82.

12. EPA *Preliminary Risk Assesment of the Developmental Toxicity Associated with Exposure to Perfluorooctanoic Acid and its Salts.* U.S. Environmental Protection Agency: Washington, DC, 2003a.

13. Ellis, D. A.; Martin, J. W.; De Silva, A. O.; Mabury, S. A.; Hurley, M. D.; Andersen, M. P. S.; Wallington, T. J., Degradation of fluorotelomer alcohols: A likely atmospheric source of perfluorinated carboxylic acids. *Environ. Sci. Technol.* **2004,** *38*, (12), 3316-3321.

14. 3M *Technical data bulletin. Environmental health, safety and regulatory (ehsr) profile of perfluorobutane sulfonate (PFBS)*; 3M: St Paul, MN, 2002.

15. Poulsen, P. B.; Jensen, A. A.; Wallstrom, E. *More environmentally friendly alternatives to PFOS-compounds and PFOA*; Environmental Project No. 10132005; Danish Ministry of the Environment, Environmental Protection Agency: 2005; pp 1 -162.

16. NICNAS, Potassium perfluorobutane sulfonate. Existing chemical hazard assessment report. *Department of Health and Ageing, National Industrial Chemicals Notification and Assessment Scheme (NICNAS), Australian Government, Sidney, Australia* **2005**.

17. 3M *Fluorochemical isomer distribution by ^{19}F NMR spectroscopy*; U.S. Environmental Protection Agency Public Docket AR226-0564. : 1997.

18. Vyas, S. M.; Kania-Korwel, I.; Lehmler, H. J., Differences in the isomer composition of perfluoroctanesulfonyl (PFOS) derivatives. *J. Environ. Sci. Health Part A-Toxic/Hazard. Subst. Environ. Eng.* **2007,** *42*, (3), 249-255.

19. Ochoa-Herrera, V.; Sierra-Alvarez, R.; Somogyi, A.; Jacobsen, N. E.; Wysocki, V. H.; Field, J. A., Reductive defluorination of perfluorooctane sulfonate. *Environ. Sci. Technol.* **2008,** *42*, (9), 3260-3264.

20. Langlois, I.; Oehme, M., Structural identification of isomers present in technical perfluorooctane sulfonate by tandem mass spectrometry. *Rapid Communications in Mass Spectrometry* **2006,** *20*, (5), 844-850.

21. Benskin, J. P.; Bataineh, M.; Martin, J. W., Simultaneous characterization of perfluoroalkyl carboxylate, sulfonate, and sulfonamide isomers by liquid chromatography-tandem mass spectrometry. *Analytical Chemistry* **2007,** *79*, (17), 6455-6464.

22. Arsenault, G.; Chittim, B.; McAlees, A.; McCrindle, R.; Riddell, N.; Yeo, B., Some issues relating to the use of perfluoro octane sulfonate (PFOS) samples as reference standards. *Chemosphere* **2008,** *70*, (4), 616-625.

23. Arsenault, G.; McCrindle, R.; Chittim, B.; McAlees, A., Separation and fluorine nuclear magnetic resonance spectroscopy (^{19}F-NMR) analysis of the individual branched isomers present in technical perfluorooctanesulfonic acid (PFOS). *Organohalogen Compounds* **2005,** *67*, 818 - 822.

24. Weber, J., 3M's Big Cleanup: Why it decided to pull the plug on its best selling stain repellant. *BusinessWeek* 2000, pp 96 - 98.

25. EPA *2010/15 PFOA Stewardship Program*; U.S. Environmental Protection Agency: Washington, DC, 2006.

26. EPA *Perfluorooctyl sulfonates: Proposed significant new use rule*; U.S. Environmental Protection Agency. Federal Register: 2000; pp 62319 -62333.

27. EPA *Perfluoroalkyl Sulfonates; Proposed Significant New Use Rule*; U.S. Environmental Protection Agency: Washington, DC, 2006; pp 12311 - 12324.

28. RPA-BRE *Perfluorooctane sulfonate - Risk reduction strategy and analysis of advantages and drawbacks. Final Report prepared for Department for Environment, Food and Rural Affairs and the Environmental Agency for England*

and Wales, Risk & Policy Analysts Limited (RPA) in association with BRE Environment: 2004; pp 1 - 266.

29. Anonymous, Environmental Protection Agency tentatively OKs PFOS for semiconductor industry. *Solid State Technology* **2002,** *45*, (32).

30. Personal communication with Tim Yeakley, Texas Instruments. In July 2006.

31. Tang, C. Y. Y.; Fu, Q. S.; Robertson, A. P.; Criddle, C. S.; Leckie, J. O., Use of reverse osmosis membranes to remove perfluorooctane sulfonate (PFOS) from semiconductor wastewater. *Environ. Sci. Technol.* **2006,** *40*, (23), 7343-7349.

32. keml *Perfluorooctane sulfonate (PFOS). Working Draft Risk Profile*; Swedish Chemicals Inspectorate (keml): 2006; pp 1 - 28.

33. Conder, J. M.; Hoke, R. A.; De Wolf, W.; Russell, M. H.; Buck, R. C., Are PFCAs bioaccumulative? A critical review and comparison with regulatory lipophilic compounds. *Environ. Sci. Technol.* **2008,** *42*, (4), 995-1003.

34. Martin, J. W.; Mabury, S. A.; Solomon, K. R.; Muir, D. C. G., Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (Oncorhynchus mykiss). *Environmental Toxicology and Chemistry* **2003,** *22*, (1), 196-204.

35. Martin, J. W.; Kannan, K.; Berger, U.; De Voogt, P.; Field, J.; Franklin, J.; Giesy, J. P.; Harner, T.; Muir, D. C. G.; Scott, B.; Kaiser, M.; Jarnberg, U.; Jones, K. C.; Mabury, S. A.; Schroeder, H.; Simcik, M.; Sottani, C.; Van Bavel, B.; Karrman, A.; Lindstrom, G.; Van Leeuwen, S., Analytical challenges hamper perfluoroalkyl research. *Environ. Sci. Technol.* **2004,** *38*, (13), 248A-255A.

36. Key, B. D.; Howell, R. D.; Criddle, C. S., Fluorinated organics in the biosphere. *Environ. Sci. Technol.* **1997,** *31*, (9), 2445-2454.

37. Beach, S. A.; Newsted, J. L.; Coady, K.; Giesy, J. P., Ecotoxicological evaluation of perfluorooctanesulfonate (PFOS). In *Reviews of Environmental Contamination and Toxicology, Vol 186*, Springer: New York, 2006; Vol. 186, pp 133-174.

38. Remde, A.; Debus, R., Biodegradability of fluorinated surfactants under aerobic and anaerobic conditions. *Chemosphere* **1996,** *32*, (8), 1563-1574.

39. Key, B. D.; Howell, R. D.; Criddle, C. S., Defluorination of organofluorine sulfur compounds by Pseudomonas sp. strain D2. *Environ. Sci. Technol.* **1998,** *32*, (15), 2283-2287.

40. Trudel, D.; Horowitz, L.; Wormuth, M.; Scheringer, M.; Cousins, I. T.; Hungerbuhler, K., Estimating consumer exposure to PFOS and PFOA. *Risk Anal.* **2008,** *28*, (2), 251-269.

41. Shoeib, M.; Harner, T.; Vlahos, P., Perfluorinated chemicals in the Arctic atmosphere. *Environ. Sci. Technol.* **2006,** *40*, (24), 7577-7583.

42. Young, C. J.; Furdui, V. I.; Franklin, J.; Koerner, R. M.; Muir, D. C. G.; Mabury, S. A., Perfluorinated acids in arctic snow: New evidence for atmospheric formation. *Environ. Sci. Technol.* **2007,** *41*, (10), 3455-3461.

43. Yamashita, N.; Kannan, K.; Taniyasu, S.; Horii, Y.; Petrick, G.; Gamo, T., A global survey of perfluorinated acids in oceans. *Marine Pollution Bulletin* **2005,** *51*, (8-12), 658-668.

44. Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J., Perfluoroalkyl acids: A review of monitoring and toxicological findings. *Toxicol. Sci.* **2007,** *99*, (2), 366-394.

45. Giesy, J. P.; Kannan, K., Perfluorochemical surfactants in the environment. *Environ. Sci. Technol.* **2002,** *36*, (7), 146A-152A.

46. Martin, J. W.; Smithwick, M. M.; Braune, B. M.; Hoekstra, P. F.; Muir, D. C. G.; Mabury, S. A., Identification of long-chain perfluorinated acids in biota from the Canadian Arctic. *Environ. Sci. Technol.* **2004,** *38*, (2), 373-380.

47. Smithwick, M.; Norstrom, R. J.; Mabury, S. A.; Solomon, K.; Evans, T. J.; Stirling, I.; Taylor, M. K.; Muir, D. C. G., Temporal trends of perfluoroalkyl contaminants in polar bears (Ursus maritimus) from two locations in the North American Arctic, 1972-2002. *Environ. Sci. Technol.* **2006,** *40*, (4), 1139-1143.

48. Van de Vijver, K. I.; Hoslbeek, L.; Das, K.; Blust, R.; Joiris, C.; De Coen, W., Occurrence of perfluorooctane sulfonate and other perfluorinated alkylated substances in harbor porpoises from the Black Sea. *Environ. Sci. Technol.* **2007,** *41*, (1), 315-320.

49. Olsen, G. W.; Church, T. R.; Miller, J. P.; Burris, J. M.; Hansen, K. J.; Lundberg, J. K.; Armitage, J. B.; Herron, R. M.; Medhdizadehkashi, Z.; Nobiletti, J. B.; Neill, E. M. O.; Mandel, J. H.; Zobel, L. R., Perfluorooctanesulfonate and other fluorochemicals in the serum of the American Red Cross Adult Blood Donors. *Environmental Health Perspectives* **2003,** *111*, (16), 1892 - 1901.

50. Calafat, A. M.; Kuklenyik, Z.; Caudill, S. P.; Reidy, J. A.; Needham, L. L., Perfluorochemicals in pooled serum samples from United States residents in 2001 and 2002. *Environ. Sci. Technol.* **2006,** *40*, (7), 2128-2134.

51. Hansen, K. J.; Clemen, L. A.; Ellefson, M. E.; Johnson, H. O., Compound-specific, quantitative characterization of organic: Fluorochemicals in biological matrices. *Environ. Sci. Technol.* **2001,** *35*, (4), 766-770.

52. Butenhoff, J. L.; Olsen, G. W.; Pfahles-Hutchens, A., The applicability of biomonitoring data for perfluorooctanesulfonate to the environmental public health continuum. *Environmental Health Perspectives* **2006,** *114*, (11), 1776-1782.

53. Yeung, L. W. Y.; So, M. K.; Jiang, G. B.; Taniyasu, S.; Yamashita, N.; Song, M. Y.; Wu, Y. N.; Li, J. G.; Giesy, J. P.; Guruge, K. S.; Lam, P. K. S., Perfluorooctanesulfonate and related fluorochemicals in human blood samples from China. *Environ. Sci. Technol.* **2006,** *40*, (3), 715-720.

54. Harada, K.; Saito, N.; Inoue, K.; Yoshinaga, T.; Watanabe, T.; Sasaki, S.; Kamiyama, S.; Koizumi, A., The influence of time, sex and geographic factors on levels of perfluorooctane sulfonate and perfluorooctanoate in human serum over the last 25 years. *Journal of Occupational Health* **2004,** *46*, (2), 141-147.

55. Harada, K.; Koizumi, A.; Saito, N.; Inoue, K.; Yoshinaga, T.; Date, C.; Fujii, S.; Hachiya, N.; Hirosawa, I.; Koda, S.; Kusaka, Y.; Murata, K.; Omae, K.; Shimbo, S.; Takenaka, K.; Takeshita, T.; Todoriki, H.; Wada, Y.; Watanabe, T.; Ikeda, M., Historical and geographical aspects of the increasing perfluorooctanoate and

perfluorooctane sulfonate contamination in human serum in Japan. *Chemosphere* **2007,** *66*, (2), 293-301.

56. Taniyasu, S.; Kannan, K.; Horii, Y.; Hanari, N.; Yamashita, N., A survey of perfluorooctane sulfonate and related perfluorinated organic compounds in water, fish, birds, and humans from Japan. *Environ. Sci. Technol.* **2003,** *37*, (12), 2634-2639.

57. Inoue, K.; Okada, F.; Ito, R.; Kato, S.; Sasaki, S.; Nakajima, S.; Uno, A.; Saijo, Y.; Sata, F.; Yoshimura, Y.; Kishi, R.; Nakazawa, H., Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: Assessment of PFOS exposure in a susceptible population during pregnancy. *Environmental Health Perspectives* **2004,** *112*, (11), 1204-1207.

58. Monroy, R.; Morrison, K.; Teo, K.; Atkinson, S.; Kubwabo, C.; Stewart, B.; Foster, W. G., Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. *Environmental Research* **2008,** *108*, (1), 56-62.

59. Apelberg, B. J.; Goldman, L. R.; Calafat, A. M.; Herbstman, J. B.; Kuklenyik, Z.; Heidler, J.; Needham, L. L.; Halden, R. U.; Witter, F. R., Determinants of fetal exposure to polyfluoroalkyl compounds in Baltimore, Maryland. *Environ. Sci. Technol.* **2007,** *41*, (11), 3891-3897.

60. Volkel, W.; Genzel-Boroviczeny, O.; Demmelmair, H.; Gebauer, C.; Koletzko, B.; Twardella, D.; Raab, U.; Fromme, H., Perfluorooctane sulphonate (PFOS) and perfluorooctanoic acid (PFOA) in human breast milk: Results of a pilot study. *Int. J. Hyg. Environ. Health.* **2008,** *211*, (3-4), 440-446.

61. Maestri, L.; Negri, S.; Ferrari, M.; Ghittori, S.; Fabris, F.; Danesino, P.; Imbriani, M., Determination of perfluorooctanoic acid and perfluorooctanesulfonate in human tissues by liquid chromatography/single quadrupole mass spectrometry. *Rapid Communications in Mass Spectrometry* **2006,** *20*, (18), 2728-2734.

62. Karrman, A.; Langlois, I.; van Bavel, B.; Lindstrom, G.; Oehme, M., Identification and pattern of perfluorooctane sulfonate (PFOS) isomers in human serum and plasma. *Environ. Int.* **2007,** *33*, (6), 782-788.

63. De Silva, A. O.; Mabury, S. A., Isolating isomers of perfluorocarboxylates in polar bears (Ursus maritimus) from two geographical locations. *Environ. Sci. Technol.* **2004,** *38*, (24), 6538-6545.

64. Loveless, S. E.; Finlay, C.; Everds, N. E.; Frame, S. R.; Gillies, P. J.; O'Connor, J. C.; Powley, C. R.; Kennedy, G. L., Comparative responses of rats and mice exposed to linear/branched, linear, or branched ammonium perfluorooctanoate (APFO). *Toxicology* **2006,** *220*, (2-3), 203-217.

65. Simcik, M. F.; Dorweiler, K. J., Ratio of perfluorochemical concentrations as a tracer of atmospheric deposition to surface waters. *Environ. Sci. Technol.* **2005,** *39*, (22), 8678-8683.

66. Hansen, K. J.; Johnson, H. O.; Eldridge, J. S.; Butenhoff, J. L.; Dick, L. A., Quantitative characterization of trace levels of PFOS and PFOA in the Tennessee River. *Environ. Sci. Technol.* **2002,** *36*, (8), 1681-1685.

67. Boulanger, B.; Peck, A. M.; Schnoor, J. L.; Hornbuckle, K. C., Mass budget of perfluorooctane surfactant in Lake Ontario. *Environ. Sci. Technol.* **2005,** *39*, (1), 74-79.

68. Sinclair, E.; Mayack, D. T.; Roblee, K.; Yamashita, N.; Kannan, K., Occurrence of perfluoroalkyl surfactants in water, fish, and birds from New York State. *Archives of Environmental Contamination and Toxicology* **2006,** *50*, (3), 398-410.

69. Saito, N.; Harada, K.; Inoue, K.; Sasaki, K.; Yoshinaga, T.; Koizumi, A., Perfluorooctanoate and perfluorooctane sulfonate concentrations in surface water in Japan. *Journal of Occupational Health* **2004,** *46*, (1), 49-59.

70. Skutlarek, D.; Exner, M.; Farber, H., Perfluorinated surfactants in surface and drinking water. *Environ. Sci. Pollut. Res.* **2006,** *13*, (5), 299-307.

71. Loewen, M.; Halldorson, T.; Wang, F. Y.; Tomy, G., Fluorotelomer carboxylic acids and PFOS in rainwater from an urban center in Canada. *Environ. Sci. Technol.* **2005,** *39*, (9), 2944-2951.

72. Harada, K.; Saito, N.; Sasaki, K.; Inoue, K.; Koizumi, A., Perfluorooctane sulfonate contamination of drinking water in the Tama River, Japan: Estimated effects on resident serum levels. *Bull. Environ. Contam. Toxicol.* **2003,** *71*, (1), 31-36.

73. Loos, R.; Wollgast, J.; Huber, T.; Hanke, G., Polar herbicides, pharmaceutical products, perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and nonylphenol and its carboxylates and ethoxylates in surface and tap waters around

Lake Maggiore in Northern Italy. *Anal. Bioanal. Chem.* **2007,** *387*, (4), 1469-1478.

74. Anonymous, Ground-water sampling for PFOA near the Washington Co. landfill. *Minnesota Pollution Control Agency, Minnesota Department of Health. St. Paul, MN* **2004**.

75. Higgins, C. P.; Field, J. A.; Criddle, C. S.; Luthy, R. G., Quantitative determination of perfluorochemicals in sediments and domestic sludge. *Environ. Sci. Technol.* **2005,** *39*, (11), 3946-3956.

76. 3M *Executive Summary: Environmental Monitoring - Multi-City Study. Water, Sludge, Sediments, POTW Effluent and Landfill Leachate Samples.* ; EPA- 226-1030a, U.S. Environmental Protection Agency, 2001.

77. Boulanger, B.; Vargo, J. D.; Schnoor, J. L.; Hornbuckle, K. C., Evaluation of perfluorooctane surfactants in a wastewater treatment system and in a commercial surface protection product. *Environ. Sci. Technol.* **2005,** *39*, (15), 5524-5530.

78. Loganathan, B. G.; Sajwan, K. S.; Sinclair, E.; Kumar, K. S.; Kannan, K., Perfluoroalkyl sulfonates and perfluorocarboxylates in two wastewater treatment facilities in Kentucky and Georgia. *Water Res.* **2007,** *41*, (20), 4611-4620.

79. Sinclair, E.; Kannan, K., Mass loading and fate of perfluoroalkyl surfactants in wastewater treatment plants. *Environ. Sci. Technol.* **2006,** *40*, (5), 1408-1414.

80. Becker, A. M.; Gerstmann, S.; Frank, H., Perfluorooctane surfactants in waste waters, the major source of river pollution. *Chemosphere* **2008,** *72*, (1), 115-121.

81. Bossi, R.; Strand, J.; Sortkjaer, O.; Larsen, M. M., Perfluoroalkyl compounds in Danish wastewater treatment plants and aquatic environments. *Environ. Int.* **2008,** *34*, (4), 443-450.

82. Guo, R.; Zhou, Q. F.; Cai, Y. Q.; Jiang, G. B., Determination of perfluorooctanesulfonate and perfluorooctanoic acid in sewage sludge samples using liquid chromatography/quadrupole time-of-flight mass spectrometry. *Talanta* **2008,** *75*, (5), 1394-1399.

83. Schultz, M. M.; Barofsky, D. F.; Field, J. A., Quantitative determination of fluorinated alkyl substances by large-volume-injection liquid chromatography tandem mass spectrometry - Characterization of municipal wastewaters. *Environ. Sci. Technol.* **2006,** *40*, (1), 289-295.

84. Tittlemier, S. A.; Pepper, K.; Edwards, L., *Journal of Agricultural food and chemistry* **2006**.

85. Shoeib, M.; Harner, T.; Wilford, B. H.; Jones, K. C.; Zhu, J. P., Perfluorinated sulfonamides in indoor and outdoor air and indoor dust: Occurrence, partitioning, and human exposure. *Environ. Sci. Technol.* **2005,** *39*, (17), 6599-6606.

86. Jones, P. D.; Hu, W. Y.; De Coen, W.; Newsted, J. L.; Giesy, J. P., Binding of perfluorinated fatty acids to serum proteins. *Environmental Toxicology and Chemistry* **2003,** *22*, (11), 2639-2649.

87. Luebker, D. J.; Hansen, K. J.; Bass, N. M.; Butenhoff, J. L.; Seacat, A. M., Interactions of flurochemicals with rat liver fatty acid-binding protein. *Toxicology* **2002,** *176*, (3), 175-185.

88. IPEN *Perfluorooctane Sulfonate. Chemical Fact Sheet*; Internation POPS Elimination Network. Body Burden Community Monitoring Handbook: 2005.

89. Case, M. T.; York, R. G.; Christian, M. S., Rat and rabbit oral developmental toxicology studies with two perfluorinated compounds. *Int. J. Toxicol.* **2001,** *20*, (2), 101-109.

90. Thibodeaux, J. R.; Hanson, R. G.; Rogers, J. M.; Grey, B. E.; Barbee, B. D.; Richards, J. H.; Butenhoff, J. L.; Stevenson, L. A.; Lau, C., Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. I: Maternal and prenatal evaluations. *Toxicol. Sci.* **2003,** *74*, (2), 369-381.

91. Olsen, G. W.; Logan, P. W.; Hansen, K. J.; Simpson, C. A.; Burris, J. M.; Burlew, M. M.; Vorarath, P. P.; Venkateswarlu, P.; Schumpert, J. C.; Mandell, J. H., An occupational exposure assessment of a perfluorooctanesulfonyl fluoride production site: Biomonitoring. *Aiha J.* **2003,** *64*, (5), 651-659.

92. Grice, M. M.; Alexander, B. H.; Hoffbeck, R.; Kampa, D. M., Self-reported medical conditions in perfluorooctanesulfonyl fluoride manufacturing workers. *J. Occup. Environ. Med.* **2007,** *49*, (7), 722-729.

93. Alexander, B. H.; Olsen, G. W.; Burris, J. M.; Mandel, J. H.; Mandel, J. S., Mortality of employees of a perfluorooctanesulphonyl fluoride manufacturing facility. *Occup. Environ. Med.* **2003,** *60*, (10), 722-729.

94. de Voogt, P.; Saez, M., Analytical chemistry of perfluoroalkylated substances. *Trac-Trends Anal. Chem.* **2006,** *25*, (4), 326-342.

95. Villagrasa, M.; de Alda, M. L.; Barcelo, D., Environmental analysis of fluorinated alkyl substances by liquid chromatography-(tandem) mass spectrometry: a review. *Anal. Bioanal. Chem.* **2006,** *386*, (4), 953-972.

96. Richardson, S. D., Environmental mass spectrometry: Emerging contaminants and current issues. *Analytical Chemistry* **2008,** *80*, (12), 4373-4402.

97. van Leeuwen, S. P. J.; de Boer, J., Extraction and clean-up strategies for the analysis of poly- and perfluoroalkyl substances in environmental and human matrices. *Journal of Chromatography A* **2007,** *1153*, (1-2), 172-185.

98. Hebert, G. N.; Odom, M. A.; Bowman, S. C.; Strauss, S. H., Attenuated total reflectance FTIR detection and quantification of low concentrations of aqueous polyatomic anions. *Analytical Chemistry* **2004,** *76*, (3), 781-787.

99. Hebert, G. N.; Odom, M. A.; Craig, P. S.; Dick, D. L.; Strauss, S. H., Method for the determination of sub-ppm concentrations of perfluoroalkylsulfonate anions in water. *Journal of Environmental Monitoring* **2002,** *4*, (1), 90-95.

100. Abe, T.; Baba, H.; Itoh, E.; Tanaka, K., Separation of perfluoroalkylsulfinic acids and perfluoroalkylsulfonic acids by ion-exclusion chromatography. *Journal of Chromatography A* **2001,** *920*, 173 - 180.

101. Martin, J. W.; Muir, D. C. G.; Moody, C. A.; Ellis, D. A.; Kwan, W. C.; Solomon, K. R.; Mabury, S. A., Collection of airborne fluorinated organics and analysis by gas chromatography/chemical ionization mass spectrometry. *Analytical Chemistry* **2002,** *74*, (3), 584-590.

102. Langlois, I.; Berger, U.; Zencak, Z.; Oehme, M., Mass spectral studies of perfluorooctane sulfonate derivatives separated by high-resolution gas chromatography. *Rapid Communications in Mass Spectrometry* **2007,** *21*, (22), 3547-3553.

103. Moody, C. A.; Kwan, W. C.; Martin, J. W.; Muir, D. C. G.; Mabury, S. A., Determination of perfluorinated surfactants in surface water samples by two independent analytical techniques: Liquid chromatography/tandem mass spectrometry and F-19 NMR. *Analytical Chemistry* **2001,** *73*, (10), 2200-2206.

104. Hori, H.; Hayakawa, E.; Yamashita, N.; Taniyasu, S.; Nakata, F.; Kobayashi, Y., High-performance liquid chromatography with conductimetric detection of perfluorocarboxylic acids and perfluorosulfonates. *Chemosphere* **2004,** *57*, (4), 273-282.

105. Schroder, H. F., Determination of fluorinated surfactants and their metabolites in sewage sludge samples by liquid chromatography with mass spectrometry and tandem mass spectrometry after pressurised liquid extraction and separation on fluorine-modified reversed-phase sorbents. *Journal of Chromatography A* **2003,** *1020*, (1), 131-151.

106. Higgins, C. P.; Luthy, R. G., Sorption of perfluorinated surfactants on sediments. *Environ. Sci. Technol.* **2006,** *40*, (23), 7251-7256.

107. Moriwaki, H.; Takagi, Y.; Tanaka, M.; Tsuruho, K.; Okitsu, K.; Maeda, Y., Sonochemical decomposition of perfluorooctane sulfonate and perfluorooctanoic acid. *Environ. Sci. Technol.* **2005,** *39*, (9), 3388-3392.

108. Hori, H.; Nagaoka, Y.; Yamamoto, A.; Sano, T.; Yamashita, N.; Taniyasu, S.; Kutsuna, S.; Osaka, I.; Arakawa, R., Efficient decomposition of environmentally persistent perfluorooctanesulfonate and related fluorochemicals using zerovalent iron in subcritical water. *Environ. Sci. Technol.* **2006,** *40*, (3), 1049-1054.

109. Yamamoto, T.; Noma, Y.; Sakai, S. I.; Shibata, Y., Photodegradation of perfluorooctane sulfonate by UV irradiation in water and alkaline 2-propanol. *Environ. Sci. Technol.* **2007,** *41*, (16), 5660-5665.

110. Carter, K. E.; Farrell, J., Oxidative destruction of perfluorooctane sulfonate using boron-doped diamond film electrodes. *Environ. Sci. Technol.* **2008,** *42*, (16), 6111-6115.

CHAPTER 2

Development of Analytical Methods to Quantify Perfluorooctane Sulfonate (PFOS) and Related Compounds in the Environment

2.1 Abstract

Analytical techniques based on ^{19}F NMR spectroscopy and HPLC-suppressed conductivity detection were developed to detect and quantify aqueous perfluorooctane sulfonate (PFOS), perfluoroctanoic acid (PFOA), and perfluorobutane sulfonate (PFBS), a short-chain PFOS homologue. Chromatographic separation of the perfluoroalkyl surfactants (PFAS) was performed using a C_{18} reversed-phase column and a mobile phase consisting of a mixture of boric acid and acetonitrile at mixing ratios ranging from 75:25 to 45:55 (v/v). The detection limit for PFOS by ^{19}F NMR was 3.6 mg L^{-1}. On the other hand, the detection limit for PFOS, PFOA and PFBS by HPLC-suppressed conductivity detection was 1 mg L^{-1}. With both techniques, linear calibration plots were obtained up to the highest concentrations tested, 150 mg L^{-1}. The detection limits were shown to improve considerably if samples were pre-concentrated by solid-phase extraction with octadecyl silane (ODS-C_{18}) cartridges. The detection limits for PFOS of pre-concentrated samples were 3.6 mg L^{-1} and 10 ug L^{-1} by ^{19}FNMR and HPLC-suppressed conductivity

detection, respectively. Comparison of these two methodologies showed that HPLC-suppressed conductivity detection should be preferred for routine quantification of these contaminants due to its simplicity, time efficiency, and accuracy. Conversely, ^{19}F NMR can be used to characterize changes in the chemical structure of fluorinated compounds due to its inherent advantage of high specificity and no matrix interferences.

The feasibility of utilizing total organic carbon (TOC) and chemical oxygen demand (COD) analysis for the quantitative detection of PFOS in aqueous samples was also investigated. Although, the TOC analysis provided reliable quantification of PFOS, PFOA and PFBS in aqueous samples (0.5 mg TOC-PFAS L^{-1}), the non-specificity is a drawback of the technique. The dichromate-based COD method was found unsuitable for the analysis of PFOS due to the incomplete oxidation of the highly stable perfluorinated compound under the conditions of this analysis.

Key words: PFAS, PFOS, PFOA, PFBS, ^{19}F NMR, HPLC, suppressed conductivity, TOC and COD.

2.2 Introduction

Perfluoroalkyl sulfonates and perfluorocarboxylic acids such as perfluoroctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been the subject of numerous studies in recent years. These perfluorinated alkyl surfactants (PFAS) are of particular interest due to their tendency to bioaccumulate in higher organisms, persistence in the environment, and toxicity [1-3]. PFOS and PFOA have been detected in blood samples in various populations [4, 5], in wildlife throughout the world [6-8], and in aqueous and solid environmental matrices [9, 10].

Aqueous environments seem to be the primary sink for PFAS [3, 11] due to the relatively high solubility of these compounds (*i.e.,* 3.4 and 0.5 g L^{-1} for PFOA and PFOS in pure water, respectively [12, 13]), their negligible vapor pressure (i.e., 1.33 x 10^{-5} and 3.31 x 10^{-4} Pa at 20ºC and 25ºC for PFOA and PFOS, respectively [12, 13]), and moderate sorption to organic matter [14, 15]. Literature studies have reported significant PFAS concentrations in surface waters [16-22]. For instance, Skutlarek and coworkers [22] measured total PFAS concentrations as high as 4,385 ng L^{-1} in the Moehne River at Heidberg, Germany. In the same study, PFOA and PFOS were detected at concentrations of 3,640 and 247 ng L^{-1}, respectively.

The analysis of perfluorinated surfactants in environmental samples has been primarily conducted by liquid chromatography coupled to mass spectrometry (LC-MS) or tandem mass spectrometry (LC-MS/MS) [23-25]. The accuracy and precision of these techniques make them very valuable for quantification of these pollutants. However, ion

suppression due to matrix effects, sample contamination and elevated analysis cost limit the application of LC-MS or LC-MS/MS for routine analysis of environmental samples [26, 27]. Gas chromatography mass spectrometry (GC-MS) has also been employed for the analysis of PFAS [25, 28, 29]. The low volatility of the perfluoroalkane sulfonates requires the application of derivatization techniques to make them amendable to GC-MS analysis. Derivatization of PFOS compounds is problematic because esterified PFOS derivatives are not very stable [23, 30], and derivatization-reaction yields are often poor [31]. The derivatization-step makes this method time-consuming and not suitable for routine monitoring of non-volatile PFAS.

PFOS and related compounds have also been quantified by ^{19}F NMR spectroscopy. Moody and colleagues [32] reported the quantification of PFOA and PFOS in surface water samples by means of this spectroscopy technique. Recently, two studies have been published on the quantification of isomers in technical PFOS by ^{19}F NMR [33, 34].

The lack of reliable commercial standards limits the accurate analysis of PFAS in aqueous samples. Perfluoroalkyl sulfonyl-based chemicals commonly produced by electrochemical fluorination processes (ECF) are often mixtures of linear and branched isomer compounds [35, 36]. Commercial PFOS samples from fine-chemical manufacturers labeled as > 98% pure have been shown to contain 20-30 % branched PFOS isomers [34, 37-39]. The quality assurance of the analysis of perfluorinated surfactants might be improved by the use of commercially available ^{13}C-labeled and deuterium-labeled standards [23, 25]. However, ^{13}C-label standards are not optimal for all

samples matrices, therefore careful selection of the internal standard for each type of matrix is required. In addition, traces of other PFAS can be found in these standards [25]. Currently, there are two mass-labeled standards for linear PFOS, ^{13}C-PFOS and $^{18}O_2$-PFOS [5, 40].

The objective of this research is to develop analytical methods relying on ^{19}F NMR spectroscopy and HPLC with suppressed conductivity detection to monitor PFOS and related compounds in aqueous environmental matrices. Quantification of perfluoroalkyl compounds was also investigated by means of TOC and COD analysis.

2.3 Materials and Methods

2.3.1 Chemicals

Perfluorooctane sulfonic acid potassium salt, PFOS (98% purity), and 1H,1H,2H,2H-tetrahydroperfluorooctane sulfonate, TH-PFOS (98%) were purchased from SynQuest Laboratories (Alachua, FL). Perfluorobutane sulfonic acid potassium salt, PFBS (98.2%), was kindly provided by the 3M Company (St. Paul, MN). Perfluorooctanoic acid, PFOA (96%), sodium fluoride (99%), chromium (III) acetylacetonate, $Cr(acac)_3$ (97%), and potassium hydrogen phthalate (99%) were obtained from Sigma-Aldrich (St. Louis, MO). 4’-(trifluoromethoxy)acetanilide, 4-TFMeAc (97%) was obtained from Matrix Scientific

(Columbia, SC). Methanol-D4 was purchased from Cambridge Isotope Laboratories (Andover, MA). HPLC-grade acetonitrile, methanol, sulfuric acid, and boric acid (99.5%) were purchased from Mallinckrodt Chemicals (Phillipsburg, NJ). All chemicals were used as received.

2.3.2 ^{19}F NMR quantification

The quantitative determination of PFOS by ^{19}F NMR was performed by a method adapted from Moody *et al.* [32]. Samples were dissolved in 0.7 mL of 90% H_2O / 10% CD_3OD or in 90% CH_3OH / 10% CD_3OD containing 4 mg mL^{-1} chromium acetylacetonate ($Cr(acac)_3$) and analyzed in 5-mm tubes. The internal standard, 4-TFMeAc, was present in all samples at a concentration of 140 mg L^{-1}, corresponding to a 1:1 molar 4-TFMeAc:PFOS ratio for the most concentrated PFOS sample. Samples containing PFOS and TH-PFOS in a 1:1 molar fluoride ratio (100 mg PFOS L^{-1}:112 mg TH-PFOS L^{-1}) were prepared as described above. Solid phase extraction (SPE) was conducted to preconcentrate analytes when required. SPE cartridges (3 mL, 500 mg ODS-C_{18}, Agilent Technologies, DE) mounted on a vacuum manifold were conditioned with 5 mL methanol, following by 5 mL of dionized water and then aqueous sample was loaded at 1 mL min^{-1}. SPE cartridges were rinsed with 5 mL of dionized water and then dried under vacuum for 3 h prior elution. Analytes were eluted with 5 mL methanol and collected in clean Nalgene flasks. Standard solutions spiked with known PFOS

concentrations were extracted in parallel to determine recovery efficiencies as discussed later.

All ^{19}F NMR spectra were acquired at 22°C on a Varian Unity-300 spectrometer operating at a ^{19}F frequency of 282.208 MHz using a 5mm 4-nucleus (^{31}P, ^{13}C, ^{19}F, ^{1}H) probe. Acquisition involved a relaxation delay of 1.44 s followed by a 90° pulse (16.2 μs) and a Hahn echo with an echo delay of 100 μs, with 16,384 complex data points and a spectral width of 33,333 Hz. The acquisition time for all samples was 32.5 min. A 10 Hz line broadening was applied before zero filling to 32,768 data points and Fourier transform. Baseline correction was performed using a 5th order polynomial, and chemical shifts were referenced to internal standard, 4-TFMeAc at -58.08 ppm [41].

2.3.3 HPLC-suppressed conductivity detection

PFOS and related compounds in aqueous samples were analyzed by a HPLC system with suppressed conductivity detector (ICS-3000 Ion Chromatography System, DIONEX, Sunnyvale, CA). The chromatograph was equipped with an autosampler (injection volume 100 μL), a pump, a degasser, a guard column (Acclaim Polar Advantage II, C_{18}, 4.3 mm i.d., 1 cm length) and a separation column (Acclaim Polar Advantage II, C_{18}, 4.6 mm i.d., 25 cm length) operating at 35 ºC. A mixture of 20 mM boric acid (pH 9.0) and 95% acetonitrile was used as the mobile phase at a flow rate of 1mL min^{-1}. The amount of boric acid varied with linear gradient program starting with 75% (v/v) at time zero and

decreasing to 45% (v/v) in 13.2 min. Blanks were continuously run to assure that the column was clean and that traces of the analyte were not carried over between samples. Solid phase extraction (SPE) was conducted to preconcentrate analytes when required as described above. Standard solutions spiked with known PFOS concentrations were extracted in parallel to determine recovery efficiencies as discussed later.

2.3.4 TOC analysis

Measurements of PFOS and derivatives in aqueous solution were determined using a Total Organic Carbon analyzer (Shimadzu TOC-V CSH/CSN system, Columbia, MD). All samples, including blanks and standards, were acidified with HCl to pH 2.20-2.50 prior to analysis.

2.4 Results and Discussion

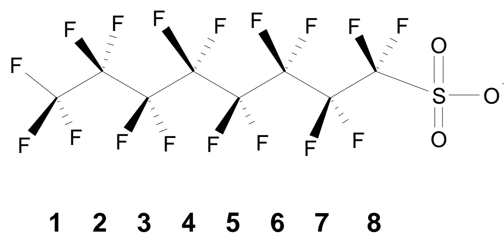
2.4.1 ^{19}F NMR assignments and quantification of PFOS

The chemical structures of PFOS and TH-PFOS including numerical labels of the carbon chains are depicted in Figure 2.1. ^{19}F NMR is a very valuable technique for structural

studies of fluorinated compounds. Figure 2.2 illustrates a typical ^{19}F NMR spectrum of aqueous PFOS sample. The spectral window was restricted to -55 ppm to -135 ppm to improve the clarity of the spectrum. Chemical shifts were referenced to the internal standard 4-TFMeAc at -58.08 ppm [41]. Moody and coworkers incorrectly assigned the resonance at -72.0 ppm to 4-TFMeAc [32]. In this study, the mentioned resonance is observed even if 4-TFMeAc is not added, suggesting the presence of fluorinated PFOS impurities. These impurities have been identified as branched PFOS isomers as described in Chapter 5 and 6. The peak at about –72 ppm is characteristic of the internal and terminal branched CF_3 of all PFOS isomers [33, 34].

Previous studies on ^{19}F NMR spectroscopy of perfluorinated compounds have clearly identified some of the key peaks in the carbon-fluorine chain [42, 43]. The peak at -80.7 ppm has been assigned to the terminal CF_3 (C1), the peak at -113.1 ppm is shown to arise from fluorine atoms in C8 adjacent to the sulfonate group, and the peak at -125.6 ppm is characteristic of the CF_2 group (C2) next to the terminal CF_3 [42]. The resonance peaks at – 120 ppm, -121.2 ppm, and -122.1 ppm correspond to intermediate CF_2 moieties in carbons atoms C3 to C7.

A)



B)

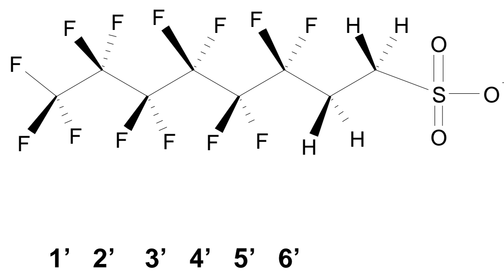


Figure 2.1 Molecular structure of perfluoroalkyl surfactants. A) PFOS and B) TH-PFOS.

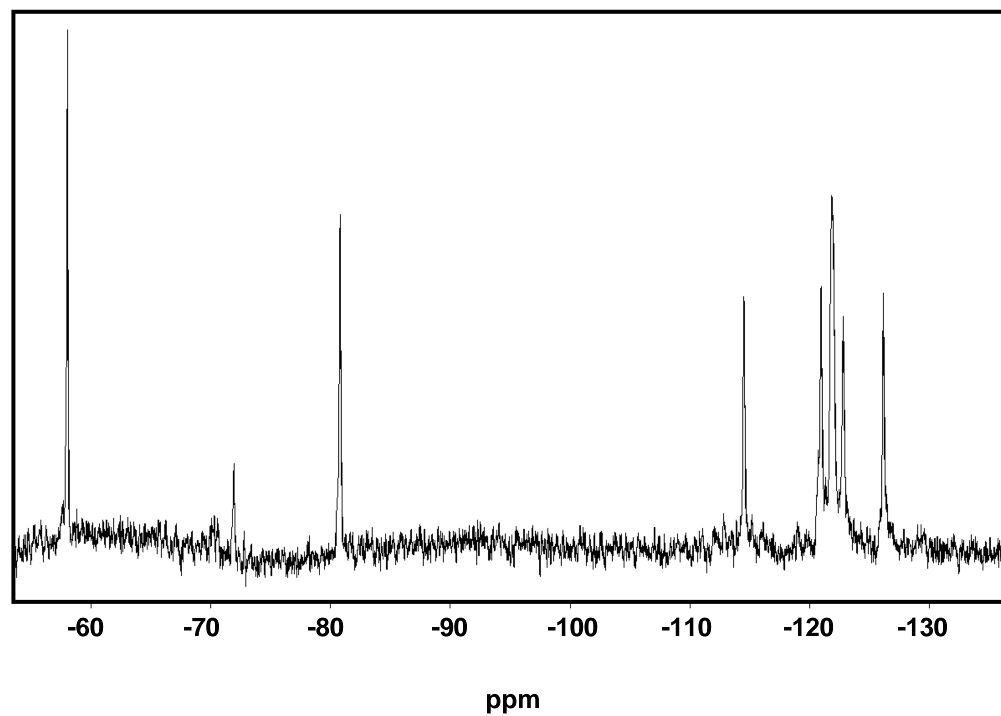


Figure 2.2 ^{19}F NMR spectrum of an aqueous PFOS sample (31.5 mg L^{-1}) relative to the internal standard, 4'-(trifluoromethoxy)acetanilide, 4-TFMeAc, (-58.08 ppm).

The spectrum of TH-PFOS, a compound structurally related to PFOS (Figure 2.1), was obtained to identify the intermediate CF_2 NMR signals of the perfluorinated compounds. The ^{19}F NMR spectra of aqueous solutions containing PFOS only and a mixture of PFOS:THPFOS in a 1:1 molar fluoride ratio are illustrated in Figure 2.3. For comparison purposes the spectral window was restricted to -77 ppm to -133 ppm.

As expected, the spectrum of the solution containing the mixture of PFOS and TH-PFOS is different from that of PFOS alone. The intensities of the peaks at -80.7 and -125.6 ppm assigned to the terminal CF_3 (C1) and the CF_2 (C7) moiety next to it, respectively, are higher in the PFOS:THPFOS solution spectrum as compared to the same peaks in the spectrum of the solution that only contains PFOS. These findings suggest that C1' and the C2' in the TH-PFOS molecule are chemically equivalent to the fluorine atoms in C1 and C2 in the PFOS molecule. These two peaks seem to appear in the same place for long chain perfluorinated compounds as suggested by Buchanan et *al.* (2005) [42].

In contrast, the intensities of the peaks assigned to the CF_2 moiety adjacent to the sulfonate group C8 (-113.1 ppm) and that at –120 ppm are reduced approximately by half in the spectrum of the mixture. The decrease in intensity suggests that the resonance at –120 ppm corresponds to the fluorine atoms in C7 in the PFOS molecule.

In the TH-PFOS molecule, the chemical environment of the fluorine atoms that are in the vicinity of the hydrogen atoms is different from that of fluorine atoms in PFOS, a compound that is fully fluorinated. Therefore, the two additional peaks present in the perfluorinated mixture at -112.9 and -122.8 ppm must correspond to fluorine atoms in

C3' to C6' in the TH-PFOS compound. So, the peak at -113.4 ppm presumably arises from the CF_2 group (C6') next to the $CH_2CH_2SO_3^-$ group. Under the experimental conditions employed in this study, no further information can be obtained to identify the intermediate fluorine atoms in the PFOS structure.

As shown in Table 2.1, our ^{19}F NMR assignments are in good agreement with those reported by other research groups [33, 34]. The slight discrepancy with the NMR signals assigned by Arsenault and coworkers [33] can be attributed to the use of a different compound as the internal standard as well as experimental/instrumental errors. The signals referenced to hexafluorobenzene (-169 ppm) are slightly shifted upfiled by 5 ppm, meaning that they were generated at higher external electric magnetic fields. Considering that the shifts were observed in all fluorine assignments, the differences are negligible.

Table 2.1 ^{19}F NMR assignments of the PFOS molecule.

Carbon #	**Resonance (ppm)**	**Literature data (ppm)**	
	Our work	**Arsenault *et. al*** [a]	**Vyas *et al.*** [b]
C1	-80.7	-86.0	-82
C2	-125.6	-131.0	-127
C3	--	-127.4	-124
C4	--	-126.6	--
C5	--	-126.4	--
C6	--	-126.3	--
C7	-120	-125.0	-121
C8	-113.1	-117.8	-115

Literature data: [a] [33] and [b] [34].

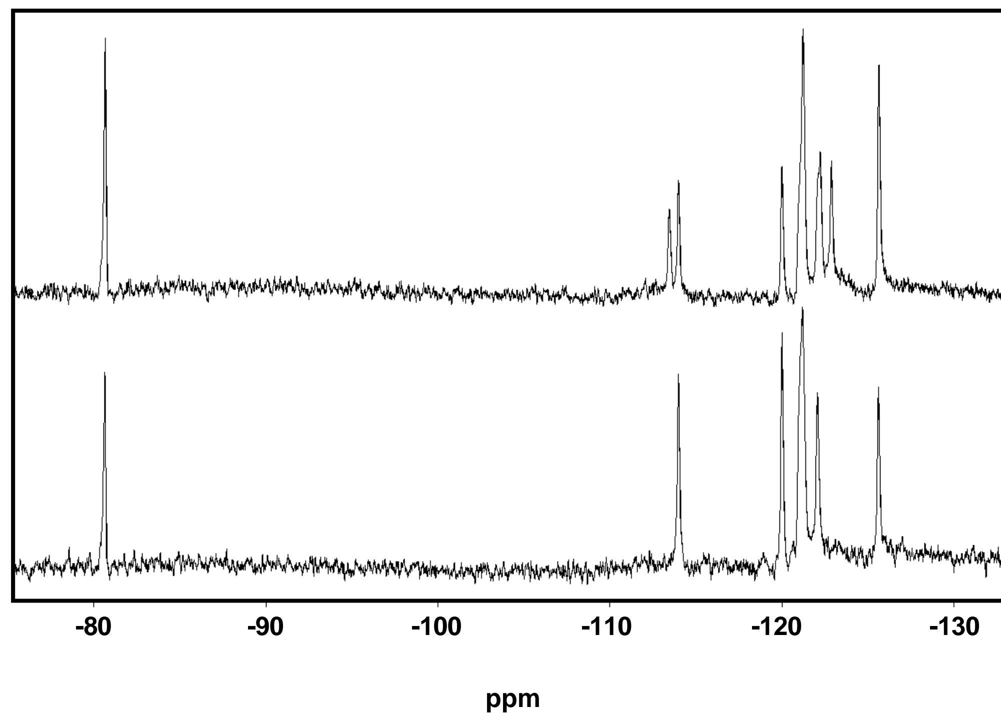


Figure 2.3 ^{19}F NMR spectra of aqueous solutions containing a mixture of PFOS and TH-PFOS in a 1:1 molar fluoride ratio (100 mg PFOS L^{-1} : 112 mg TH-PFOS L^{-1}) (upper panel) and PFOS only solution (100 mg L^{-1}) (lower panel).

^{19}F NMR quantification of PFOS in aqueous samples was conducted according to the protocol described in the *Materials and Methods* section. A known concentration of the internal standard, 4-TFMeAc, was added to all samples. Quantification was possible by integrating the area of the PFOS signal relative to the area of the internal standard. Although calibration curves were obtained for each PFOS peak in the ^{19}F NMR spectrum, the area of the largest peak, which corresponds to the terminal CF_3, was used for quantification purposes (Figure 2.4). Linear calibration curves ($r^2 > 0.98$) using known PFOS concentrations ranging from 10 to 140 mg L^{-1} were employed.

The detection limit of ^{19}F NMR based on a signal-to-noise ratio of 3 was found to be 12.5 mg PFOS L^{-1} in aqueous samples. This limit could be significantly improved when a solvent of less polarity-polarizability character is employed. In samples concentrated by SPE procedures based on ODS-C_{18} cartridges using methanol as solvent, PFOS was detected at concentrations as low as 3.6 mg L^{-1}, which is 3.5-fold lower than the detection limit obtained in aqueous samples. This could be attributed to the interactions of the molecule with the solvent. Hence, since H_2O interactions with the fluorine atoms of PFOS are stronger than those with CH_3OH, a higher coupling between the ^{19}F nuclei spins will result in the aqueous solvent, producing an increased diversification of F atoms in the molecule and, thus, higher detection limits [44]. The recovery efficiency of this procedure was 100.8% $\pm$ 5.4% for triplicate aqueous samples of 100 mg L^{-1} of PFOS standard.

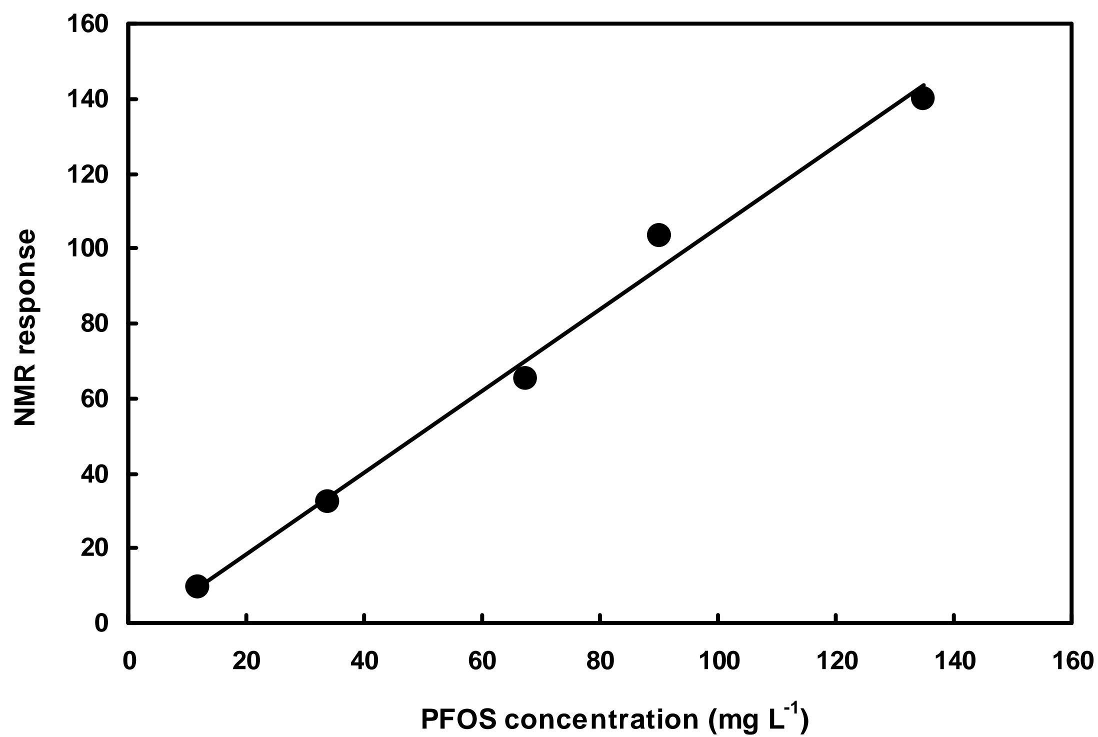


Figure 2.4 Calibration curve of aqueous PFOS based on the terminal CF_3 peak by ^{19}F NMR ($r^2 > 0.98$).

Quantification of PFOS by NMR spectroscopy was accurate and precise. However, the sensitivity of the technique is low and quantification of PFOS will generally require sample preconcentration, e.g., by SPE extraction. Nonetheless, alternative techniques such as LC-MS or LC-MS/MS also often require sample pretreatment by SPE because the concentrations of PFOS in environmental samples are generally in the low ppm range (µg L^{-1}) [23, 24, 45].

Moody *et al.* [32] reported higher detection limits than those obtained in this study by one order of magnitude, 0.25 mg L^{-1} for concentrated samples. However, the acquisition time used in our study was relatively short, 32.5 min, and the sensitivity of the F-NMR sytem used (a Varian Unity-300 spectrometer) was limited compared to that in the study conducted by Moody and coworkers.

^{19}F NMR spectroscopy is a valuable technique to provide structural information of perfluoroalkyl compounds due to its ability to respond to different electronic environments with changes in the chemical shift. Unfortunately, ^{19}F NMR cannot be used for routine monitoring of PFOS because data acquisition and data processing are very time-consuming compared to other analytical methods such as LC-MS and LC-MS/MS.

2.4.2 HPLC-suppressed conductivity detection

A HPLC method that relies on suppressed conductivity detection has been developed to separate and detect aqueous PFOS and related perfluoroalkyl compounds in

environmental samples. Chromatographic separation was conducted with a C_{18} reverse-phase column and a mobile phase consisting of a mixture of boric acid and acetonitrile with various mixing ratios.

Figure 2.5 shows a chromatogram obtained for a standard containing 25 mg L^{-1} commercial PFOS. Well-resolved peaks and reproducible results were achieved by this chromatography method. Moreover, various PFOS isomers were separated with this method. The peak at 9.9 min is assigned to the linear PFOS anion and the two little peaks eluting before the major peak at 9.3 and 9.5 min correspond to PFOS isomers. These isomers are structural isomers of PFOS which have the same molecular weight as PFOS but are branched perfluoroalkyl sulfonates [38, 46]. Detailed information on the identification of branched PFOS isomers is provided in Chapter 5.

The purity of the PFOS salt was calculated by relating the area of the linear PFOS peak to the sum of the areas of all three peaks, assuming that the response factors for branched and linear isomers are equivalent. The latter assumption cannot be confirmed experimentally due to the lack of authentic isomer standards. Linear PFOS was found to be 75.4% pure [38]. Previous studies have reported the purity of PFOS commercial standards (labeled as >98%) in the range of 70 to 80% [34, 37, 39].

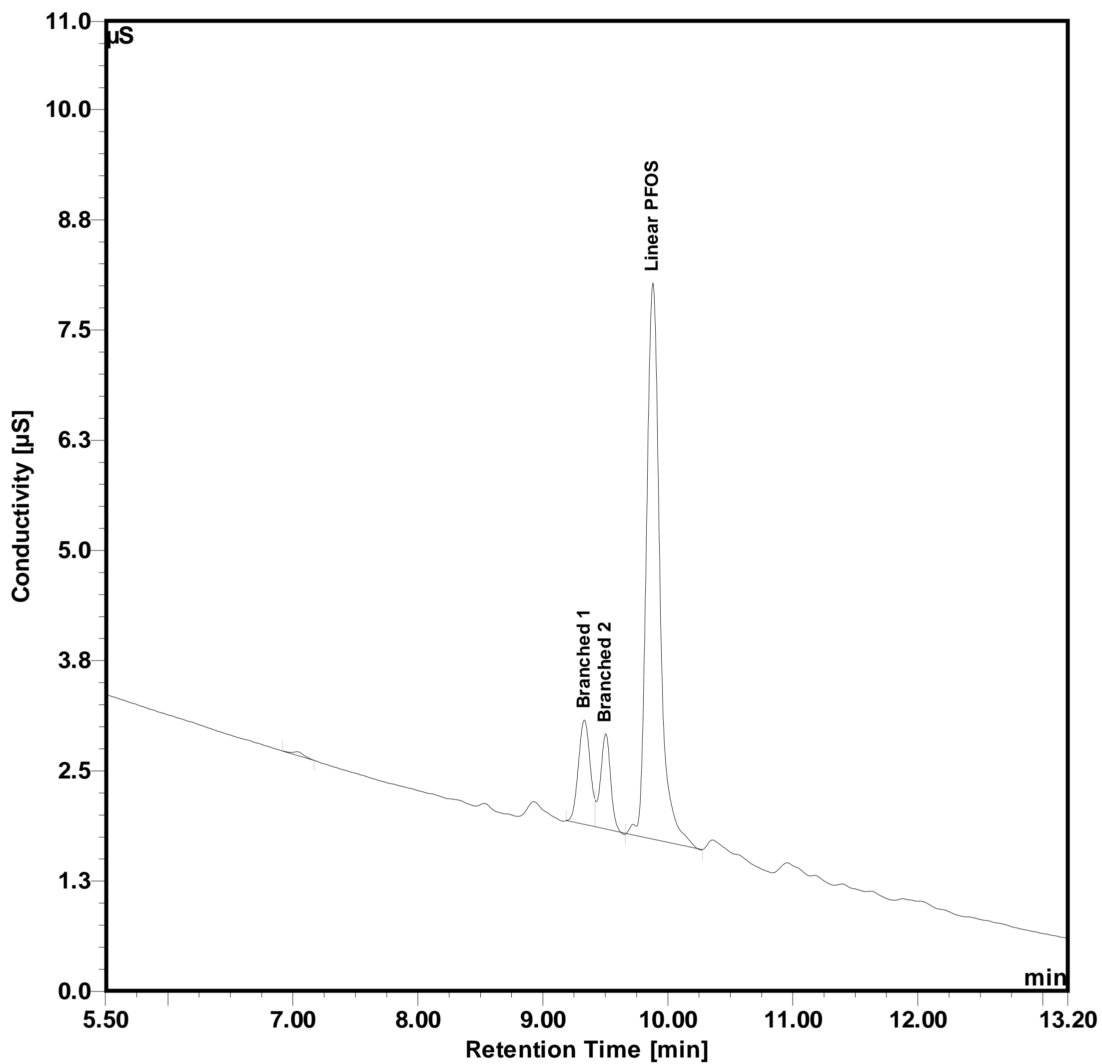


Figure 2.5 HPLC-suppressed conductivity detection chromatogram of aqueous PFOS (25 mg L^{-1}).

The quantitative determination of low-ppm concentration of aqueous PFOS was effectively conducted by HPLC-suppressed conductivity detection as described in the *Materials and Methods* section. . The total concentration of perfluorinated compounds in aqueous samples was obtained by linear calibration curves ($r^2 > 0.99$) using known concentrations of PFOS, PFBS and PFOA ranging from 0 to 150 mg L^{-1}. The detection limit of PFOS, PFBS and PFOA was 1 mg L^{-1}. A calibration curve for PFOS in aqueous samples is shown in Figure 2.6. These results are consistent with those obtained by Hori *et al.* [47] by conductimetric detection. A detection limit of 0.66 to 1.0 mg PFOS L^{-1} was reported utilizing a ODS column and a methanol:phosphate gradient with linear calibration graphs up to 100 mg PFOS L^{-1}. The new detection limits for samples pre-concentrated by SPE using ODS-C_{18} cartridges and methanol as solvent increased significantly. PFOS was detected at concentrations as low as 10 μg L^{-1}. Blanks samples spiked with known concentrations of PFOS (25 mg L^{-1}) were extracted in parallel to determine the recovery efficiency which was 102% $\pm$ 9.4% for triplicate samples.

The use of a reverse-phase C_{18} column provided good resolution for the perfluorinated alkyl substances. A mixture of PFBS, PFOA and PFOS in a 1:1:1 ratio was successfully separated as shown in Figure 2.7. Standard samples of each perfluorinated compound were run in parallel to identify their retention times. The peaks eluted based on number of carbons and molecular weight. PFBS, 4-carbon chain with a molecular weight of 299.08 g mol^{-1}, appeared first followed by PFOA, a 8-carbon chain compound with a molecular weight of 414.07 g mol^{-1}, and finally PFOS, 8-carbon compound with a molecular weight of 499.12 g mol^{-1}. The response factors determined for these

perfluorinated compounds differed considerably. The response factor was calculated by dividing the area of analyte by its concentration. The response factors for PFBS, PFOA and linear PFOS were 0.104, 0.067, and 0.039, respectively.

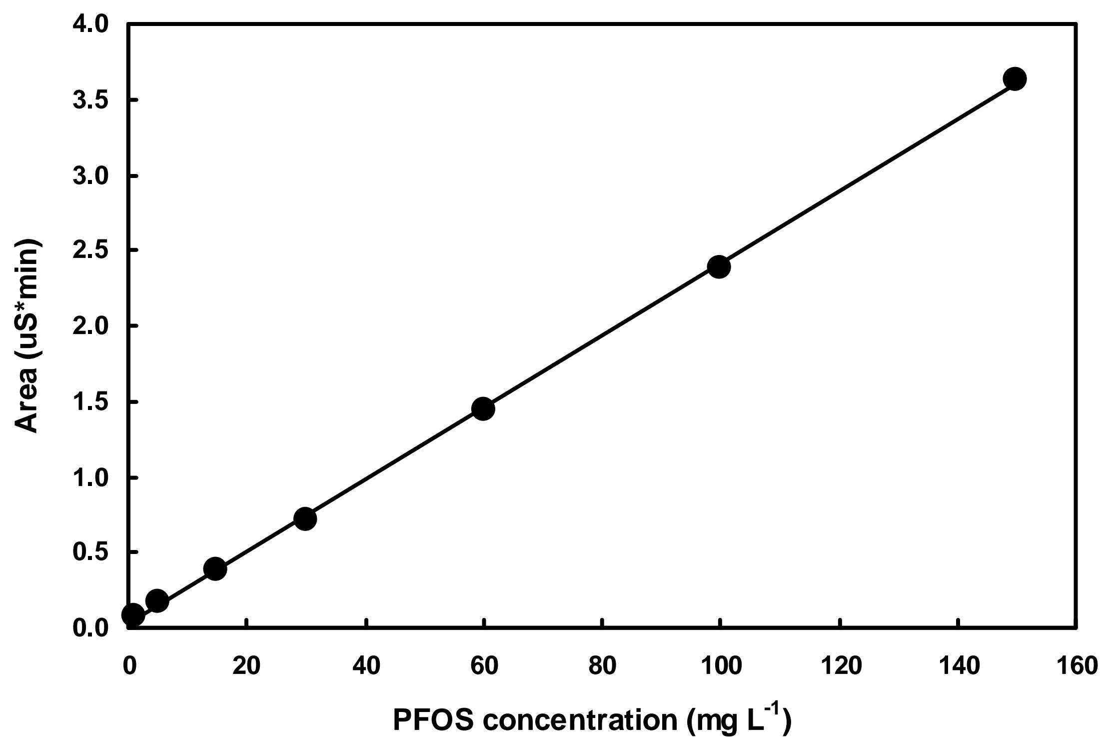


Figure 2.6 Calibration curve of aqueous PFOS by HPLC suppressed conductivity detection ($r^2 > 0.99$).

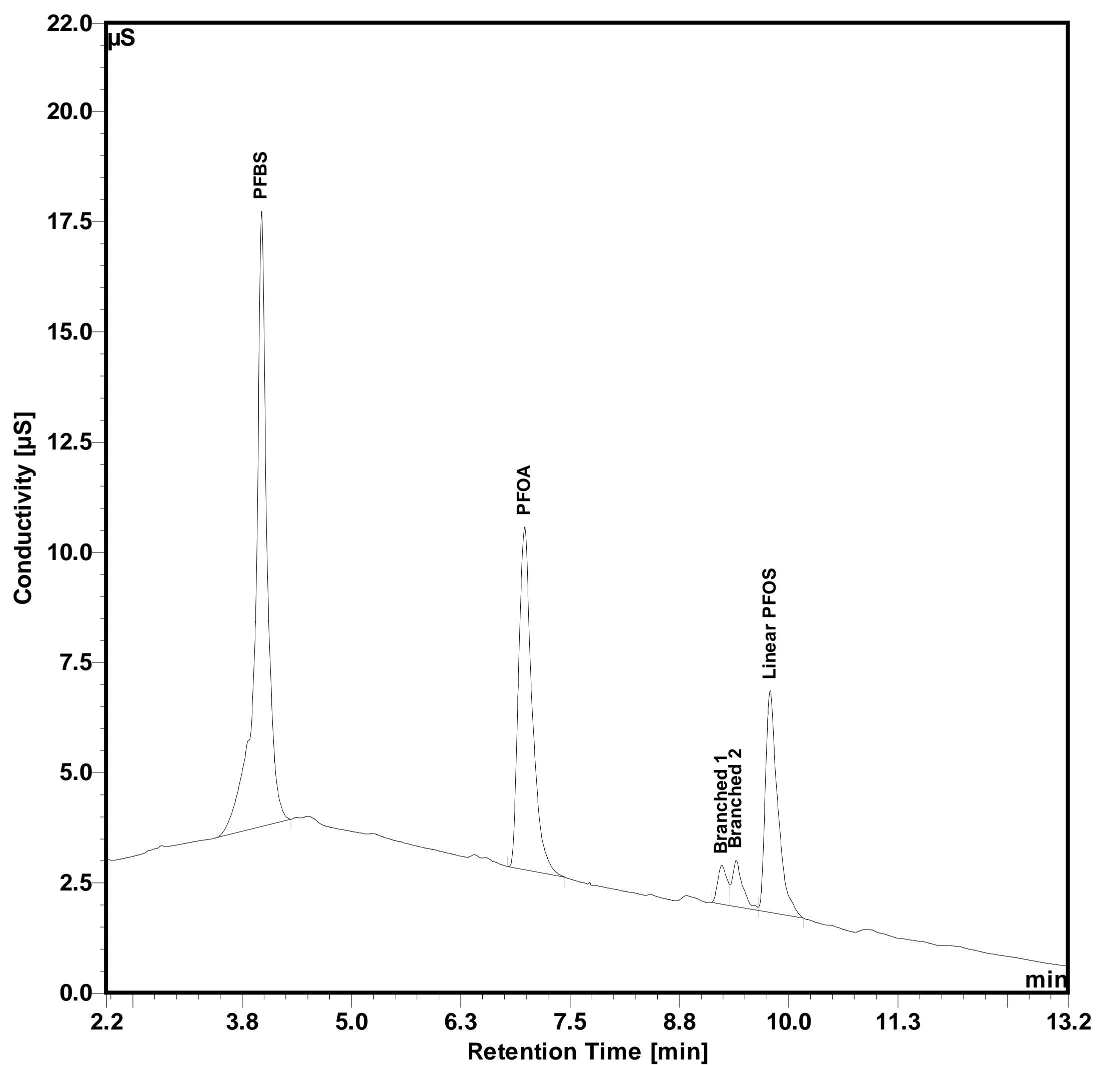


Figure 2.7 HPLC-suppressed conductivity detection chromatograph of an aqueous solution containing PFBS, PFOA, and PFOS (21 mg L^{-1} each).

HPLC based on suppressed conductivity detection is a simple, rapid and efficient method for the separation and detection of perfluorinated compounds. Since it does not require sample pretreatment, the analysis times are significantly reduced compared to those required in ^{19}F NMR spectroscopy. Besides, this technique is less expensive and time consuming than LC-MS/MS. The good separation achieved by the reverse phase C_{18} column allows detection of PFOS isomers and calculation of the purity of PFOS commercial standard. This chromatography method can be successfully utilized for monitoring PFAS on a routine basis in aqueous solutions. Moreover, the high sensitivity of the technique makes it appropriate for the determination and quantification of low-ppm amounts of perfluoroalkyl surfactants in real environmental samples. Detection of PFAS concentrations in the ppb-range is feasible if the samples are preconcentrated by solid-phase extraction with cartridges based on ODS-C_{18} columns.

2.4.3 Quantification of PFOS by TOC and COD analysis

The feasibility of utilizing TOC and COD analysis for the quantitative detection of PFOS in aqueous samples was also investigated in this study. Instrument calibration was performed using potassium hydrogen phthalate standards ranging from 0.40 to 25 mg TOC L^{-1}. The coefficient of determination (r^2) for each calibration was > 0.99. Calibration lines obtained using potassium hydrogen phthalate and PFAS standards were

nearly identical confirming the suitability of TOC measurements for PFAS quantification.

Figure 2.8 shows a calibration curve for PFOS in aqueous samples by total organic carbon (TOC) analysis. Linear calibration curves ($r^2 > 0.99$) using known concentrations of PFOS ranging from 0.5 to 19.2 mg TOC-PFOS L^{-1}, equivalent to 2.5 to 100.0 mg PFOS L^{-1}, were obtained. The detection limit was 2.5 mg PFOS L^{-1} equivalent to 0.5 mg L^{-1} of PFOS as TOC. A significant improve in the detection limit can be obtained by using a high-sensitivity catalyst; PFOS concentrations in the µg L^{-1} range can be detected. Although the TOC analysis provides reliable quantification of PFOS in environmental samples, the lack of selectivity is a drawback of the technique. The method is not compound specific since all organic carbons are detected.

PFOS solutions containing 180 and 250 mg L^{-1} were reacted with the dichromate solution for 2 h at 150ºC according to the COD protocol described elsewhere [48]. Blank solutions lacking PFOS were also analyzed. No oxidation of PFOS was detected under these experimental conditions. The fact that the dichromate-based COD method was not suitable for the analysis of PFOS was likely due to the well-known chemical stability of perfluorinated compounds. The strength of the carbon-fluorine bond is responsible for the recalcitrant nature of these pollutants [36]. This finding is surprising considering that dichromate is a strong oxidant and the COD assay is the standard method for the analysis of organic matter in wastewaters. However, highly persistent organic compounds such as benzene and pyridines are also not oxidized in the COD test [49].

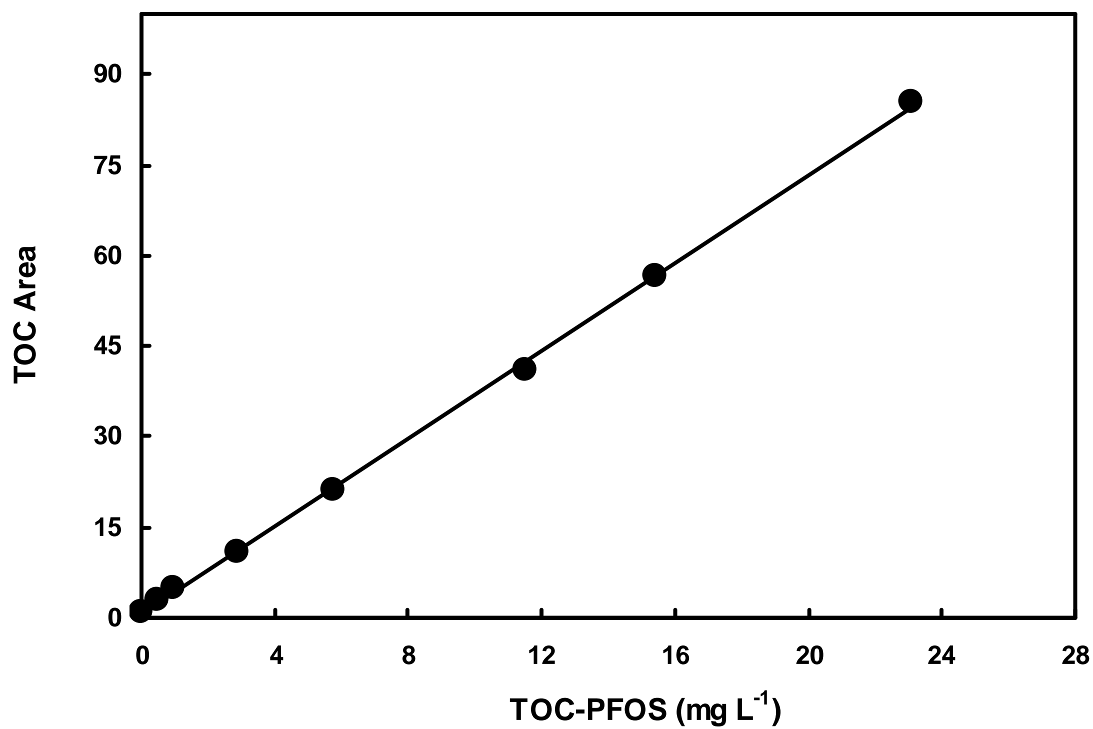


Figure 2.8 Calibration curve of aqueous PFOS by TOC analysis ($r^2 > 0.99$).

2.5 Conclusions

The detection and quantification of PFOS and related compounds in aqueous samples by two independent methodologies, *i.e.* HPLC-suppressed conductivity detection and ^{19}F NMR spectroscopy, was investigated. In terms of sensitivity and analysis time, the HPLC-suppressed conductivity detection method offers an advantage over ^{19}F NMR as a technique for the quantification of perfluorinated sulfonates and carboxylates. However, F-NMR spectroscopy is a powerful technique to gain information on the chemical environment of fluorine atoms in the perfluorinated chemicals. The presence of branched PFOS isomers in the PFOS commercial standard was confirmed by these two independent methods. Analysis of PFOS and derivatives can also be conducted by total organic carbon analyzer when no matrix interferences are present, *i.e.*, organic matter. The COD method was not suitable for the analysis of PFOS in aqueous environments; the compound was not oxidized under these conditions, confirming its well-known chemical stability.

2.6 Acknowledgments

We would like to thank the Semiconductor Research Corporation/Sematech Engineering Research Center for Environmentally Benign Semiconductor Manufacturing for financial support. We are thankful to Dr. Neil Jacobsen from the Department of Chemistry, University of Arizona, for his support with ^{19}F NMR analysis.

2.7 References

1. Houde, M.; Martin, J. W.; Letcher, R. J.; Solomon, K. R.; Muir, D. C. G., Biological monitoring of polyfluoroalkyl substances: A review. *Environ. Sci. Technol.* **2006,** *40*, (11), 3463-3473.
2. Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J., Perfluoroalkyl acids: A review of monitoring and toxicological findings. *Toxicol. Sci.* **2007,** *99*, (2), 366-394.
3. Conder, J. M.; Hoke, R. A.; De Wolf, W.; Russell, M. H.; Buck, R. C., Are PFCAs bioaccumulative? A critical review and comparison with regulatory lipophilic compounds. *Environ. Sci. Technol.* **2008,** *42*, (4), 995-1003.
4. Kannan, K.; Corsolini, S.; Falandysz, J.; Fillmann, G.; Kumar, K. S.; Loganathan, B. G.; Mohd, M. A.; Olivero, J.; Van Wouwe, N.; Yang, J. H.; Aldous, K. M., Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. *Environ. Sci. Technol.* **2004,** *38*, (17), 4489-4495.
5. Butenhoff, J. L.; Olsen, G. W.; Pfahles-Hutchens, A., The applicability of biomonitoring data for perfluorooctanesulfonate to the environmental public health continuum. *Environmental Health Perspectives* **2006,** *114*, (11), 1776-1782.
6. Giesy, J. P.; Kannan, K., Global distribution of perfluorooctane sulfonate in wildlife. *Environ. Sci. Technol.* **2001,** *35*, (7), 1339-1342.

7. Martin, J. W.; Smithwick, M. M.; Braune, B. M.; Hoekstra, P. F.; Muir, D. C. G.; Mabury, S. A., Identification of long-chain perfluorinated acids in biota from the Canadian Arctic. *Environ. Sci. Technol.* **2004,** *38*, (2), 373-380.

8. Tao, L.; Kannan, K.; Kajiwara, N.; Costa, M. M.; Fillmann, G.; Takahashi, S.; Tanabe, S., Perfluorooctanesulfonate and related fluorochemicals in albatrosses, elephant seals, penguins, and Polar Skuas from the Southern Ocean. *Environ. Sci. Technol.* **2006,** *40*, (24), 7642-7648.

9. Higgins, C. P.; Field, J. A.; Criddle, C. S.; Luthy, R. G., Quantitative determination of perfluorochemicals in sediments and domestic sludge. *Environ. Sci. Technol.* **2005,** *39*, (11), 3946-3956.

10. Senthilkumar, K.; Ohi, E.; Sajwan, K.; Takasuga, T.; Kannan, K., Perfluorinated compounds in river water, river sediment, market fish, and wildlife samples from Japan. *Bulletin of Environmental Contamination and Toxicology* **2007,** *79*, (4), 427-431.

11. Prevedouros, K.; Cousins, I. T.; Buck, R. C.; Korzeniowski, S. H., Sources, fate and transport of perfluorocarboxylates. *Environ. Sci. Technol.* **2006,** *40*, (1), 32-44.

12. EPA *Preliminary Risk Assesment of the Developmental Toxicity Associated with Exposure to Perfluorooctanoic Acid and its Salts,* U.S. Environmental Protection Agency: Washington, DC, 2003a.

13. Brooke, D.; Footitt, A.; Nwaogu, T. A., Environmental risk evaluation report: Perfluorooctanesulphonate (PFOS). *Environment Agency, Chemicals Assessment Section, Wallingford, UK* **2004**.

14. Ochoa-Herrera, V.; Sierra-Alvarez, R., Removal of prefluorinated surfactants by sorption onto granular activated carbon, zeolite and sludge. *Chemosphere* **2008,** *72*, 1588 - 1593.

15. Higgins, C. P.; Luthy, R. G., Sorption of perfluorinated surfactants on sediments. *Environ. Sci. Technol.* **2006,** *40*, (23), 7251-7256.

16. Saito, N.; Harada, K.; Inoue, K.; Sasaki, K.; Yoshinaga, T.; Koizumi, A., Perfluorooctanoate and perfluorooctane sulfonate concentrations in surface water in Japan. *Journal of Occupational Health* **2004,** *46*, (1), 49-59.

17. So, M. K.; Taniyasu, S.; Yamashita, N.; Giesy, J. P.; Zheng, J.; Fang, Z.; Im, S. H.; Lam, P. K. S., Perfluorinated compounds in coastal waters of Hong Kong, South China, and Korea. *Environ. Sci. Technol.* **2004,** *38*, (15), 4056-4063.

18. Yamashita, N.; Kannan, K.; Taniyasu, S.; Horii, Y.; Petrick, G.; Gamo, T., A global survey of perfluorinated acids in oceans. *Mar. Pollut. Bull.* **2005,** *51*, (8-12), 658-668.

19. Loos, R.; Wollgast, J.; Huber, T.; Hanke, G., Polar herbicides, pharmaceutical products, perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and nonylphenol and its carboxylates and ethoxylates in surface and tap waters around Lake Maggiore in Northern Italy. *Anal. Bioanal. Chem.* **2007,** *387*, (4), 1469-1478.

20. Konwick, B. J.; Tomy, G. T.; Ismail, N.; Peterson, J. T.; Fauver, R. J.; Higginbotham, D.; Fisk, A. T., Concentrations and patterns of perfluoroalkyl acids in Georgia, USA surface waters near and distant to a major use source. *Environmental Toxicology and Chemistry* **2008,** *27*, (10), 2011-2018.

21. Sinclair, E.; Mayack, D. T.; Roblee, K.; Yamashita, N.; Kannan, K., Occurrence of perfluoroalkyl surfactants in water, fish, and birds from New York State. *Archives of Environmental Contamination and Toxicology* **2006,** *50*, (3), 398-410.

22. Skutlarek, D.; Exner, M.; Farber, H., Perfluorinated surfactants in surface and drinking water. *Environ. Sci. Pollut. Res.* **2006,** *13*, (5), 299-307.

23. Villagrasa, M.; de Alda, M. L.; Barcelo, D., Environmental analysis of fluorinated alkyl substances by liquid chromatography-(tandem) mass spectrometry: a review. *Anal. Bioanal. Chem.* **2006,** *386*, (4), 953-972.

24. Richardson, S. D., Environmental mass spectrometry: Emerging contaminants and current issues. *Analytical Chemistry* **2008,** *80*, (12), 4373-4402.

25. de Voogt, P.; Saez, M., Analytical chemistry of perfluoroalkylated substances. *Trac-Trends Anal. Chem.* **2006,** *25*, (4), 326-342.

26. Hernandez, F.; Sancho, J. V.; Pozo, O. J., Critical review of the application of liquid chromatography/mass spectrometry to the determination of pesticide residues in biological samples. *Anal. Bioanal. Chem.* **2005,** *382*, (4), 934-946.

27. Barcelo, D.; Petrovic, M., Challenges and achievements of LC-MS in environmental analysis: 25 years on. *Trac-Trends Anal. Chem.* **2007,** *26*, (1), 2-11.

28. Martin, J. W.; Muir, D. C. G.; Moody, C. A.; Ellis, D. A.; Kwan, W. C.; Solomon, K. R.; Mabury, S. A., Collection of airborne fluorinated organics and analysis by gas chromatography/chemical ionization mass spectrometry. *Analytical Chemistry* **2002,** *74*, (3), 584-590.

29. Jahnke, A.; Ahrens, L.; Ebinghaus, R.; Berger, U.; Barber, J. L.; Temme, C., An improved method for the analysis of volatile polyfluorinated alkyl substances in environmental air samples. *Anal. Bioanal. Chem.* **2007,** *387*, (3), 965-975.

30. Moody, C. A.; Field, J. A., Perfluorinated surfactants and the environmental implications of their use in fire-fighting foams. *Environ. Sci. Technol.* **2000,** *34*, (18), 3864-3870.

31. Langlois, I.; Berger, U.; Zencak, Z.; Oehme, M., Mass spectral studies of perfluorooctane sulfonate derivatives separated by high-resolution gas chromatography. *Rapid Communications in Mass Spectrometry* **2007,** *21*, (22), 3547-3553.

32. Moody, C. A.; Kwan, W. C.; Martin, J. W.; Muir, D. C. G.; Mabury, S. A., Determination of perfluorinated surfactants in surface water samples by two independent analytical techniques: Liquid chromatography/tandem mass spectrometry and F-19 NMR. *Analytical Chemistry* **2001,** *73*, (10), 2200-2206.

33. Arsenault, G.; McCrindle, R.; Chittim, B.; McAlees, A., Separation and fluorine nuclear magnetic resonance spectroscopy (^{19}F-NMR) analysis of the individual branched isomers present in technical perfluorooctanesulfonic acid (PFOS). *Organohalogen Compounds* **2005,** *67*, 818 - 822.

34. Vyas, S. M.; Kania-Korwel, I.; Lehmler, H. J., Differences in the isomer composition of perfluoroctanesulfonyl (PFOS) derivatives. *J. Environ. Sci. Health Part A-Toxic/Hazard. Subst. Environ. Eng.* **2007,** *42*, (3), 249-255.

35. 3M *Fluorochemical isomer distribution by ^{19}F NMR spectroscopy*; U.S. Evironmental Protection Agency Public Docket AR226-0564. : 1997.

36. Kissa, E., *Fluorinated surfactants and repellants*. Marcel Dekker, Inc.: New York, 2001.

37. Arsenault, G.; Chittim, B.; McAlees, A.; McCrindle, R.; Riddell, N.; Yeo, B., Some issues relating to the use of perfluoro octane sulfonate (PFOS) samples as reference standards. *Chemosphere* **2008,** *70*, (4), 616-625.

38. Ochoa-Herrera, V.; Sierra-Alvarez, R.; Somogyi, A.; Jacobsen, N. E.; Wysocki, V. H.; Field, J. A., Reductive defluorination of perfluorooctane sulfonate. *Environ. Sci. Technol.* **2008,** *42*, (9), 3260-3264.

39. Martin, J. W.; Kannan, K.; Berger, U.; De Voogt, P.; Field, J.; Franklin, J.; Giesy, J. P.; Harner, T.; Muir, D. C. G.; Scott, B.; Kaiser, M.; Jarnberg, U.; Jones, K. C.; Mabury, S. A.; Schroeder, H.; Simcik, M.; Sottani, C.; Van Bavel, B.; Karrman, A.; Lindstrom, G.; Van Leeuwen, S., Analytical challenges hamper perfluoroalkyl research. *Environ. Sci. Technol.* **2004,** *38*, (13), 248A-255A.

40. Murakami, M.; Takada, H., Perfluorinated surfactants (PFSs) in size-fractionated street in Tokyo. *Chemosphere* **2008,** *73*, 1172 - 1177.

41. Tugnait, M.; Lenz, E. M.; Phillips, P.; Hofmann, M.; Spraul, M.; Lindon, J. C.; Nicholson, J. K.; Wilson, I. D., The metabolism of 4-trifluoromethoxyaniline and

[C-13]-4-trifluoromethoxyacetanilide in the rat: detection and identification of metabolites excreted in the urine by NMR and HPLC-NMR. *Journal of Pharmaceutical and Biomedical Analysis* **2002,** *28*, (5), 875-885.

42. Buchanan, G. W.; Munteanu, E.; Dawson, B. A.; Hodgson, D., Concerning the origin of F-19-F-19 NMR COSY and NOESY connections in the spectra of perfluorooctanoic acid, R-F-palmitic acid-F-13 and diethyl perfluorosuberate. *Magnetic Resonance in Chemistry* **2005,** *43*, (7), 528-534.

43. Takasaki, M.; Kimura, K.; Kawaguchi, K.; Abe, A.; Katagiri, G., Structural analysis of a perfluorosulfonate ionomer in solution by F-19 and C-13 NMR. *Macromolecules* **2005,** *38*, (14), 6031-6037.

44. Bruch, M. D., *NMR Spectroscopy Techniques*. CRC Press: Boca Raton, FL, 1996.

45. Hebert, G. N.; Odom, M. A.; Craig, P. S.; Dick, D. L.; Strauss, S. H., Method for the determination of sub-ppm concentrations of perfluoroalkylsulfonate anions in water. *Journal of Environmental Monitoring* **2002,** *4*, (1), 90-95.

46. Langlois, I.; Oehme, M., Structural identification of isomers present in technical perfluorooctane sulfonate by tandem mass spectrometry. *Rapid Communications in Mass Spectrometry* **2006,** *20*, (5), 844-850.

47. Hori, H.; Hayakawa, E.; Yamashita, N.; Taniyasu, S.; Nakata, F.; Kobayashi, Y., High-performance liquid chromatography with conductimetric detection of perfluorocarboxylic acids and perfluorosulfonates. *Chemosphere* **2004,** *57*, (4), 273-282.

48. AHPA, *Standard Methods for the Examination of Water and Wastewater.* 21st ed.; American Public Health Association: Washington, DC, 2005.

49. Mara, D.; Horan, N. J., *Handbook of Water and Wastewater Microbiology*. Academic Press: 2003; p 819.

CHAPTER 3

Application of LC-MS/MS Methods to Determine the Fate of Perfluorinated Chemicals in Municipal Wastewater Treatment Plants in Arizona

3.1 Abstract

Perfluorinated chemicals (PFCs) are emerging pollutants that have been used for half a century in a wide variety of industrial processes and consumer-based products. PFCs have received a lot of attention in the last eight years due to their detection in environmental and biological matrices as well as concerns regarding their persistence and toxicity. Recent studies indicate that municipal wastewater treatment plants are point sources of PFCs. The fluorochemicals may enter the environment through effluent discharge or land application of biosolids. The significance of PFCs discharges from wastweter treatment plants in Arizona was investigated in this research.

A quantitative method consisting of acid wash-solvent extraction of the PFCs followed by solid phase extraction (SPE) clean-up and concentration was successfully developed to assess the presence of PFCs in environmental solid matrices. LC-MS/MS was employed to detect and quantify PFCs in sludge samples. Selected ion monitoring (SIM) analysis was used to improve detection limits for the perfluorinated compounds.

The detection limits of the PFCs evaluated in this study were 50 μg L^{-1} for perfluorooctane sulfonate (PFOS), perfluorodecano sulfonate (PFDS) and perfluorodecanoic acid (PFDA), and 200 μg L^{-1} for perfluorohexane sulfonate (PFHXs) and perfluorooctanoic acid (PFOA). PFOS (77 $\pm$ 5 ng g^{-1} sludge dry weight) was the only perfluorinated compound detected in municipal sewage sludge samples from Tucson, Arizona.

Key words: PFCs, PFOS, PFOA, adsorption, wastewater sludge, LC-MS/MS and SPE.

3.2 Introduction

Perfluorinated chemicals (PFCs) are emerging environmental pollutants that have been used for decades in the manufacturing of products that repel oil, stain, grease and water [1, 2]. Among these compounds, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are subject of increased research due to recent reports of their detection in biological and environmental matrices as well as concerns regarding their persistence and toxicity [3-8]. PFOS and PFOA are the end degradation products of a number of PFCs of commercial applications [9, 10]

Little is known concerning the environmental sources and pathways of exposure of perfluorinated compounds. Wastewater treatment plants (WWTP) have been identified as point sources of PFCs in the environment [11-13]. However, the presence of perfluorinated compounds in wastewaters is still not well understood. These chemicals are likely to be introduced into WWTP through domestic and industrials discharges generated during the production, use and disposal of consumer and industrial products such as fire-fighting foams, surface protectors, etc. [14, 15].

Sewage sludge is the main sink of PFOS, PFOA and related PFCs in municipal wastewater treatment plants as these compounds are expected to partition into biosolids [12, 15]. Furthermore, biosorption of perfluorooctane sulfonate has been reported by various wastewater treatment sludges [16]. Recent studies show PFOS and related PFCs have been detected in sewage sludge from several wastewater treatment plants at ng g^{-1} levels in different countries. In the U.S., a study conducted by 3M in 2,001 revealed that

PFOS concentrations in publicly-owned treatment works (POWTs) sludge in Alabama, Tennessee, Georgia and Florida were in the range of 58 to 3,120 ng g^{-1} [17]. Higgins and colleagues [12] quantified PFCs levels in sewage sludge and in sediments impacted by sewage discharges in California. The survey detected total PFCs levels ranging from 5 to 3,370 ng g^{-1} in domestic sludge including perfluorosulfonates, perfluorooctanesulfonamide and perfluorocarboxylates. Sinclair [18] reported perfluoroosulfonates and perfluorocarboxylates at concentrations of 10 to 241 ng g^{-1} in sewage sludge samples of six WWTP in the state of New York. Loganathan and coworkers [19] reported PFOS and PFOA at concentrations of 2.5 – 990 ng g^{-1} in sludge samples obtained from municipal WWTP in Kentucky and Georgia. In Germany, PFOS and PFOA were detected in sludge samples at levels ranging from 5 to 120 ng g^{-1} [20]. Guo *et al.* [21] quantified PFOS and PFOA in sewage sludge samples in China and the concentrations range from 278 to 5,383 ng g^{-1}. A recent study conducted by Bossi *et al.* [22] reported perfluorinated compounds in Danish WWTPs at concentrations similar to those measured in other countries, 0.4 – 74.1 ng g^{-1}.

Elevated concentrations of PFOS have been found in industrial wastewaters of PFCs manufacturing plants and other activities that utilize PFCs [23-27]. PFOS concentrations as high as 1650 mg L^{-1} were detected in semiconductor effluents [28]. PFCs are extensively used in the growing semiconductor industry sector in the State, in (forest) fire fighting operations, and in a wide variety of other industrial, commercial and consumer applications. Currently, there is no data on the occurrence of PFCs in environmental samples nor in municipal wastewater treatment plants (WWTP) in

Arizona. Moreover, Arizona's population centers expand and the demand for water increases, reuse of treated wastewater is expected to become more prevalent, which could increase the potential for environmental contamination with PFCs. Information on the occurrence of PFCs in sewage sludge is also of importance for Arizona because biosolids are used as soil amendments throughout the State.

Safe water resources are of strategic importance for Arizona to meet the rapidly increasing demand for potable water. Therefore, understanding the occurrence and fate of these emerging pollutants in Arizona's wastewater treatment plants could be critical to protect water supplies. Utilities and government agencies will be able to utilize information gained from this study to determine the need for implementing measures to prevent the spread of PFCs in the environment.

The objective of this research is to determine the significance of PFCs discharges from selected municipal wastewater treatment plants by applying analytical methods for the detection and quantification of PFCs in environmental samples based on LC-MS/MS and assessing PFCS levels in sewage wastewater in POTWs in Tucson, Arizona. The chemical structures of the perfluorinated compounds evaluated in this study are depicted in Figure 3.1.

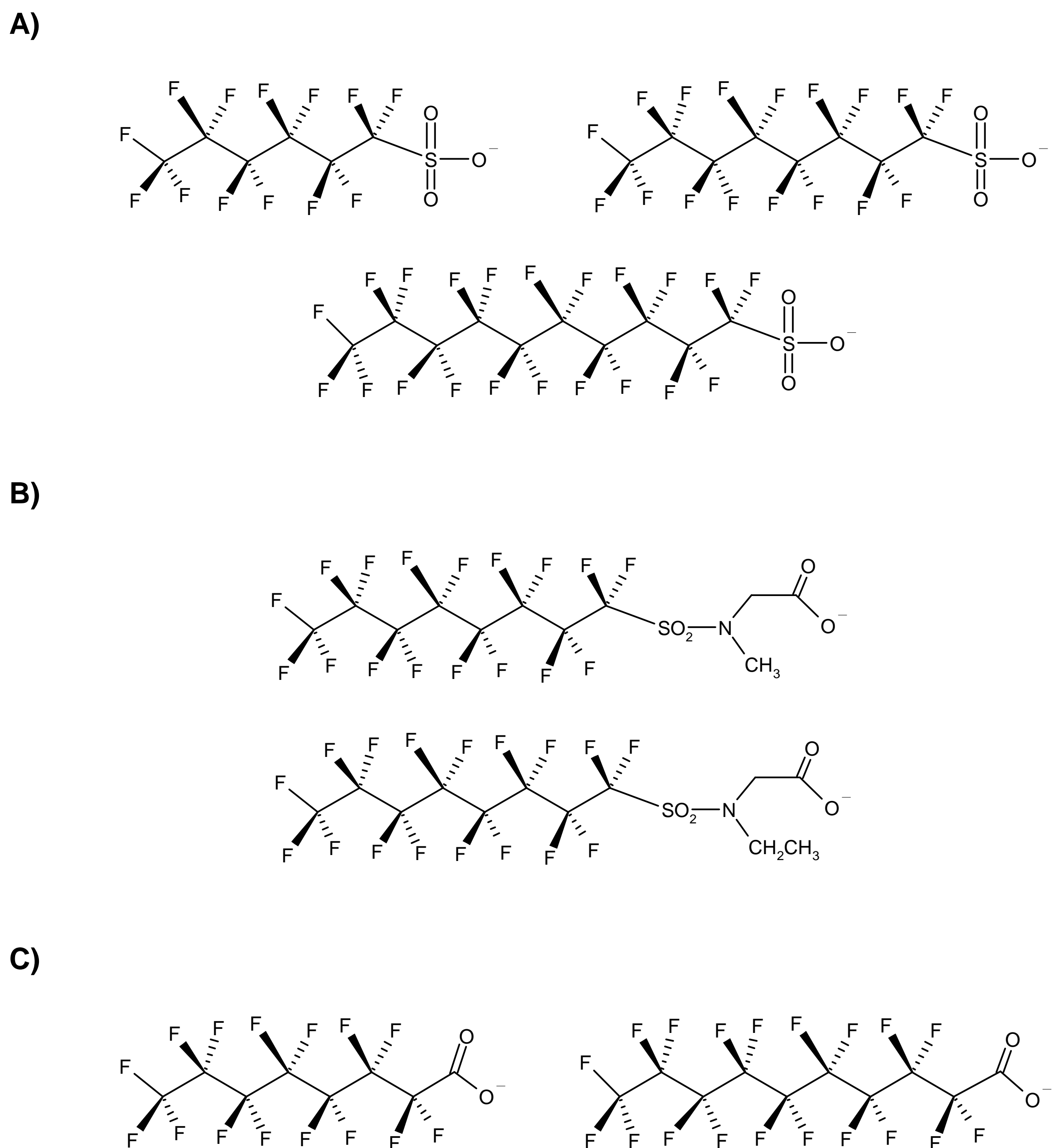


Figure 3.1 Chemical structures of perfluorinated compounds evaluated in this study. A) Perfluorosulfonates: perfluorohexane sulfonate (PFHXs), perfluorooctane sulfonate (PFOS) and perfluorodecano sulfonate (PFDS). B) Perfluorosulfonamides: 2-(N-methylperfluorooctanesulfonamido) acetate (*N*-MeFOSAA) and 2-(N-ethylperfluorooctanesulfonamido) acetate (*N*-EtFOSAA). C) Perfluorocarboxylates: perfluorooctanoic acid (PFOA) and perfluorodecanoic acid (PFDA).

3.3 Materials and Methods

3.3.1 Chemicals

Perfluorooctane sulfonic acid potassium salt, PFOS (98% purity), was purchased from SynQuest Laboratories (Alachua, FL). Sodium perfluorohexane sulfonate, PFHXs (98%), sodium perfluorodecano sulfonate, PFDS (98%) and perfluorodecanoic acid, PFDA (98%) were obtained from Wellington Laboratories (Ontario, Canada). Perfluorooctanoic acid, PFOA (96%) and sodium fluoride (99%) were obtained from Sigma-Aldrich (St. Louis, MO). Solid phase extraction (SPE) cartridges, 3mL, 500 mg ODS-C_{18} were supplied by Agilent Technologies (New Castle, DE). Methanol (HPLC grade) was purchased from Burdikc & Jackson (Muskegon, MI). Acetic acid, glacial (ACS grade) was obtained from EMD chemicals (Gibbstown, NJ). All chemicals were used as received.

3.3.2 Sludge extractions

Sludge extractions were conducted by the method described by Higgins and coworkers [12]. Anaerobically digested sewage sludge (ADS) from two different municipal WWTPs in Tucson, Arizona, Ina Road WWTP and Roger Road WWTP, were evaluated in this

study. Sewage sludge samples were dried in an oven at 70ºC overnight and air-dried for 1 day. Samples were ground and homogenized using a mortar and pestle. 100 mg of homogenized sludge were transferred to a Nalgene bottle and 7.5 mL of 1% acetic acid solution was added. Each sample was vortexed for 10 min and sonicated for 30 min at 60ºC. Following sonication, samples were centrifuged (10,000 rpm, 25 min) and the wash was decanted in a second Nalgene flask. A volume of 1.7 mL of a methanol/1% acetic acid (90/10 v/v) solution was added to the original vial to extract the PFCs. Before centrifugation, samples were vortexed for 10 min and then sonicated for 30 min at 60ºC. The extract was decanted in a third Nalgene flask. This procedure was conducted two more times and all washes and extracts were combined. A second PFCs extraction of Ina Road WWTP sewage sludge was conducted following the procedure described above with minor modifications. Briefly, 75 mL of 1% acetic acid solution was added to 6,000 mg of homogenized air-dried sludge followed by 20 mL methanol / 1% acetic acid (90:10, v/v) extraction mixture. All wash and extract vials were centrifuged (10,000 rpm, 25 min) to avoid clogging of the SPE column. Each sludge sample was extracted and analyzed in triplicate.

3.3.3 Sample clean-up

Solid phase extraction (SPE) was conducted to pre-concentrate and clean-up wash and extract samples. SPE cartridges (3 mL, 500 mg ODS-C_{18}, Agilent Technologies, DE)

mounted on a vacuum manifold were conditioned with 6 mL methanol, followed by 6 mL of 1% acetic acid solution. The desired volume of wash or extract was loaded at 1 mL min^{-1}. SPE cartridges were rinsed with 4 mL of deionized water and then centrifuged (4000 rpm, 25 min) prior elution. Analytes were eluted with 4 mL methanol and collected in clean Nalgene flasks. The eluent was concentrated under nitrogen by a 8-fold. In the second extraction when significant amounts of Ina Road WWTP sewage sludge were employed, the sample was loaded in 3 different columns and each column was washed with 20 mL of deionized water. All eluents were combined and finally concentrated under nitrogen by a 10-30-fold. The extracts were stored at 4°C for LC-MS/MS analysis. Prior analysis, the samples were 10-fold diluted to reduce matrix interferences and bring concentration to the right LC-MS/MS measurement range.

3.3.4 LC-MS/MS

Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) analysis was adapted from a method described by Higgins *et al.* [12]. LC-MS/MS measurements were performed on a Magic 2002 (Michrom Biosciences, Inc.) ThermoFisher (Finnigan) LCQ Classic HPLC-MS system. Chromatographic separation was conducted on a MagicMS C_{18} micobore column (5 µm, 200Å, 1 x 150 mm). The mobile phase consisted of (A) ammonium acetate buffer (10mM) and (B) methanol. A gradient program with 5% B to 90% B in 35 min was used to elute the components of the samples with a flow rate of 50

μL min^{-1}, temperature 40ºC, and injection volume of 25 μL. Negative ionization was employed to detect fluorinated sulfonates and carboxylates. Selected ion monitoring (SIM) was used to improve detection limit for the perfluorinated compounds. Standard solutions were run first to determine peak intensity ratios for the selected ions (m/z 399, 413, 499, 513, and 599). A m/z window of +/- 3 around the selected m/z value was used in the SIM experiments. Tandem MS/MS was also applied to get structural information on selected ions (e.g., on m/z 499, $CF_3(CF_2)_7SO_3^-$). Helium was used as a collision gas and a 35% relative collision energy was applied in the MS/MS experiments. MS/MS spectra were recorded within a mass range of m/z ranging from 75 to 1000 using a scan time of 0.2 s. Blanks samples were used to monitor instrument background and were continuously run after three sludge samples.

3.3.5 Quantification

Selected ion monitoring was employed to enhance sensitivity during quantification. Standards of the perfluorinated compounds were run in parallel to validate the analysis. Since chemical characteristics influence the ionization process, PFDS was used as an internal standard for PFOS, both compounds belong to the perfluoroalkyl sulfonate family and chemically are very similar.

Samples were spiked with a known concentration of the internal standard, PFDS, 0.5 mg L^{-1}. Peaks were averaged and the areas were calculated by multiplying the height

by the peak width at the half-height. Quantification was possible by relating the area of the PFOS peak to the area of the internal standard. The ratio of areas of PFOS and PFDS in an equimolar standard solution was employed to correct for the differences in response factors of the compounds. The precision of the method was determined by analyzing sludge samples in triplicate and calculating the standard deviation.

3.4 Results and Discussion

3.4.1 Application of LC-MS/MS for the detection and quantification of PFOS and related compounds

A method based on reverse-phased high-performance liquid chromatography with negative electrospray tandem mass spectrometry (LC-MS/MS) described by Higgins *et al.* [12] was employed to detect PFCs in solid environmental matrices. Figure 3.2 shows a chromatogram obtained for a mixed standard solution containing 5 mg L^{-1} of PFHXs, PFOA, PFOS, PFDA and PFDS in methanol and chromatograms of standard solutions of each compound (5 mg L^{-1}) analyzed individually. The MS/MS spectra of the same mixed standard solution are shown in Figure 3.3.

As expected for a reverse-phased C_{18} column, perfluorinated chemicals were separated based on the perfluoroalkyl chain length. Distinct peaks are evident for the mixed standard solution of the perfluorinated chemicals (Figure 3.2). PFHXs (C_6, 399.10 g/mol) eluted first, followed by PFOA (C_8, 413 g/mol), PFOS (C_8 499.12 g/mol), PFDA (C_{10}, 514.09 g/mol), and last PFDS (C_{10} 599.13 g/mol). The retention times for the different compounds are 15.8, 19.2, 22.5 (21.4 and 23.4), 25.1 and 27 min, respectively.

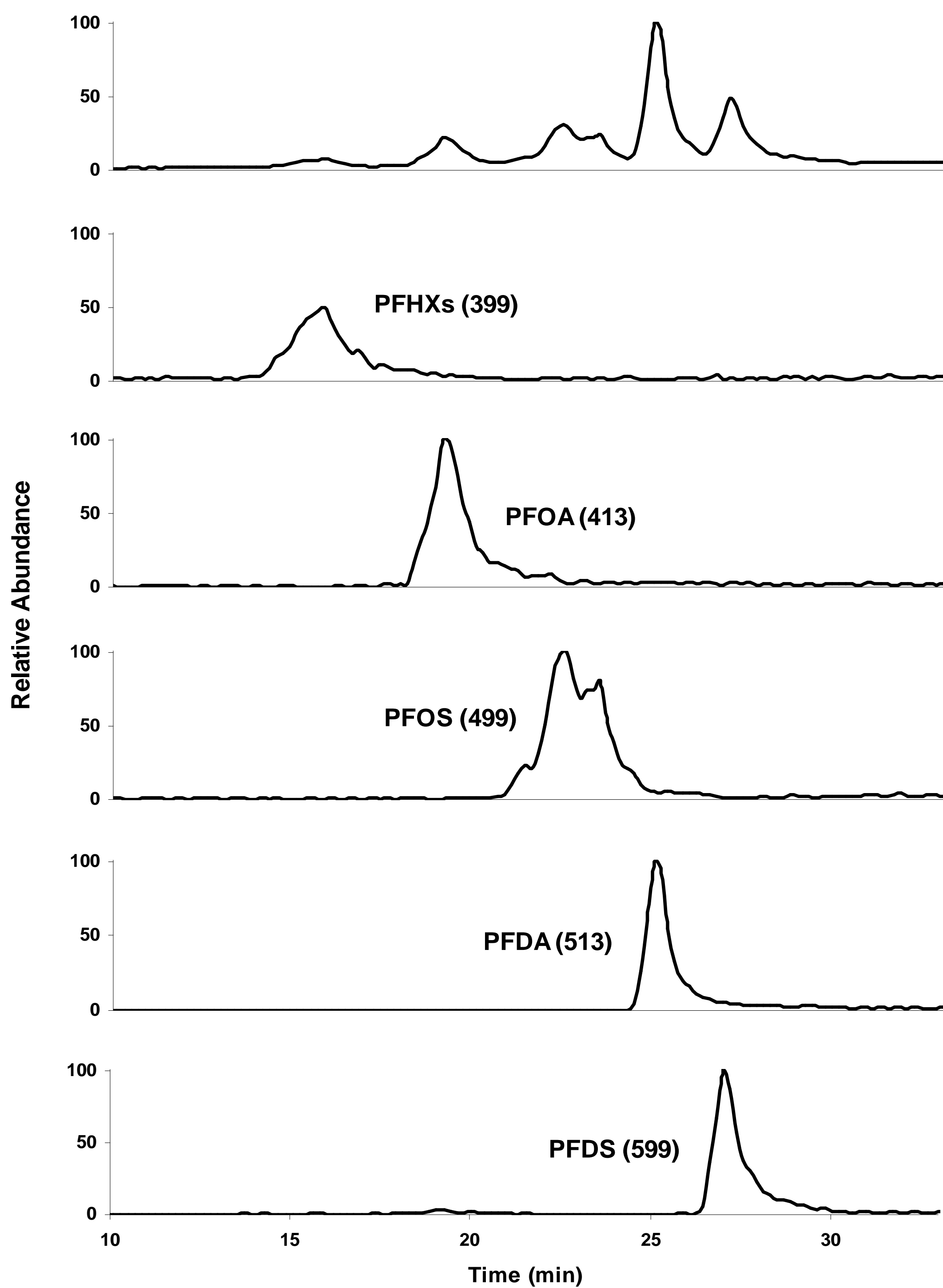


Figure 3.2 LC-MS chromatograms of 5 mg L^{-1} of PFHXs, PFOA, PFOS, PFDA and PFDS in a mixed standard solution (top) and individual standard solutions.

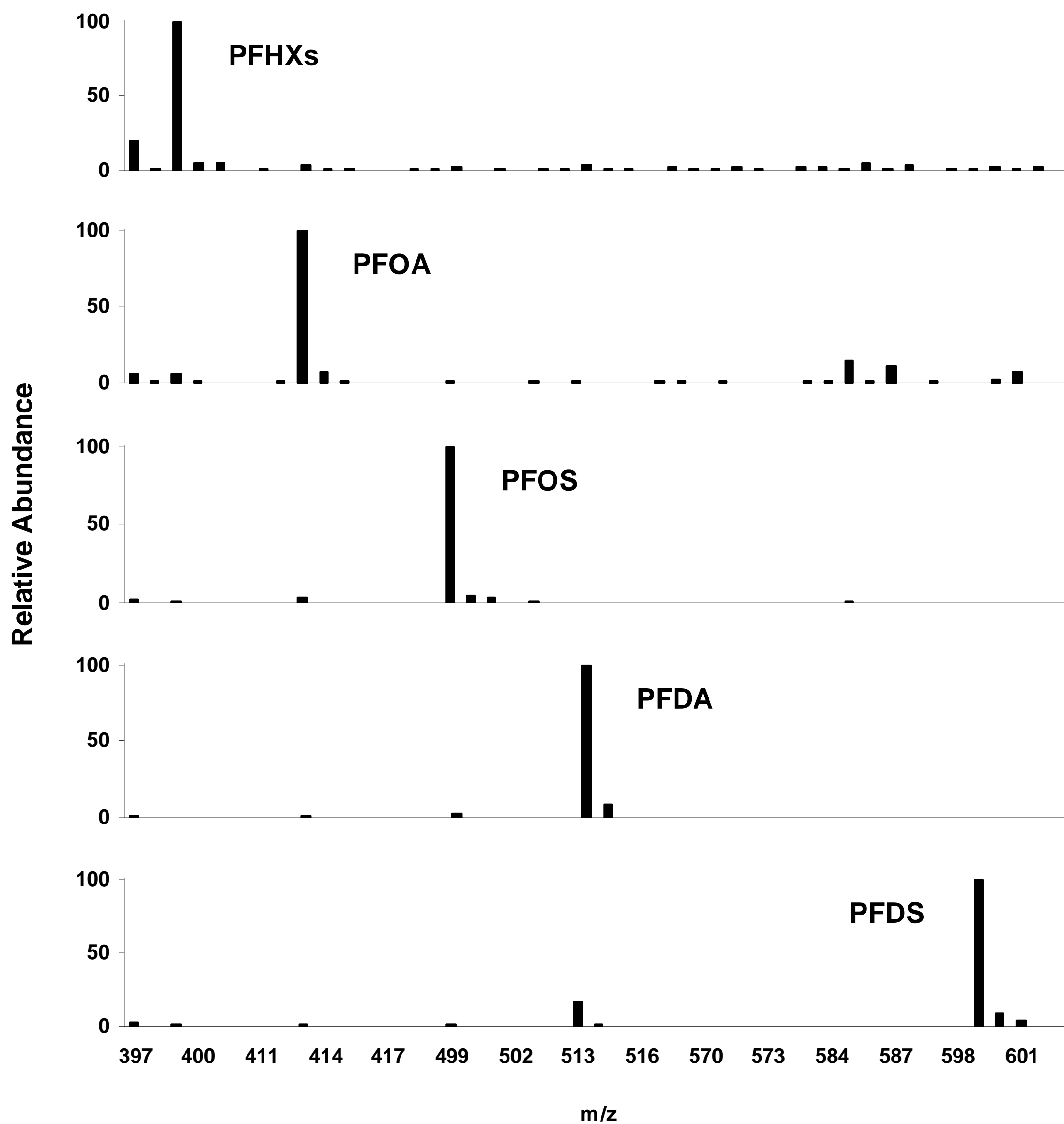


Figure 3.3 Negative ESI/MS spectra of a mixed standard solution containing 5 mg L^{-1} of PFHXs, PFOA, PFOS, PFDA and PFDS obtained under SIM analysis.

Technical PFOS is a mixture of linear and branched structural isomers. The PFOS salt employed in this study contains 75.4% linear PFOS [29]. In the LC-MS chromatograms of PFOS (Figure 3.2), the peak detected at 22.5 min corresponds to the linear PFOS isomer and the peaks detected before and after at 21.4 and 23.4 min, respectively, correspond to structural isomers.

The response factor of the different PFCs was calculated by dividing the peak area by the concentration of the analyte. The response factor of PFHXs, PFOA, PFOS, PFDA and PFDS were calculated to be 15, 21, 32, 14 and 14, respectively. The response factors of perfluorinated sulfonates and carboxylates with chain lengths of 6 and 10 carbons were about the same. However in the case of PFOA and PFOS (8-carbon chain), these values were higher and significantly different and not related to the perfluorocarbon chain. Similarly, the response factors of these two compounds are different from those of their homologues perfluorosulfonates and perfluorocarboxylates.

The detection limits of the PFCs chemicals evaluated in this study differed among compounds. The estimated detection limit based on a signal-to-noise ratio of 3 was calculated to be 50 μg L^{-1} for PFOS, PFDA and PFDS as compared to 200 μg L^{-1} in the case of PFHXs and PFOA. Literature studies on the quantification of PFCs in environmental samples by LC-MS/MS reported detection limits in the order of ng to μg L^{-1} range [30, 31]. In most cases, TOF-MS and Q-TOF-MS are the detectors employed in the analysis of environmental samples. These detectors have shown a significant increased resolution capability and have become commercially available in the past five years [32].

The higher detection limits obtained in this study can be primarily attributed to instrument limitations. The LC-MS/MS spectrometer employed in our investigation was acquired several years ago and does not provide very good sensitivities as demonstrated above. In order to overcome theses limitations, preconcentration steps by SPE procedures utilizing large samples volumes are required to obtain reliable measurements.

3.4.2 Assessment of PFC levels in wastewater treatment sludge

The presence of perfluorinated chemicals in samples of anaerobically digested sewage sludge obtained from two different WWTPs from Tucson, Arizona was evaluated in this study. LC-MS/MS quantification of PFCs in sewage sludge was performed according to the protocol described in the *Materials and Methods* section. Briefly, an acid wash-solvent extraction cycle followed by a SPE sample clean-up and concentration procedure were conducted to assess the levels of PFCs in municipal sludge. Two extractions were carried out, the first one employing 100 mg and a second one using 6,000 mg of dried sewage sludge. In both cases, the wash and the extract were analyzed. In the first extraction, no PFCs were detected, either in the wash or in the extract, suggesting that the compounds were not present or their concentrations were lower than the detection limits. In the second extraction using 6000 mg of Ina Road WWTP anaerobically digested sewage sludge, PFOS was detected only in the extract and no PFCs were detected in the

wash. The analytical results obtained in the second extraction are presented below in more detail.

Recent studies have reported the presence of PFOS- and PFOA-related compounds as well as perfluorooctanesulfonamides in sewage sludge samples [12, 15, 17]. For instance, Higgins and collaborators [12] reported that the concentration of perfluorosulfonamides are often exceeding PFOS concentrations in municipal sewage sludge samples. *N*-MeFOSAA and *N*-EtFOSAA were detected in WWTP discharges in the San Francisco Bay Area at concentrations ranging from less than 6 to 544 ng g^{-1}. Based on these studies, selected ion monitoring (SIM) was employed to detect the presence of three classes of PFCs in the extract obtained following extraction of municipal sewage sludge. Perfluorosulfonates: PFHXs, PFOS, PFDS, perfluorooctanesulfonamides: *N*-MeFOSAA, and *N*-EtFOSSA and the perfluroorocarboxylates, PFOA and PFDA (Figure 3.1).

Figure 3.4 shows a LC-MS chromatogram and MS/MS spectrum obtained under SIM analysis of an extract sample spiked with 500 μg PFDS L^{-1} as the internal standard. The retention time of PFDS was 27 min. PFOS was the only perfluorinated compound detected under the experimental conditions evaluated in this study. PFOS concentration in Ina Road ADS was 77 $\pm$ 5 ng g^{-1} sludge dry weight (dwt). The levels of PFOS determined here are within the range of those determined in related studies around the country [12, 17-19] . PFOS appears to be the dominant PFC in sewage sludge samples. Data collected by 3M in 2001 indicated that PFOS concentrations in municipal sludge from Alabama, Tennessee, Georgia and Florida exceed PFOA and FOSA

(perfluorooctanesulfonamide) concentrations by one order of magnitude [17]. In sewage sludge samples from Kentucky, PFOS concentrations were 2 to 5-fold higher than those of PFOA [19]. These results are also consistent with previous work on the presence of perfluorinated chemicals in the environment. In wildlife samples throughout the world, PFOS was the only compound detected [3, 33, 34]. PFOS was the most abundant PFC found in fish and birds from New York State [35]. PFOS is also the major perfluorinated contaminant present in human blood. PFOS concentrations in human sera samples from different countries were at least 2-fold greater than those of PFOA and about one order of magnitude higher than PFHXs concentrations [4, 5, 36-38].

The fact that PFOS was the only compound detected in the sewage sludge samples analyzed in this study could be attributed to several aspects, inefficient extraction recoveries from sludge, inadequate retention or elution times during SPE clean-up, instrument limitations and most likely matrix-derived analyte signal suppression effects [39]. In electrospray ionization (ESI) tandem mass spectrometry, the intensity of an analyte ion signal is a function of both the concentration of the analyte and the total ions present in solution. In heterogeneous matrices such as sewage sludge, the variability and amount of organic matter present can result into suppression effects of the analyte signal [30, 39]. In fact, in the extraction of sludge (6,000 mg), linear alkylbenzene sulfonates (LAS) coeluted with high molecular weight PFCs (*N*-MeFOSAA, *N*-EtFOSAA and PFDS) in significant concentrations (Figure 3.4). Therefore, it could be possible that these perfluorinated chemicals might be present but that they were not detected due the matrix interferences.

Employing ionization techniques in which the ionization process takes place in the gas phase could alleviate the matrix effects problems encountered by ESI. Takino and coworkers [40] developed a liquid chromatography method based on atmospheric pressure photoionization mass spectrometry (AAPI) for the detection of perfluorinated surfactants in environmental samples. The advantage of using the later ionization technique as compared to ESI is the absence of matrix effects. However, the detection limits of AAPI are higher than those of obtained by LC with ESI (tandem) MS. Further research needs to be conducted to establish if other PFCs than PFOS are present in sewage sludge in Arizona. AAPI MS/MS could offer a good alternative for the analysis of environmental samples by liquid chromatography.

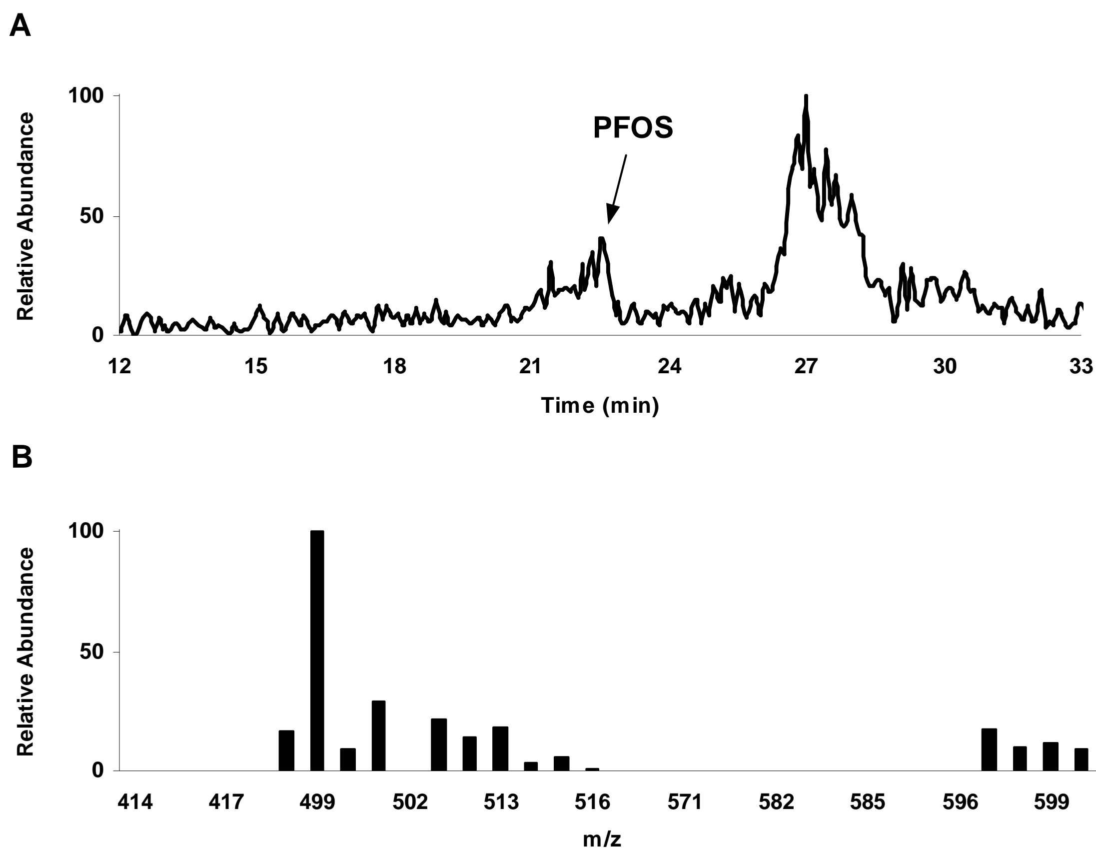


Figure 3.4 SIM analysis of a sewage sludge sample of Ina Road WWTP from Tucson, Arizona. A) Typical LC-MS chromatogram and, B) MS/MS spectrum of extract sludge sample spiked with 0.5 mg PFDS L^{-1}.

3.5 Conclusions

The presence of perfluorinated chemicals in sludge samples was determined by applying a quantitative method consisting of liquid solvent extraction followed by solid phase extraction (SPE) clean-up/pre-concentration and injection into LC-MS/MS. The technique was shown to be a valuable for the analysis of PFCs in aqueous and solid environmental samples. However, its moderate sensitivity, ionization suppression effects and matrix interferences such as coelution of other chemicals present in the samples are drawbacks of the method and can compromise the quantitative analysis.

Among the perfluorosulfonate, perfluorosulfonamide and perfluorocarboxylate compounds evaluated in this study, PFOS was the only PFC detected in sewage wastewater in publicly-owned treatment works (POTWs) in Tucson, Arizona. This finding is significant since they provide insights into understanding the sources and pathways of PFCs in the environment. To our knowledge this is the first report of the occurrence of PFCs in sewage sludge in Arizona.

3.6 Acknowledgements

This study was conducted with financial support from National Institutes for Water Resources (NIWR) – U.S. Geological Survey (USGS) (State Water Resources Research Institute Program, grant # 2006AZ132B). We are grateful to Dr. Arpad Somogyi,

Supervisor of Mass Spectroscopy Facility of the Chemistry Department, University of Arizona, for his support and invaluable advice with LC-MS/MS.

3.7 References

1. Schultz, M. M.; Barofsky, D. F.; Field, J. A., Fluorinated alkyl surfactants. *Environ. Eng. Sci.* **2003,** *20*, (5), 487-501.
2. 3M *Environmental and health assesment of Perfluorooctane sulfonate and its salts.*; U.S. Evironmental Protection Agency Public Docket AR226-1486.: Washington, DC, 2003.
3. Giesy, J. P.; Kannan, K., Global distribution of perfluorooctane sulfonate in wildlife. *Environ. Sci. Technol.* **2001,** *35*, (7), 1339-1342.
4. Hansen, K. J.; Clemen, L. A.; Ellefson, M. E.; Johnson, H. O., Compound-specific, quantitative characterization of organic: Fluorochemicals in biological matrices. *Environ. Sci. Technol.* **2001,** *35*, (4), 766-770.
5. Kannan, K.; Corsolini, S.; Falandysz, J.; Fillmann, G.; Kumar, K. S.; Loganathan, B. G.; Mohd, M. A.; Olivero, J.; Van Wouwe, N.; Yang, J. H.; Aldous, K. M., Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. *Environ. Sci. Technol.* **2004,** *38*, (17), 4489-4495.

6. Houde, M.; Martin, J. W.; Letcher, R. J.; Solomon, K. R.; Muir, D. C. G., Biological monitoring of polyfluoroalkyl substances: A review. *Environ. Sci. Technol.* **2006,** *40*, (11), 3463-3473.

7. Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J., Perfluoroalkyl acids: A review of monitoring and toxicological findings. *Toxicol. Sci.* **2007,** *99*, (2), 366-394.

8. Conder, J. M.; Hoke, R. A.; De Wolf, W.; Russell, M. H.; Buck, R. C., Are PFCAs bioaccumulative? A critical review and comparison with regulatory lipophilic compounds. *Environ. Sci. Technol.* **2008,** *42*, (4), 995-1003.

9. Ellis, D. A.; Mabury, S. A.; Martin, J. W.; Muir, D. C. G., Thermolysis of fluoropolymers as a potential source of halogenated organic acids in the environment. *Nature* **2001,** *412*, (6844), 321-324.

10. Dinglasan, M. J. A.; Ye, Y.; Edwards, E. A.; Mabury, S. A., Fluorotelomer alcohol biodegradation yields poly- and perfluorinated acids. *Environ. Sci. Technol.* **2004,** *38*, (10), 2857-2864.

11. Boulanger, B.; Peck, A. M.; Schnoor, J. L.; Hornbuckle, K. C., Mass budget of perfluorooctane surfactant in Lake Ontario. *Environ. Sci. Technol.* **2005,** *39*, (1), 74-79.

12. Higgins, C. P.; Field, J. A.; Criddle, C. S.; Luthy, R. G., Quantitative determination of perfluorochemicals in sediments and domestic sludge. *Environ. Sci. Technol.* **2005,** *39*, (11), 3946-3956.

13. Schultz, M. M.; Barofsky, D. F.; Field, J. A., Quantitative determination of fluorinated alkyl substances by large-volume-injection liquid chromatography tandem mass spectrometry - Characterization of municipal wastewaters. *Environ. Sci. Technol.* **2006,** *40*, (1), 289-295.

14. Prevedouros, K.; Cousins, I. T.; Buck, R. C.; Korzeniowski, S. H., Sources, fate and transport of perfluorocarboxylates. *Environ. Sci. Technol.* **2006,** *40*, (1), 32-44.

15. Boulanger, B.; Vargo, J. D.; Schnoor, J. L.; Hornbuckle, K. C., Evaluation of perfluorooctane surfactants in a wastewater treatment system and in a commercial surface protection product. *Environ. Sci. Technol.* **2005,** *39*, (15), 5524-5530.

16. Ochoa-Herrera, V.; Sierra-Alvarez, R., Removal of prefluorinated surfactants by sorption onto granular activated carbon, zeolite and sludge. *Chemosphere* **2008,** *72*, 1588 - 1593.

17. 3M *Executive Summary: Environmental Monitoring - Multi-City Study. Water, Sludge, Sediments, POTW Effluent and Landfill Leachate Samples.* ; EPA- 226-1030a, U.S. Environmental Protection Agency, 2001.

18. Sinclair, E.; Kannan, K., Mass loading and fate of perfluoroalkyl surfactants in wastewater treatment plants. *Environ. Sci. Technol.* **2006,** *40*, (5), 1408-1414.

19. Loganathan, B. G.; Sajwan, K. S.; Sinclair, E.; Kumar, K. S.; Kannan, K., Perfluoroalkyl sulfonates and perfluorocarboxylates in two wastewater treatment facilities in Kentucky and Georgia. *Water Res.* **2007,** *41*, (20), 4611-4620.

20. Becker, A. M.; Gerstmann, S.; Frank, H., Perfluorooctane surfactants in waste waters, the major source of river pollution. *Chemosphere* **2008,** *72*, (1), 115-121.

21. Guo, R.; Zhou, Q. F.; Cai, Y. Q.; Jiang, G. B., Determination of perfluorooctanesulfonate and perfluorooctanoic acid in sewage sludge samples using liquid chromatography/quadrupole time-of-flight mass spectrometry. *Talanta* **2008,** *75*, (5), 1394-1399.

22. Bossi, R.; Strand, J.; Sortkjaer, O.; Larsen, M. M., Perfluoroalkyl compounds in Danish wastewater treatment plants and aquatic environments. *Environ. Int.* **2008,** *34*, (4), 443-450.

23. Moody, C. A.; Hebert, G. N.; Strauss, S. H.; Field, J. A., Occurrence and persistence of perfluorooctanesulfonate and other perfluorinated surfactants in groundwater at a fire-training area at Wurtsmith Air Force Base, Michigan, USA. *J. Environ. Monit.* **2003,** *5*, (2), 341-345.

24. Skutlarek, D.; Exner, M.; Farber, H., Perfluorinated surfactants in surface and drinking water. *Environ. Sci. Pollut. Res.* **2006,** *13*, (5), 299-307.

25. Richardson, S. D., Water analysis: Emerging contaminants and current issues. *Analytical Chemistry* **2007,** *79*, (12), 4295-4323.

26. Poulsen, P. B.; Jensen, A. A.; Wallstrom, E. *More environmentally friendly alternatives to PFOS-compounds and PFOA*; Environmental Project No. 10132005; Danish EPA: 2005; pp 1 -162.

27. Ju, X. D.; Jin, Y. H.; Sasaki, K.; Saito, N., Perfluorinated surfactants in surface, subsurface water and microlayer from Dalian Coastal waters in China. *Environ. Sci. Technol.* **2008,** *42*, (10), 3538-3542.

28. Tang, C. Y. Y.; Fu, Q. S.; Robertson, A. P.; Criddle, C. S.; Leckie, J. O., Use of reverse osmosis membranes to remove perfluorooctane sulfonate (PFOS) from semiconductor wastewater. *Environ. Sci. Technol.* **2006,** *40*, (23), 7343-7349.

29. Ochoa-Herrera, V.; Sierra-Alvarez, R.; Somogyi, A.; Jacobsen, N. E.; Wysocki, V. H.; Field, J. A., Reductive defluorination of perfluorooctane sulfonate. *Environ. Sci. Technol.* **2008,** *42*, (9), 3260-3264.

30. Villagrasa, M.; de Alda, M. L.; Barcelo, D., Environmental analysis of fluorinated alkyl substances by liquid chromatography-(tandem) mass spectrometry: a review. *Anal. Bioanal. Chem.* **2006,** *386*, (4), 953-972.

31. de Voogt, P.; Saez, M., Analytical chemistry of perfluoroalkylated substances. *Trac-Trends Anal. Chem.* **2006,** *25*, (4), 326-342.

32. Richardson, S. D., Environmental mass spectrometry: Emerging contaminants and current issues. *Analytical Chemistry* **2008,** *80*, (12), 4373-4402.

33. 3M, Technical data bulletin. Environmental health, safety and regulatory (ehsr) profile of perfluorobutane sulfonate (PFBS). *3M, St Paul, MN.* **2002**.

34. Martin, J. W.; Smithwick, M. M.; Braune, B. M.; Hoekstra, P. F.; Muir, D. C. G.; Mabury, S. A., Identification of long-chain perfluorinated acids in biota from the Canadian Arctic. *Environ. Sci. Technol.* **2004,** *38*, (2), 373-380.

35. Sinclair, E.; Mayack, D. T.; Roblee, K.; Yamashita, N.; Kannan, K., Occurrence of perfluoroalkyl surfactants in water, fish, and birds from New York State. *Archives of Environmental Contamination and Toxicology* **2006,** *50*, (3), 398-410.

36. Harada, K.; Koizumi, A.; Saito, N.; Inoue, K.; Yoshinaga, T.; Date, C.; Fujii, S.; Hachiya, N.; Hirosawa, I.; Koda, S.; Kusaka, Y.; Murata, K.; Omae, K.; Shimbo, S.; Takenaka, K.; Takeshita, T.; Todoriki, H.; Wada, Y.; Watanabe, T.; Ikeda, M., Historical and geographical aspects of the increasing perfluorooctanoate and perfluorooctane sulfonate contamination in human serum in Japan. *Chemosphere* **2007,** *66*, (2), 293-301.

37. Butenhoff, J. L.; Olsen, G. W.; Pfahles-Hutchens, A., The applicability of biomonitoring data for perfluorooctanesulfonate to the environmental public health continuum. *Environmental Health Perspectives* **2006,** *114*, (11), 1776-1782.

38. Yeung, L. W. Y.; So, M. K.; Jiang, G. B.; Taniyasu, S.; Yamashita, N.; Song, M. Y.; Wu, Y. N.; Li, J. G.; Giesy, J. P.; Guruge, K. S.; Lam, P. K. S., Perfluorooctanesulfonate and related fluorochemicals in human blood samples from China. *Environ. Sci. Technol.* **2006,** *40*, (3), 715-720.

39. Ferguson, P. L.; Iden, C. R.; Brownawell, B. J., Analysis of alkylphenol ethoxylate metabolites in the aquatic environment using liquid chromatography-electrospray mass spectrometry. *Analytical Chemistry* **2000,** *72*, (18), 4322-4330.

40. Takino, M.; Daishima, S.; Nakahara, T., Determination of perfluorooctane sulfonate in river water by liquid chromatography/atmospheric pressure

photoionization mass spectrometry by automated on-line extraction using turbulent flow chromatography. *Rapid Communications in Mass Spectrometry* **2003,** *17*, (5), 383-390.

CHAPTER 4

Microbial Toxicity and Biodegradability of Perfluorooctane Sulfonate (PFOS) and Related Compounds

4.1 Abstract

Perfluorooctane sulfonate (PFOS) and related perfluoroalkyl surfactants (PFAS) are emerging contaminants which find important applications in consumer and industrial applications such as photolithography operations of semiconductor manufacturing. The objective of the research reported here is to investigate the microbial degradation of PFOS and related short and long-chain (per) fluorinated compounds by microorganisms present in industrial and municipal sludge, and in sediments. The microbial toxicity of the (per) fluorinated compounds towards methanogenic microorganism was determined to avoid inhibition of these compounds during the reductive dehalogenation bioassays. The microbial toxicity and anaerobic biodegradability of PFOS and perfluorobutane sulfonate (PFBS) solutions treated electrochemically for different periods of time was also evaluated in this study. None of the compounds tested were toxic to the methanogenic activity of anaerobic wastewater treatment sludge. In contrast, hydrogenotrophic methanogens were inhibited by PFOS and PFBS solutions subjected to electrochemical

treatment for several periods of time. Perfluorinated alkyl surfactants were shown to be highly resistant to microbial degradation and these compounds were not reductively dehalogenated even after very long periods of incubation (> 2 years). PFOS-related compounds with a shorter alkyl chain (e.g., PFBS) and partially defluorinated PFOS molecules (e.g., TH-PFOS, 1H,1H,2H,2H-perfluorooctane sulfonic acid) were also resistant to biodegradation. No evidence of the microbial degradation of electrolyzed PFOS and PFBS samples was obtained after more than 1 y of incubation.

Key words: PFOS, PFBS, TH-PFOS, short-chain PFAS, microbial toxicity, reductive dehalogenation

4.2 Introduction

Perfluorooctane sulfonate (PFOS) and related chemicals are fully fluorinated surfactants where all the hydrogen atoms have been replaced by fluorine atoms. These compounds are a unique class of chemicals different from hydrocarbon surfactants because they repel water, oil, and grease [1]. Due to their excellent chemical and physical properties, these anthropogenic surfactants are commonly used in the manufacturing of surface coatings, paper packing products, pesticides and insecticides, fire fighting foams, photolithography, and manufacturing of semiconductors, among others [2].

PFOS has raised public concern due to its high bioaccumulative character, environmental persistence, and toxicity [3-7]. PFOS and related perfluorinated surfactants have been detected in human samples, wildlife biota, and environmental matrices worldwide [2, 8-11]. Concentrations of PFOS ranging from 3 to 3,160 ng L^{-1} were detected in municipal-, industrial-, surface-, and ground waters in USA [12-18], Canada [19], Germany [20], Italy [21], China [22], and Japan [23].

Treatment of PFOS in wastewater is challenging due to outstanding stability of the compound, and its dual hydrophobic-oleophobic nature [24]. Currently, there is no evidence of the chemical or biological degradation of PFOS and derivatives under ambient conditions [24]. No photolytic degradation of PFOS has been observed at 25ºC. Similarly, no hydrolysis of PFOS has been observed at pH values of 1.5, 5, 7, 9 and 11 at 50ºC [25]. The recalcitrant nature of PFOS and related compounds has been attributed to the strength of the C-F bond [1].

PFOS does not appear to undergo degradation under aerobic or anaerobic conditions [24-26]. Key and coworkers [27] reported that PFOS was not degraded by *Pseudomonas* sp. strain D2 in aerobic assays. Likewise, no evidence of PFOS degradation was obtained after 54 days of incubation in a study conducted by 3M to evaluate the biodegradability of the perfluorinated compound using anaerobic wastewater treatment sludge and pure cultures [24].

Trichloroethylene (TCE) and perchloroethylene (PCE) are members of the highly chlorinated compounds family which are widely known to be biodegraded by means of reductive dehalogenation [28]. Certain microorganisms can rapidly utilize the organohalogens as electron acceptors in an energy yielding reaction known as halorespiration. The anaerobic metabolism of halogenated compounds proceeds through sequential reductive dehalogenation in which hydrogen replaces the halogen-ion as illustrated in Figure 4.1. Literature data on the reductive dehalogenation of fluorinated compounds show that chlorofluorocarbons (CFC, Freon's) and hydrochlorofluorocarbons (HCFC) are susceptible to anaerobic biotransformation [29]. There is a good possibility that microorganisms capable of degrading chlorinated compounds by reductive dehalogenation might be able to degrade fluorinated surfactants as well. However, no studies have been published to date considering the biological reductive dehalogenation of PFOS and related surfactants.

The main objective of this work is to investigate the feasibility of microbial reductive dehalogenation for the removal of PFOS and related (per)fluoroalkyl surfactants in industrial effluents. The characterization of the fate of the incomplete

destruction products of PFOS and PFBS from electrochemical treatment in conventional biological wastewater treatment systems will also be investigated.

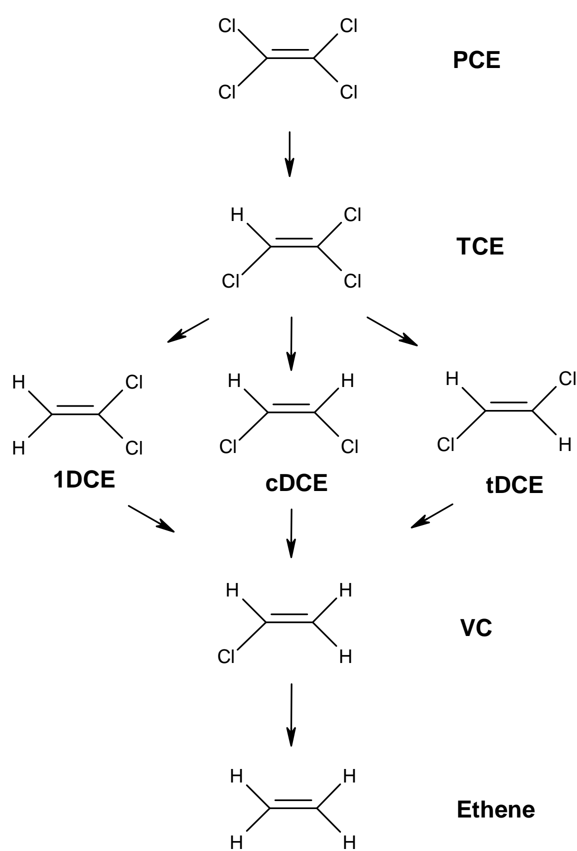


Figure 4.1 Sequential reduction of PCE to ethane by anaerobic reductive dechlorination (adapted from reference [29]).

4.3 Materials and Methods

4.3.1 Chemicals

Perfluorooctane sulfonic acid potassium salt, PFOS (98% purity), 1H,1H,2H,2H-perfluorooctane sulfonic acid, TH-PFOS (98%), pentafluoropropionic acid, PFPA (97%), 3H-tetrafluoropropionic acid, 3H-PFPA (97%), 3,3,3-trifluoropropionic acid, 2H,2H-PFPA (97%), 2,2-bis(trifluoromethyl)propionic acid, $2CH_3$,$2CF_3$-PFPA (97%), perfluoropentanoic acid, PFPeA (97%), 5H-perfluoropentanoic acid, 5H-PFPeA (97%) and 1H,1H,2H,2H,3H,3H-perfluoropentanoic acid, 1H,1H,2H,2H,3H,3H-PFPeA (96%) were purchased from SynQuest Laboratories (Alachua, FL). Perfluorobutane sulfonic acid potassium salt, PFBS (98.2%) was kindly provided by 3M (St. Paul, MN). Trifluoroacetic acid, TFA (99%), vitamin B_{12} (99%), chromium acetylacetonate, $Cr(acac)_3$ (97%), and sodium fluoride (99%) were supplied by Sigma-Aldrich (St. Louis, MO). N_2/CO_2 and H_2/CO_2 gases (80/20, v/v) were delivered from US Air (Phoenix, AZ, USA). Methane (>99.99%) was obtained from Scotty Specialty Gases (San Bernardino, CA, USA). Methanol-D4 (99%) was purchased from Cambridge Isotope Laboratories (Andover, MA). 4'-(trifluoromethoxy)-acetanilide, 4-TFMeAc (97%) was obtained from Matrix Scientific (Columbia, SC). HPLC-grade acetonitrile, methanol, sulfuric acid and boric acid (99.5%) were purchased from Mallinckrodt Chemicals (Philipsburg, NJ). All chemicals were used as received.

4.3.2 Sludge and sediment samples

Activated sludge, anaerobic granular sludge and anaerobically digested sewage sludge (ADS) from industrial and municipal wastewater treatment works as well as biomass from various installations receiving PFOS-containing wastewaters were employed in the microbial toxicity and degradation experiments. The anaerobic granular sludge was obtained from an industrial anaerobic reactor treating alcohol distillery wastewater (Nedalco, The Netherlands). The ADS sample was obtained from the Ina Road municipal wastewater treatment plant in Tucson, AZ. The activated sludge and anaerobically digested sewage sludges were obtained from publicly owned treatment works (POTWs) in Austin, TX. Activated sludge and sediments were obtained from industrial wastewater treatment plants operated by IBM and 3M companies, respectively.

The content of total suspended solids (TSS) in the anaerobic granular sludge, Ina ADS, TX activated sludge, TX ADS, IBM activated sludge and 3M sediments was 7.45, 17.20, 2.37, 33.33, 11.54 and 43.41, respectively. The organic matter content of the sludges was estimated by determination of their content in volatile suspended solids (VSS). The VSS content in the anaerobic granular sludge, Ina ADS, TX activated sludge, TX ADS, IBM activated sludge and 3M sediments was 6.98, 11.76, 1.60, 23.09, 5.71 and 15.49%.

4.3.3 Electrolyzed PFOS and PFBS samples

Electrolyzed PFOS and PFBS samples were provided by Dr. Jim Farrell, University of Arizona. Solutions of PFOS (0.43 mM, pH 5.5) were subjected to electrolysis in a batch reactor at a current of 10 mA for time periods ranging from 0 to 24 h. The batch reactor had an anode surface area of 1 cm^2 and a solution volume of 350 mL, yielding a surface area to solution volume ratio of 2.86 x 10^{-3} cm^2 mL^{-1} [30]. PFBS electrolysis experiments were performed in a flow-through boron-doped diamond (BDD) electrode reactor at a flow rate of 100 mL min^{-1}. PFBS solution (0.40 mM) was electrolyzed at a current density of 2.5 mA cm^{-2}. Aliquots of the electrolyzed solution (250 mL) were collected after 24, 48, 72 and 96 h. An exogenous electrolyte was not added neither to the PFOS nor PFBS solution. Electrolyzed samples were denoted based on the electrolysis time. For instance, PFOS-6h represents the PFOS solution subjected to electrochemical treatment for 6 h.

4.3.4 Culture media

The anaerobic basal mineral medium used in methanogenic bioassays contained the following chemicals (in mg L^{-1}): K_2HPO_4 (250); $CaCl_2$•2 H_2O (10); $MgSO_4$•7 H_2O (100); NH_4Cl (280); NaHCO3 (3,000); yeast extract (50), and 1 mL L^{-1} of trace element solution. The trace element solution contained (in mg L^{-1}): H_3BO_3 1 (50), $FeCl_2$•4 H_2O

(2,000), $ZnCl_2$ (50), $MnCl_2$•$4H_2O$ (50), $(NH_4)6Mo_7O_{24}$•$4H_2O$ (50), $AlCl_3$•6 H_2O (90), $CoCl_2$•6 H_2O (2,000), $NiCl_2$•6 H_2O (50), $CuCl_2$•2 H_2O (30), $NaSeO_3$•5 H_2O (100), EDTA (1,000), resazurin (200) and 36% HCl (1 mL L^{-1}). The basal medium was adjusted to pH 7.2 with HCl or NaOH, as required. PFOS, TH-PFOS and PFBS were dissolved in the basal medium to obtain the desired concentrations.

4.3.5 Microbial toxicity assays

Maximum specific hydrogenotrophic methanogenic activities measurements were conducted in an orbital shaker (Innova 4300, New Brunswick Scientific, Edison, NJ) at 150 rpm at 30±2ºC. The anaerobic granular sludge was pre-incubated overnight with 150 mL of basal medium to ensure microbial adaptation to the medium conditions. In all toxicity experiments, 1.5 g VSS $L^{-1}$of the sludge were added to serum flasks (165 mL) containing the basal medium and the compound. Perfluorinated compounds were directly dissolved in 25 mL of the basal medium. In the bioassays conducted with electrolyzed PFOS and PFBS solutions, 20 mL of the samples were added to 5 mL of basal medium concentrated by 5-fold. In the toxicity bioassays of short-chain perfluorocarboxylic compounds, 15 mL of 500 mg L^{-1} of C_3- and C_5-perfluorinated compounds were added to 15 mL of basal medium (2-fold concentrated). All flasks were sealed with butyl rubber stoppers and aluminum crimp seals, and their headspace was flushed with N_2:CO_2 gas (80:20, v/v). Subsequently, H_2 was supplied by pressurizing the bottles with 15 kPa of

H_2/CO_2 (80:20 v/v). The flasks were equilibrated for one to three hours prior GC measurements of the headspace. Triplicate substrate controls were based on assays where no perfluorinated compound (short and long-chain) or electrolyzed sample was added.

The methane content in the headspace of each flask was determined periodically until 80% or more of the substrate in the controls was depleted. The maximum specific methanogenic activities (mg CH4-COD g^{-1} VSS day^{-1}) were calculated from the slope of the cumulative methane production and biomass concentration versus time (day), as the mean value of triplicate assays. The initial concentrations of F^- causing 20%, 50% and 80% reduction in activity compared to an uninhibited control were referred to as IC_{20}, IC_{50} and IC_{80}, respectively.

4.3.6 Microbial degradation

All degradation bioassays were incubated in a climate-controlled chamber at 30±2 °C under static conditions. Biodegradation experiments were carried out in duplicate or triplicate serum flasks (165 mL) supplied with 50 mL of culture medium containing 200 mg L^{-1} of the target perfluorinated compound. Test solutions with (partly) electrolyzed PFOS or PFBS were diluted by 2.5-fold to minimize microbial inhibition during the biodegradability assays. Microbial inoculum (1.5 g VSS L^{-1} or 5% (v/v) of sludge) was added to each vial. Flasks were then flushed with N_2:CO_2 gas (80:20, v/v), sealed with butyl rubber stoppers and aluminum crimp seals. In full treatment assays, hydrogen gas

(H_2) was supplied as the electron donor to promote reductive defluorination by pressurizing the bottles with 15 kPa of H_2/CO_2 (80:20 v/v) of PFOS, TH-PFOS, PFBS and electrolyzed PFOS and PFBS samples. Oxygen gas (O_2) as $He_2/O_2/CO_2$ (60:20:20 v/v) was supplied as the electron acceptor to promote aerobic degradation of short-chain fluorinated carboxylates Aseptic controls (lacking inoculum) were run in parallel to correct for the possible release of fluoride by abiotic reaction. Sludge controls (no perfluorinated compound or short-chain fluorinated compounds or electrolyzed sample added) and killed-sludge controls (by autoclaving) were also set up to determine background fluoride concentrations and to quantify the compounds removal by sorption to the biomass, respectively.

Routine analyses of fluoride were conducted using a VWR SympHony fluoride-selective combination electrode High-performance liquid chromatography with suppressed conductivity detection and ^{19}F-NMR spectroscopy were also performed periodically to determine if the perfluorinated alkyl surfactants and the products of PFOS/PFBS electrolysis are susceptible to microbial defluorination.

4.3.7 GC-FID

The methane content in the headspace of the serum flasks was determined by gas chromatography (GC) using an HP5290 Series II system (Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector (GC-FID). The GC was fitted with a DB7 FFAP column (J&W Scientific, Palo Alto, CA) capillary column. The temperature of the column, the injector port and the detector was 140, 180 and 275°C, respectively. Helium was used as the carrier gas at a flow rate of 9.3 mL min^{-1} and a split flow of 32.4 mL min^{-1}. Headspace samples (100 µL) were injected in the GC-FID using a pressure-lock gas syringe. Methane standards varying from 0 to 10% were prepared to calculate the methane production.

4.3.8 HPLC-suppressed conductivity detection

Analysis of PFOS and related compounds as well as electrolyzed PFOS and PFBS samples was conducted by liquid chromatography with conductivity detection (Dionex ICS-3000, Sunnyvale, CA). The system was equipped an Acclaim Polar Advantage II C_{18}-guard column (4.3 mm i.d., 1 cm length) and an Acclaim Polar Advantage II C_{18}-analytical column (4.6mm i.d., 25 cm length), both from Dionex. The temperature of the thermostated column and the compartment was maintained at 35 ºC. The mobile phase consisted of a mixture of 20 mM boric acid (pH 8.0) and 95% acetonitrile flowing at a

rate of 1mL min^{-1}. The ratio of boric acid to acetonitrile varied with linear gradient program as follows: 0 min 75:15 (v/v) to 45:55 (v/v) at 13.2 min. The volume of sample injected on the column was 100 µL. Blanks were continuously run to assure that the column was clean and traces of the analyte were not carried over between samples. The total aqueous concentration of PFOS and PFBS in the bioassays was obtained by linear calibration curves ($r^2 > 0.99$) using known concentrations of the respective compounds ranging from 0.5– 25 mg L^{-1}. The detection limit of PFOS and PFBS was 1 mg L^{-1}.

4.3.9 ^{19}F-NMR

^{19}F NMR analysis was conducted employing the same procedure described in our previous publication [31]. Briefly, samples were dissolved in 10% deuterated methanol containing 4 mg mL^{-1} of chromium acetylacetonate ($Cr(acac)_3$) as the relaxation agent and then spiked with 140 mg L^{-1} of the internal standard, 4'-(trifluoromethoxy)-acetanilide, 4-TFMeAc. ^{19}F NMR spectra were acquired on a Varian Unity-300 spectrometer using a 4-nucleus (^{31}P, ^{13}C, ^{19}F, ^{1}H) probe.

4.3.10 Other analytical determinations

Fluoride in liquid samples was determined using a VWR SympHony fluoride-selective combination electrode. Samples were diluted with TISAB buffer (Total Ionic Strength Adjustment Buffer) to maintain the ionic strength constant during the measurements. All other determinations (e.g., VSS and TSS content) were conducted according to Standard Methods [32].

4.4 Results and Discussion

Microbial toxicity

4.4.1 Microbial toxicity of PFOS and related compounds

The inhibitory effect of PFOS and related fluorinated compounds including, perfluorobutane sulfonate (PFBS), a fluorinated derivative proposed as an environmentally-benign alternative to PFOS [33], and *1*H,*1H*,2*H*,2*H*-perfluorooctane sulfonic acid (TH-PFOS), a partially defluorinated PFOS-derivative, on methanogenic microorganisms in anaerobic wastewater treatment sludge was evaluated. Figure 4.2 shows an illustrative example of the time course of the methane formation during the microbial toxicity assay amended with 0, 50, 125, 250 and 500 mg L^{-1} of PFOS. The highest concentration tested is close to the solubility limit for PFOS. The solubility of PFOS in pure water is 500 mg L^{-1} [24]. The activity of H_2-utilizing methanogens was calculated from the slopes of cumulative methane production versus time plots (Figure 4.2). The activities of samples containing the fluorinated compound were normalized to that of the control treatment in which no contaminant was added. The methanogenic IC_{20}, IC_{50} and IC_{80} values determined for the various fluorinated compounds are summarized in Table 4.1.

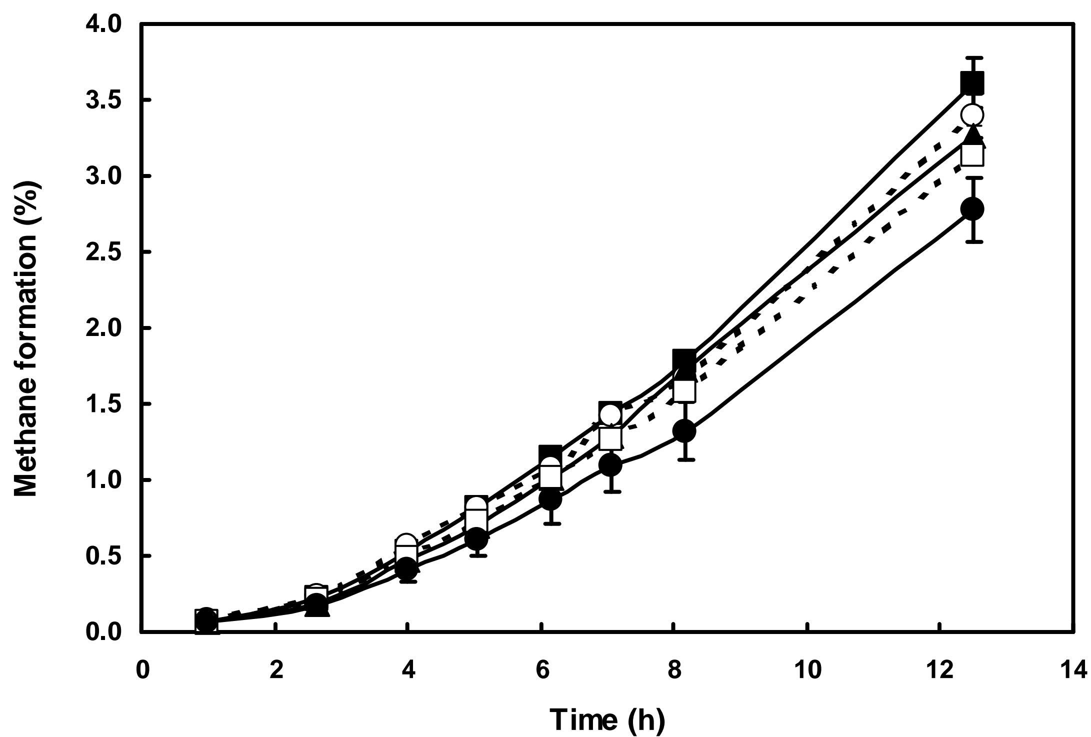


Figure 4.2 Time course of the methane formation by a methanogenic microbial consortium in anaerobic granular sludge (1.5 g VSS L^{-1}) in the presence of increasing concentrations of PFOS (in mg L^{-1}): (■) 0; (○) 50; (▲) 125; (□) 250, and (●) 500.

PFOS caused a small decrease in the hydrogenotrophic methanogenic activity (20% compared to the control) when present at 500 mg L^{-1} (Table 4.1). None of the other compounds tested (*i.e.,* PFBS and TH-PFOS) were found to cause significant microbial inhibition in these short-term bioassays at concentrations as high as 200 mg L^{-1}. These results are in agreement with studies reported on the effects of PFAS to microorganisms in sewage sludge. A PFOS concentration of 870 mg L^{-1} caused 39% microbial inhibition towards activated sludge microbes [24]. Similarly, sewage sludge microorganisms were very tolerant to high PFBS concentrations as demonstrated by the reported IC_{50} value of 1000 mg L^{-1} [34].

Literature data show that PFBS does not exert any toxicity to fish and aquatic invertebrates such as daphnids and mysid shrimp [34]. Likewise, PFOS was reported to cause little or not toxicity to aquatic organisms. PFOS was not toxic in the Microtox® assay conducted with *Photobacterium phosphoreum* at the highest concentration tested (> 250 mg L^{-1}) after 30 min of exposure [35]. On the other hand, PFOS was shown to be a low to moderate inhibitor of some aquatic biomonitoring species. Acute inhibition studies conducted with *Daphnia magna* indicated that the concentrations of PFOS causing 50% mortality after 48 h of exposure (48h-LC_{50}) ranged from 58 to 130 mg L^{-1} [25].

The information obtained from the microbial toxicity experiments was used to ensure sub-toxic concentrations of the tested compounds in the anaerobic biodegradation assays. Inhibition must be avoided as it might preclude degradation of compounds that would otherwise be susceptible to microbial attack.

Table 4.1 Microbial toxicity of PFOS and related perfluoroalkyl sulfonates to hydrogenotrophic methanogens in anaerobic sludge. IC_{20}, IC_{50} and IC_{80} are the concentrations of the perfluoroalkyl surfactants causing 20, 50 and 80% decrease in the activity of the methanogenic microorganisms, respectively.

Compound	**IC_{20}**	**IC_{50}**	**IC_{80}**
		mg L^{-1}	
PFOS	485	> 500 [a]	> 500 [a]
PFBS	> 200 [b]	> 200 [b]	> 200 [b]
TH-PFOS	> 200 [b]	> 200 [b]	> 200 [b]

[a,b] Not toxic at the highest concentration tested, 500 and 200 mg L^{-1}, respectively.

4.4.2 Microbial toxicity of short-chain (per) fluorinated compounds

Short-chain (per) fluorinated compounds are of environmental importance because they are possible substitutes of long-chain perfluorinated compounds (*i.e.* perfluorooctanoic acid (PFOA)) in industrial applications. PFAS with six or less fluorocarbon chains have different physico-chemical properties as compared to the long-chain perfluorinated compounds. They have higher aqueous solubility, lower tendency to sorb to organic matter and are not considered bioaccumulative [5, 6].

The influence of the carbon chain length as well as the partial defluorination character of short-chain fluorinated compounds on the activity of microbes was also investigated in this study. The methanogenic inhibition of homologues series of partially and fully fluorinated carboxylic acids ranging from C_2 to C_5 were evaluated in batch assays (Figure 4.3). TFA, a two carbon chain perfluorinated carboxylic acid was not toxic to hydrogenotrophic methanogens even at concentrations as high as 1,140 mg L^{-1}. Likewise, C_3-carboxylates: PFPA, 3H-PFPA, 2H,2H-PFPA and $2CH_3$,$2CF_3$-PFPA, and C_5-carboxylates: PFPeA, 5H-PFPeA and 1H,1H,2H,2H,3H,3H-PFPeA did not show an inhibitory effect on the activity of hydrogenotrophic methanogens in anaerobic granular sludge at aqueous concentrations of 250 mg L^{-1}. Thus, the length of the carbon chain and the partial defluorination of short-chain perfluorocarboxylic acids did not have an adverse effect on the methanogenic activity of wastewater treatment microorganisms.

These findings are consistent with literature studies on the microbial toxicity of perfluorocarboxylic acids. For instance, Emptage and coworkers [36] reported that TFA

exhibited no toxicity on the microbial activity of hydrogenotrophic and acetoclastic methanogens at the same aqueous concentrations examined in this research. In contrast, the acute toxicity of long-chain perfluorocarboxylates to marine bacteria (*Vibrio fischeri*) was directly related to the perfluorocabon chain length [37]. The values causing a 50% response (EC_{50}) of perfluorohexanoic (C_6), perfluoroheptanoic (C_7), perfluorooctanoic (C_8) and perfluorononanoic (C_9) acids were 1,339, 1099, 571 and 533 mg L^{-1}, respectively. Since, the acute toxicity values are significantly higher than those found in the environment; perfluorinated compounds are considered low inhibitors of aquatic organisms. Nevertheless, the same trend has been observed on the bioaccumulation potential of long-chain perfluorocarboxylates, the longer the perfluorocarbon chain the more bioaccumulative the compound is [6, 38]. Further research is needed to establish the toxic effect of perfluorocarboxylates with carbon chains higher than five on the activity of microorganisms present in wastewater sludge.

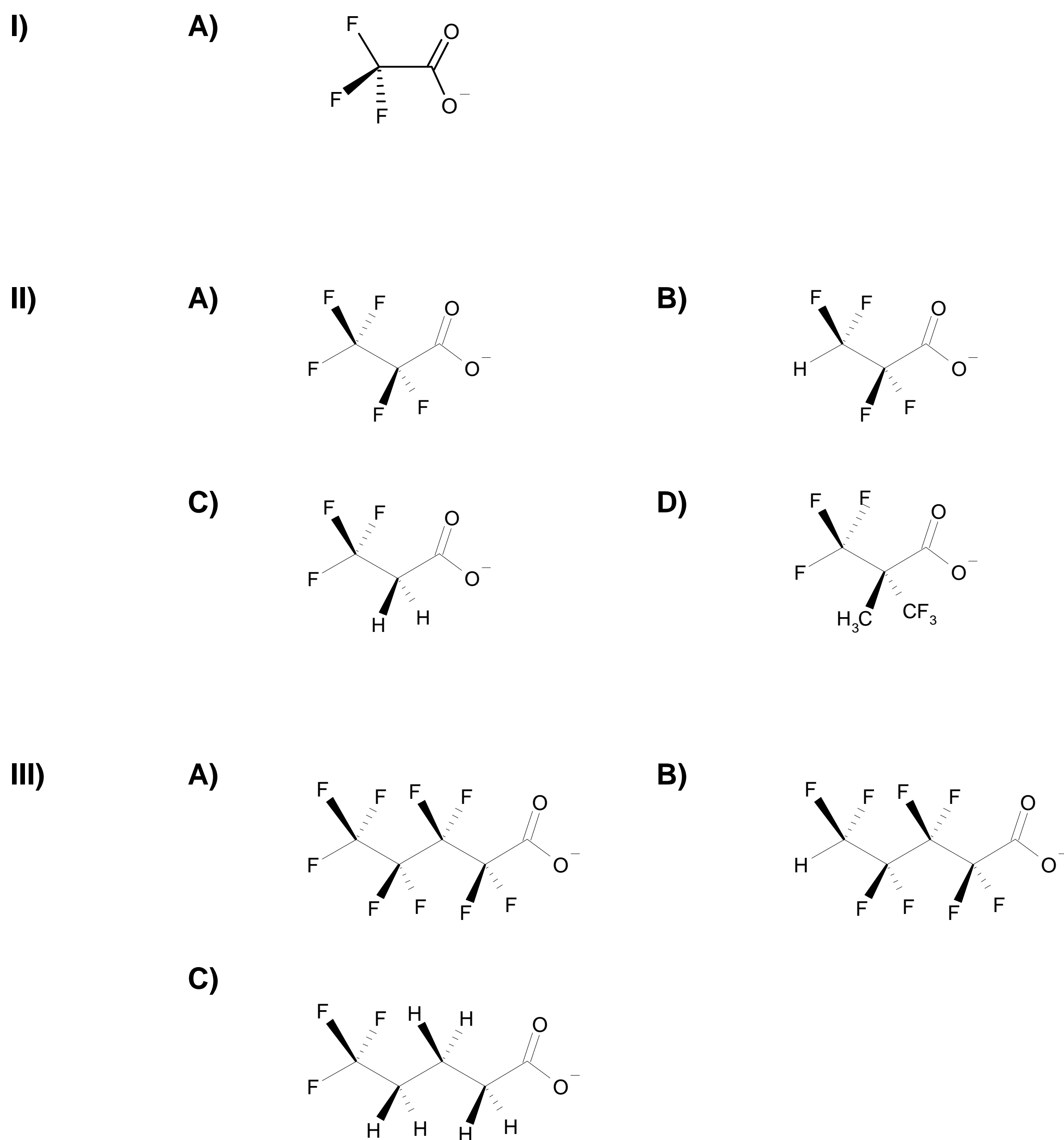


Figure 4.3 Chemical structures of short-chain perfluorocarboxylates. I) C_2: A) trifluoroacetic acid. II) C_3: A) pentafluoropropionic acid, PFPA, B) 3H-tetrafluo-ropropionic acid, 3H-PFPA, C) 3,3,3-trifluoropropionic acid, 2H,2H-PFPA and D) 2,2-bis(trifluoromethyl)propionic acid, $2CH_3,2CF_3$-PFPA. II) C_5: A) perfluoropentanoic acid, PFPeA, B) 5H-perfluoropentanoic acid, 5H-PFPeA and C) 1H,1H,2H,2H,3H,3H-perfluoropentanoic acid, 1H,1H,2H,2H,3H,3H-PFPeA.

4.4.3 Microbial toxicity of electrolyzed PFOS and PFBS samples

A feasible method for the removal of PFOS from wastewater streams could be the coupling of electrochemical and biological treatment processes. The application of electrochemical process followed by biological systems has shown to be very effective in the degradation of a variety of recalcitrant compounds [39, 40]. The contaminant is electrochemically degraded into less persistent counterparts that are subsequently biodegraded to benign products such as water and carbon dioxide. Combined electrochemical-biological treatment often provides significant energy and chemical savings when compared to a single stage processes relying on electrochemical treatment.

PFOS and PFBS solutions were subjected to electrochemical treatment as described in the *Materials and Methods* section. Electrolysis experiments performed in in batch reactors using a rotating disk BDD electrode indicated that PFOS can be rapidly removed from water. Electrochemical treatment resulted in a gradual decrease in the concentration of total organic carbon (TOC), 80% removal after 24 hours (Figure 4.4). The concentration of PFOS in solution also decreased with electrolysis time, albeit at a slower rate compared to the TOC. The decrease in PFOS content was accompanied by release of fluoride ion (Table 4.2), which accounted for approximately 7 moles or fluoride per mole of PFOS removed.

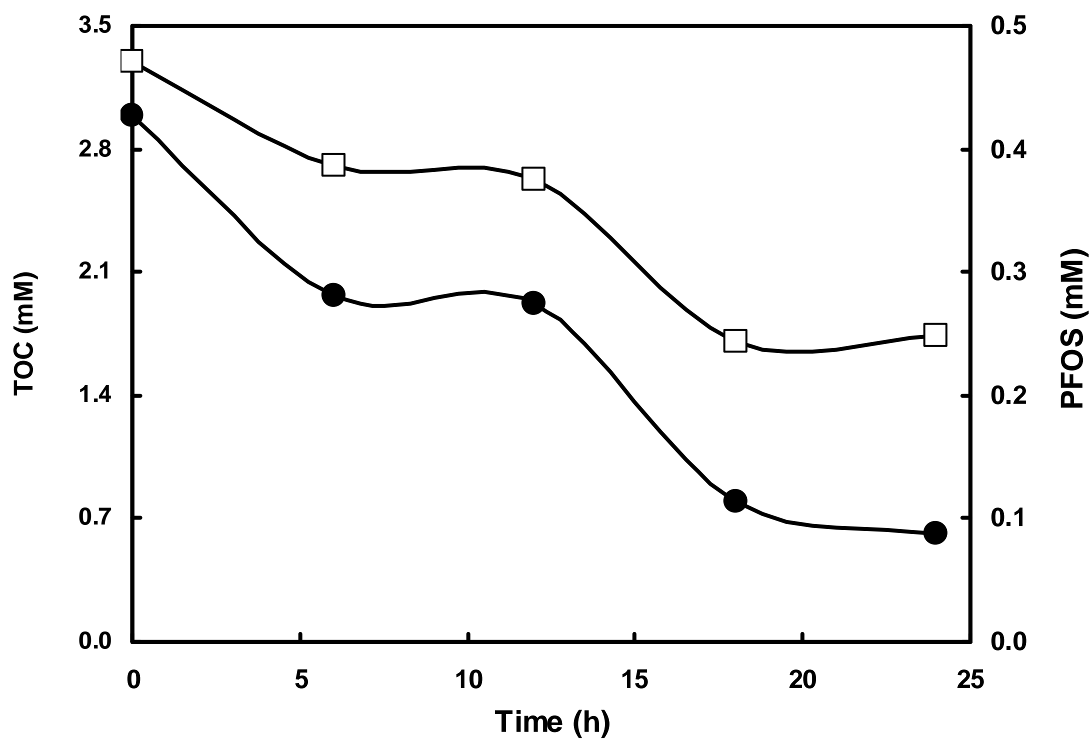


Figure 4.4 Concentrations of total organic carbon (TOC) and PFOS as a function of time during the electrochemical of PFOS in a BDD batch reactor operated at a current of 10 mA. Legend: (□) TOC and (●) PFOS.

PFBS does undergo electrochemical attack at considerably lower rates than PFOS as shown in Figure 4.5. The concentration of both total organic carbon and PFBS decreased with electrolysis time. Results from this study have demonstrated that PFBS is completely degraded after 72 hours of exposure in the flow-through reactor. As much as 60 mg L^{-1} of fluoride, equivalent to the removal of 9 moles of fluoride per mol of PFBS degraded were released to the reaction medium in a solution subjected to 96 hours of electrochemical treatment (Table 4.2).

Table 4.2 Fluoride release during the electrochemical treatment of PFOS and PFBS after different periods of time.

PFAS sample	**F^- release (mg L^{-1})**
PFOS - 0 h	0.0
PFOS - 6 h	11.7
PFOS - 12 h	16.1
PFOS - 18 h	38.1
PFOS - 24 h	45.1
PFBS - 0 h	0.0
PFBS - 24 h	19.2
PFBS - 48 h	42.0
PFBS - 72 h	57.6
PFBS - 96 h	60.5

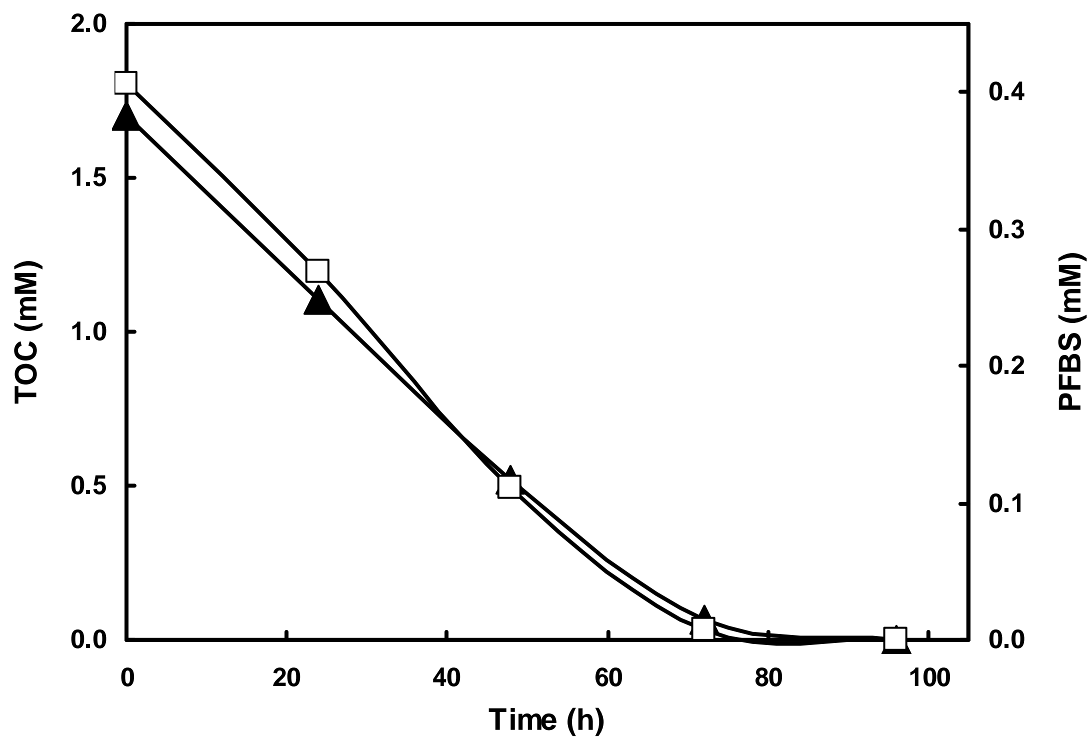


Figure 4.5 Concentrations of total organic carbon (TOC) and PFBS as a function of time during the electrochemical of PFBS in BDD flow-through reactor operated with a current density of 2.5 mA cm^{-2}. Legend: (□) TOC and (▲) PFBS.

Figures 4.6 and 4.7 show the ^{19}F-NMR spectra of electrolyzed PFOS and PFBS samples, respectively. No evidence of the presence of other fluorinated compounds than PFOS and PFBS in the reaction mixtures was found after 18 or 24 h of electrochemical treatment. These results are in agreement with the information obtained from the sample providers. Dr Farrell and collaborators [30] reported that the detected products of the electrochemical oxidation of PFOS consisted on sulfate, fluoride, and trace levels of trifluoroacetic acid (TFA). The authors suggested that presence of carbon dioxide, major reaction product, was indicated by the decrease in pH from 4 to 2.3. However, no quantification of CO_2 was provided.

The ^{19}F NMR spectra of treated PFOS and PFBS samples only show a new peak with a shift of approximately -121 ppm which corresponds to the fluoride ion. All chemical shifts were referenced to the internal standard 4'-(trifluoromethoxy)-acetanilide 4-TFMeAc (-58.08 ppm) [41].

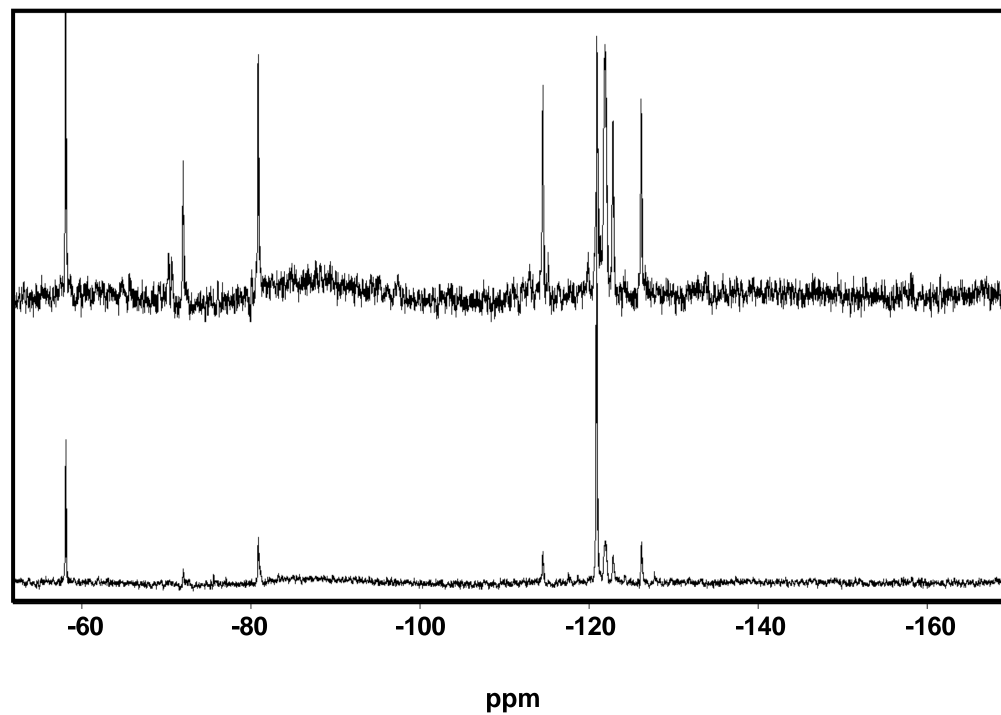


Figure 4.6 ^{19}F-NMR spectra of technical PFOS (214 mg L^{-1}) (upper panel) and PFOS subjected to electrochemical treatment for 18 h (lower panel) in a BDD batch reactor operated at a current of 10 mA.

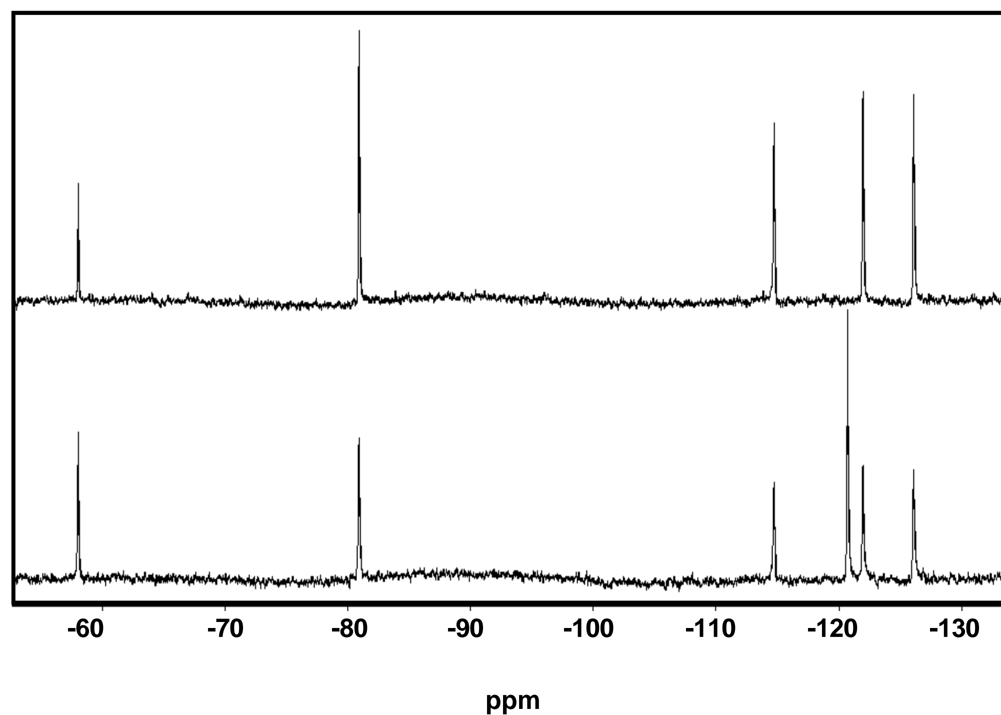


Figure 4.7 ^{19}F-NMR spectra of PFBS (120 mg L^{-1}) and PFBS subjected to electrochemical attack for 24 h in a BDD flow-through reactor operated with a current density of 2.5 mA cm^{-2}.

The impact of electrochemical treatment on the toxicity of PFOS and PFBS towards methanogenic microorganisms in anaerobic sludge was tested in laboratory assays. Electrochemical treatment was found to increase the microbial toxicity of PFOS (Figure 4.8). The untreated PFOS solution (0.43 mM equivalent to 214 mg L^{-1}) caused moderate inhibition of the hydrogenotrophic metabolic activity of the methanogenic inoculum (30% reduction compared to the uninhibited control). In contrast, exposure to the electrolyzed PFOS solutions led to a significant decrease in the microbial activity ranging from 58 to 70% reduction in the activity of hydrogenotrophic methanogens compared to the inhibited control. Additional experiments performed with the PFOS solution subjected to electrolysis for 24 h (data not shown) indicated that the toxicity decreased rapidly with sample dilution. The microbial inhibition of a solution diluted two-fold was only 31%. In the case of PFBS, the electrochemical treatment did not have an impact into the microbial activity of the electrolyzed samples (Figure 4.9). The PFBS- and electrolyzed PFBS samples did not inhibit the activity of hydrogenotrophic methanogens after 42 h of exposure. PFBS samples electrolyzed for 72 h or longer only caused a minor decrease in the methanogenic activity (24% compared to the control).

The negative effect of the electrolyzed PFOS samples on the activity of methanogens is not well understood. Fluoride ions released by electrochemical treatment probably contributed to the observed microbial inhibition. Fluoride has recently been shown to inhibit methanogenic microorganisms in anaerobic sludge [42]. The concentration of fluoride causing 50% inhibition (IC_{50}) of acetate-utilizing methanogens ranged from 18 to 43 mg L^{-1}. H_2-utilizing methanogens were more tolerant to fluoride, as

indicated by IC_{50} values ranging from 82 to 430 mg L^{-1}. Electrochemical attack of PFOS and PFBS for 24 and 96 h, respectively, released fluoride concentrations ranging from 45 to 61 mg L^{-1} (Table 4.3). These concentrations are relatively similar, suggesting that degradation products other than fluoride might contribute to the enhanced inhibitory effects determined in the electrolyzed PFOS samples. Further research is needed to identify the degradation products generated during electrochemical treatment of PFOS and their impact on methanogenic microorganisms.

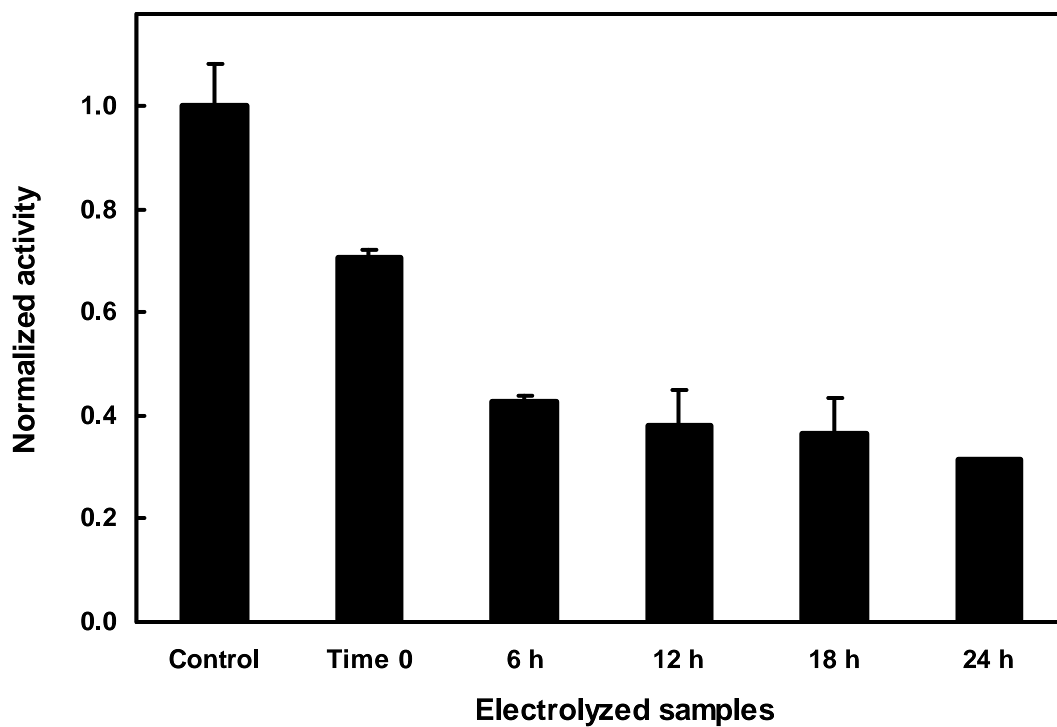


Figure 4.8 Normalized hydrogenotrophic methanogenic activities of PFOS samples electrolyzed for 6, 12, 18 and 24 h. Error bars (shown if larger than the symbols) represent standard deviations of duplicate assays.

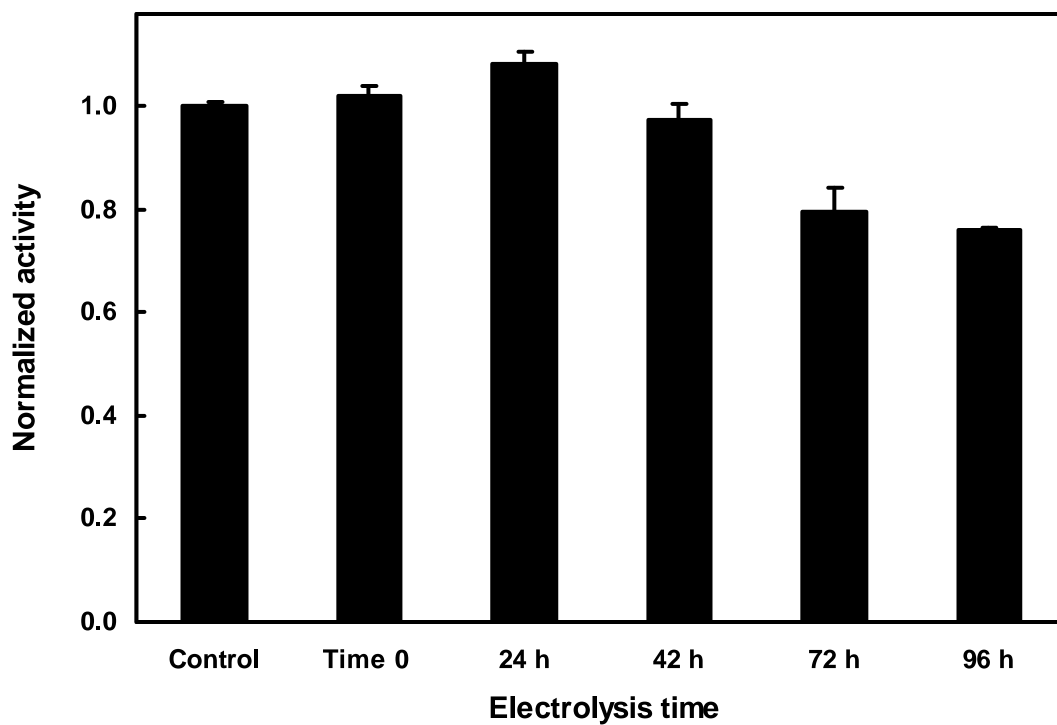


Figure 4.9 Normalized hydrogenotrophic methanogenic activities of PFBS samples subjected to electrochemical attack for different time periods. Error bars (shown if larger than the symbols) represent standard deviations of duplicate assays.

Microbial degradation

4.4.4 Anaerobic microbial degradation of PFOS and related compounds

Microbial reductive dehalogenation experiments were set up to determine the ability of municipal and industrial microbial inocula to degrade PFOS, PFBS and TH-PFOS. The highly oxidized character of these perfluoroalkyl surfactants is expected to make them more prone to microbial attack in anaerobic reducing environments as compared to aerobic conditions [29]. Therefore, bioassays were inoculated with anaerobic sediments or sludge samples obtained from a variety of sources, including inocula previously exposed to fluorinated compounds (e.g. biosolids from wastewater treatment plants receiving PFOS-containing semiconductor wastewaters).

Table 4.3 summarizes the biodegradability potential of fluorinated sulfonates to reductive dehalogenation with different inocula sources based on fluoride measurements. This fluoride values corresponds to data corrected with the control samples, abiotic, sludge and killed-sludge controls. As illustrated in the table, no fluoride release as a result of the degradation of the fluorinated compounds has been obtained in any of the bioassays after > 2 years of incubation. In agreement with our results, no biodegradation of PFOS has been reported in several studies conducted with a variety of microbial sources and different exposure times [3, 9, 26, 43]. For instance, no biotransformation of PFOS was observed with activated sludge under anaerobic and aerobic conditions after

56 d and 20 weeks of exposure, respectively [25]. Similarly, no biodegradation of PFBS and TH-PFOS with sludge and sediments under aerobic and anaerobic conditions was observed after 9 to 40 weeks of incubation [44]. However, it is interesting to note that TH-PFOS was reported to be biodegradable in previous studies conducted under aerobic conditions [27].

Selected liquid samples were also analyzed using ^{19}F-NMR and HPLC-IC with conductivity detection. These techniques can serve to detect changes in the fluorine substitution pattern and to identify putative microbial metabolites of the parent compounds, respectively. In agreement with our previous results, no indication was obtained that PFOS, PFBS, and TH-PFOS were reductively dehalogenated by the anaerobic consortia.

Technical PFOS is a mixture of linear and branched structural isomers, with the latter making up 20 to 30% of the total mass [45, 46]. Work conducted in the frame of this study has shown that branched PFOS isomers are more susceptible to biomimetic reductive dehalogenation than the linear PFOS isomer [31]. These results suggest that branching may facilitate the microbial degradation of perfluorinated compounds. Currently, the microbial transformation of branched PFOS isomers in anaerobic environments is being investigated. After 4 months of incubation, no evidence of biodegradation of branched PFOS isomers has been obtained (data not shown).

Table 4.3 Fluoride release corrected with control samples of PFOS, PFBS and TH-PFOS (200 mg L^{-1}) in biodegradability assays inoculated with different municipal and industrial sludge and sediments.

Compound	Sludge/sediments*	Incubation period (years)	F^- release corrected (mg L^{-1})
PFOS	Anaerobic granular sludge	3.4	0.02 $\pm$ 0.01
PFOS	Ina ADS sludge	3.3	0.09 $\pm$ 0.02
PFOS	Tx activated sludge	3.2	0.12 $\pm$ 0.02
PFOS	Tx ADS sludge	3.2	0.02 $\pm$ 0.01
PFOS	IBM activated sludge	2.8	0.50 $\pm$ 0.13
PFOS	3M sediments	2.7	0.05 $\pm$ 0.01
TH-PFOS	Ina ADS sludge	3.1	0.03 $\pm$ 0.01
TH-PFOS	Tx ADS sludge	3.0	0.06 $\pm$ 0.02
TH-PFOS	IBM activated sludge	2.1	0.50 $\pm$ 0.06
TH-PFOS	3M sediments	2.1	0.04 $\pm$ 0.01
PFBS	Ina ADS sludge	3.1	0.01 $\pm$ 0.0
PFBS	Tx ADS sludge	3.0	0.01 $\pm$ 0.0
PFBS	IBM activated sludge	2.7	0.43 $\pm$ 0.10
PFBS	3M sediments	2.7	0.02 $\pm$ 0.10

* Tx activated sludge and TS ADS sludge: activated sludge and anaerobically digested sewage sludge from POTWs in Austin, TX. IBM activated sludge: activated sludge from an industrial wastewater treatment plant operated by IBM Company. 3M sediments: sediments from an industrial wastewater treatment plant operated by 3M Company.

4.4.5 Microbial degradation of short-chain perfluorocarboxylates

The biodegradability of C_3 and C_5 (per) fluorinated carboxylic acids was evaluated in batch assays under aerobic conditions. The biodegradation of TFA was evaluated utilizing anaerobic sludge. Preliminary results indicate that none of the short-chain fluorinated compounds was susceptible to microbial transformation. No biotic degradation of PFPA, 3H-PFPA, 2H,2H-PFPA, $2CH_3$,$2CF_3$-PFPA, PFPeA, 5H-PFPeA and 1H,1H,2H,2H,3H,3H-PFPeA was observed after 2 months of exposure to municipal activated sludge. In the case of TFA, the compound was found to be very recalcitrant after more than 2 years of incubation. Supporting our results, no biodegradation of TFA has been observed under aerobic/anaerobic conditions in a vast majority of studies [47]. Only two research groups have reported biotransformation of TFA [48]. TFA was microbially degraded under methanogenic and sulfur conditions with marine sediments and cometabollically degraded in anaerobic environments after 90 days of incubation. These results suggest that microorganism capable of degrading TFA are very specific and not distributed in common environments.

In general, perfluorinated compounds appear to be very persistence to biotransformation. Even short-chain and hydrogen-substituted carboxylates were not susceptible to microbial degradation after long periods of incubation. These results confirm the outstanding resistance of PFOS and related perfluorinated compounds to microbial attack [24].

4.4.6 Anaerobic microbial degradation of electrolyzed PFOS and PFBS samples

The susceptibility to microbial degradation of products from the electrolysis of PFOS and PFBS was investigated in anaerobic batch bioassays. Electrolyzed PFOS and PFBS samples collected at regular intervals for up to 24 and 100 h, respectively, were tested.

For PFOS, the concentration of total organic carbon (TOC) detected in solution at the different times of electrolysis exceeded the residual surfactant concentration, suggesting some accumulation of electrolysis products in solution (Figure 4.4). Nonetheless, fluorinated compounds other than PFOS were not detected by ^{19}F-NMR, mass spectroscopy, or HPLC-suppressed conductivity detection in the sample electrolyzed for 18 h as demonstrated above (Figure 4.6).

In the case of PFBS, the residual concentration of surfactant and TOC in solution was nearly the same independently of the electrolysis time, indicating complete mineralization of the compound by electrolysis. After 72 h of electrolysis, only traces of both TOC and surfactant were detected. These results are in agreement with ^{19}F-NMR measurements which showed that PFBS was the only fluorinated compound detected in all the electrolyzed solutions (Figure 4.7).

Electrolyzed PFOS and PFBS samples have a high fluoride background as a result of the electrochemical treatment (Table 4.2). These samples were diluted by 2.5 fold for the biodegradation experiments for two reasons to avoid microbial inhibition and reduce the fluoride residual. Table 4.4 summarizes the results of the microbial degradation of the electrolysis products of PFOS and PFBS corrected with the values obtained in the control

samples. No fluoride release as a result of the microbial treatment was obtained after long periods of incubation. The results indicate that electrolyzed PFOS and PFBS samples were not degraded after approximately 1.5 and 1.1 years of biological treatment.

Table 4.4 Fluoride release corrected with control samples during the microbial degradation of electrolyzed PFOS and PFBS samples in bioassays inoculated with anaerobic wastewater treatment sludge after 1.5 and 1.1 years of incubation, respectively.

PFAS sample	**F^- residual (mg L^{-1})**	**F^- release corrected (mg L^{-1})**
PFOS - 0 h	0.0	0.02 ± 0.01
PFOS - 6 h	4.7	0.03 ± 0.01
PFOS - 12 h	6.4	0.02 ± 0.01
PFOS - 18 h	15.2	0.06 ± 0.01
PFOS - 24 h	18.1	0.10 ± 0.01
PFBS - 0 h	0.0	0.02 ± 0.01
PFBS - 24 h	7.7	0.03 ± 0.01
PFBS - 48 h	16.8	0.02 ± 0.01
PFBS - 72 h	23.1	0.06 ± 0.01
PFBS - 96 h	24.2	0.10 ± 0.01

Monitoring the presence of fluorinated compounds in the liquid medium confirmed our previous results on the resistance of electrolyzed PFOS and PFBS samples to microbial degradation. The concentration of PFOS and PFBS in the treatment samples did not decrease with time (data not shown). Sorption of the perfluoralkyl compounds to biomass was registered during the initial 30 d of the incubation. Work conducted in our laboratory has shown that PFOS shows a low to moderate affinity for anaerobic sludge [17].

Regardless of the duration of the electrochemical treatment, the products of the electrolysis of PFOS and PFBS were found to be highly resistant to microbial degradation after 1.5 and 1.1 years of incubation, respectively. These results are in agreement with fluoride release and ^{19}F-NMR measurements in which no evidence that microbial attack led to changes in the fluorine substitution pattern of the organic molecules in solution was obtained.

4.5 Conclusions

PFOS, PFBS and TH-PFOS caused no or only minor methanogenic toxicity. No evidence has been obtained after > 2 years that the perfluorinated compounds are biodegraded by sludge obtained from various industrial and municipal wastewater treatment processes under anaerobic conditions.

C_2, C_3 and C_5 partially and fully fluorinated carboxylic acids are not toxic to hydrogenotrophic methanogens. Hence, the perfluorocarbon chain and the partial defluorination of the short-chain perfluorocarboxylates do not interfere with the activity of wastewater sludge microbes. These compounds are also very resistant to microbial degradation after 2 months of incubation.

Electrochemical treatment was found to increase the microbial toxicity of PFOS towards methanogenic microorganisms in anaerobic wastewater treatment sludge. In contrast, electrochemical treatment of PFBS did not have an impact on the methanogenic activity of the microbial consortium. PFOS and PFBS samples subjected to electrochemical treatment up to 24 and 96 h, respectively, were not susceptible to anaerobic biodegradation after more than 1.5 and 1.1 years of incubation.

4.6 Acknowledgments

This research was funded by the Semiconductor Research Corporation/Sematech Engineering Research Center for Environmentally Benign Semiconductor Manufacturing. We are thankful to Dr. Jim Farrell from the Department of Chemical and Environmental Engineering, University of Arizona, for providing the electrolyzed PFOS and PFBS samples.

4.7 References

1. Kissa, E., *Fluorinated Surfactants and Repellants*. Marcel Dekker, Inc.: New York, 2001.
2. EPA *3M Company Environmental and health assessment of Perfluorooctane sulfonate and its salts.*; U.S. Environmental Protection Agency Public Docket AR226-1486. : Washington, DC, 2003.
3. Schultz, M. M.; Barofsky, D. F.; Field, J. A., Fluorinated alkyl surfactants. *Environ. Eng. Sci.* **2003,** *20*, (5), 487-501.
4. *Perfluoroalkyl Sulfonates; Proposed Significant New Use Rule*; U.S. Environmental Protection Agency: 2006; pp 12311 - 12324.
5. Houde, M.; Martin, J. W.; Letcher, R. J.; Solomon, K. R.; Muir, D. C. G., Biological monitoring of polyfluoroalkyl substances: A review. *Environ. Sci. Technol.* **2006,** *40*, (11), 3463-3473.
6. Conder, J. M.; Hoke, R. A.; De Wolf, W.; Russell, M. H.; Buck, R. C., Are PFCAs bioaccumulative? A critical review and comparison with regulatory lipophilic compounds. *Environ. Sci. Technol.* **2008,** *42*, (4), 995-1003.
7. Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J., Perfluoroalkyl acids: A review of monitoring and toxicological findings. *Toxicol. Sci.* **2007,** *99*, (2), 366-394.

8. Butenhoff, J. L.; Olsen, G. W.; Pfahles-Hutchens, A., The applicability of biomonitoring data for perfluorooctanesulfonate to the environmental public health continuum. *Environ. Health Perspect.* **2006,** *114*, (11), 1776-1782.

9. Giesy, J. P.; Kannan, K., Global distribution of perfluorooctane sulfonate in wildlife. *Environ. Sci. Technol.* **2001,** *35*, (7), 1339-1342.

10. Kannan, K.; Corsolini, S.; Falandysz, J.; Fillmann, G.; Kumar, K. S.; Loganathan, B. G.; Mohd, M. A.; Olivero, J.; Van Wouwe, N.; Yang, J. H.; Aldous, K. M., Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. *Environ. Sci. Technol.* **2004,** *38*, (17), 4489-4495.

11. Hansen, K. J.; Clemen, L. A.; Ellefson, M. E.; Johnson, H. O., Compound-specific, quantitative characterization of organic: Fluorochemicals in biological matrices. *Environ. Sci. Technol.* **2001,** *35*, (4), 766-770.

12. Sinclair, E.; Kannan, K., Mass loading and fate of perfluoroalkyl surfactants in wastewater treatment plants. *Environ. Sci. Technol.* **2006,** *40*, (5), 1408-1414.

13. Boulanger, B.; Vargo, J. D.; Schnoor, J. L.; Hornbuckle, K. C., Evaluation of perfluorooctane surfactants in a wastewater treatment system and in a commercial surface protection product. *Environ. Sci. Technol.* **2005,** *39*, (15), 5524-5530.

14. Higgins, C. P.; Field, J. A.; Criddle, C. S.; Luthy, R. G., Quantitative determination of perfluorochemicals in sediments and domestic sludge. *Environ. Sci. Technol.* **2005,** *39*, (11), 3946-3956.

15. *3M Executive Summary: Environmental Monitoring - Multi-City Study. Water, Sludge, Sediments, POTW Effluent and Landfill Leachate Samples.* ; U.S. Environmental Protection Agency, EPA- 226-1030a, 2001.

16. Plumlee, M. H.; Larabee, J.; Reinhard, M., Perfluorochemicals in water reuse. *Chemosphere* **2008,** *72*, (10), 1541-1547.

17. Ochoa-Herrera, V.; Sierra-Alvarez, R., Removal of prefluorinated surfactants by sorption onto granular activated carbon, zeolite and sludge. *Chemosphere* **2008,** *72*, 1588 - 1593.

18. Becker, A. M.; Gerstmann, S.; Frank, H., Perfluorooctane surfactants in waste waters, the major source of river pollution. *Chemosphere* **2008,** *72*, (1), 115-121.

19. Moody, C. A.; Martin, J. W.; Kwan, W. C.; Muir, D. C. G.; Mabury, S. C., Monitoring perfluorinated surfactants in biota and surface water samples following an accidental release of fire-fighting foam into Etohicoke Creek. *Environ. Sci. Technol.* **2002,** *36*, (4), 545-551.

20. Skutlarek, D.; Exner, M.; Farber, H., Perfluorinated surfactants in surface and drinking water. *Environ. Sci. Pollut. Res.* **2006,** *13*, (5), 299-307.

21. Loos, R.; Locoro, G.; Huber, T.; Wollgast, J.; Christoph, E. H.; De Jager, A.; Gawlik, B. M.; Hanke, G.; Umlauf, G.; Zaldivar, J. M., Analysis of perfluorooctanoate (PFOA) and other perfluorinated compounds (PFCs) in the River Po watershed in N-Italy. *Chemosphere* **2008,** *71*, (2), 306-313.

22. Ju, X. D.; Jin, Y. H.; Sasaki, K.; Saito, N., Perfluorinated surfactants in surface, subsurface water and microlayer from Dalian Coastal waters in China. *Environ. Sci. Technol.* **2008,** *42*, (10), 3538-3542.

23. Saito, N.; Harada, K.; Inoue, K.; Sasaki, K.; Yoshinaga, T.; Koizumi, A., Perfluorooctanoate and perfluorooctane sulfonate concentrations in surface water in Japan. *Journal of Occupational Health* **2004,** *46*, (1), 49-59.

24. Brooke, D.; Footitt, A.; Nwaogu, T. A., Environmental risk evaluation report: Perfluorooctanesulphonate (PFOS). *Environment Agency, Chemicals Assessment Section, Wallingford, UK* **2004**.

25. Beach, S. A.; Newsted, J. L.; Coady, K.; Giesy, J. P., Ecotoxicological evaluation of perfluorooctanesulfonate (PFOS). In *Reviews of Environmental Contamination and Toxicology, Vol 186*, Springer: New York, 2006; Vol. 186, pp 133-174.

26. Remde, A.; Debus, R., Biodegradability of fluorinated surfactants under aerobic and anaerobic conditions. *Chemosphere* **1996,** *32*, (8), 1563-1574.

27. Key, B. D.; Howell, R. D.; Criddle, C. S., Defluorination of organofluorine sulfur compounds by Pseudomonas sp. strain D2. *Environ. Sci. Technol.* **1998,** *32*, (15), 2283-2287.

28. Smidt, H.; de Vos, W. M., Anaerobic microbial dehalogenation. *Annu. Rev. Microbiol.* **2004,** *58*, 43-73.

29. Field, J. A.; Sierra-Alvarez, R., Biodegradability of chlorinated solvents and related chlorinated aliphatic compounds. *Reviews in Environmental Science & Bio/Techonology* **2004,** *3*.

30. Carter, K. E.; Farrell, J., Oxidative destruction of perfluorooctane sulfonate using boron-doped diamond film electrodes. *Environ. Sci. Technol.* **2008,** *42*, (16), 6111-6115.

31. Ochoa-Herrera, V.; Sierra-Alvarez, R.; Somogyi, A.; Jacobsen, N. E.; Wysocki, V. H.; Field, J. A., Reductive defluorination of perfluorooctane sulfonate. *Environ. Sci. Technol.* **2008,** *42*, (9), 3260-3264.

32. APHA., *Standard methods for the examination of water and wastewater*. 20th ed.; American Public Health Association: Washington D.C., 1998.

33. *3M Technical data bulletin. Environmental health, safety and regulatory (ehsr) profile of perfluorobutane sulfonate (PFBS)*; St Paul, MN, 2002.

34. NICNAS, Potassium perfluorobutane sulfonate. Existing chemical hazard assessment report. *Department of Health and Ageing, National Industrial Chemicals Notification and Assessment Scheme (NICNAS), Australian Government, Sidney, Australia* **2005**.

35. OECD *Co-operation on existing chemicals: Hazard assesment of perfluorooctane sulfonate (PFOS) and its salts.* ; JT00135607; Organisation for Economic Co-operation and Development (OCED), Environment Directorate, Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology: 2002.

36. Emptage, M.; Tabinowski, J.; Odom, J. M., Effect of fluoroacetates on methanogenesis in samples from selected methanogenic environments. *Environ. Sci. Technol.* **1997,** *31*, (3), 732-734.

37. Mulkiewicz, E.; Jastorff, B.; Skladanowski, A. C.; Kleszczynski, K.; Stepnowski, P., Evaluation of the acute toxicity of perfluorinated carboxylic acids using eukaryotic cell lines, bacteria and enzymatic assays. *Environmental Toxicology and Pharmacology* **2007,** *23*, (3), 279-285.

38. Martin, J. W.; Mabury, S. A.; Solomon, K. R.; Muir, D. C. G., Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (Oncorhynchus mykiss). *Environmental Toxicology and Chemistry* **2003,** *22*, (1), 196-204.

39. Chiang, L. C.; Chang, J. E.; Tseng, S. C. In *Electrochemical oxidation pretreatment of refractory organic pollutants*, 1997; 1997; pp 123-130.

40. Torres, R. A.; Sarria, V.; Torres, W.; Peringer, P.; Pulgarin, C., Electrochemical treatment of industrial wastewater containing 5-amino-6-methyl-2-benzimidazolone: toward an electrochemical-biological coupling. *Water Res.* **2003,** *37*, (13), 3118-3124.

41. Tugnait, M.; Lenz, E. M.; Phillips, P.; Hofmann, M.; Spraul, M.; Lindon, J. C.; Nicholson, J. K.; Wilson, I. D., The metabolism of 4-trifluoromethoxyaniline and [C-13]-4-trifluoromethoxyacetanilide in the rat: detection and identification of metabolites excreted in the urine by NMR and HPLC-NMR. *J. Pharm. Biomed. Anal.* **2002,** *28*, (5), 875-885.

42. Ochoa-Herrera, V.; Banihani, Q.; Leon, G.; Field, J. A.; Sierra-Alvarez, R., Toxicity of fluoride to microorganisms in biological wastewater treatment systems. *In preparation* **2008**.

43. Lange, C. *The 35-day aerobic biodegradation study of PFOS.*; 3M: Minneapolis, MN, 2001.

44. PEFORCE *Scientific report of the Europian project "Perfluorinated organic compounds in the European environment"*; 2006.

45. 3M *Fluorochemical Isomer Distribution by 19F-NMR Spectroscopy*; U.S. Environmental Protection Agency Public Docket AR226-0564. : 1997.

46. Vyas, S. M.; Kania-Korwel, I.; Lehmler, H. J., Differences in the isomer composition of perfluoroctanesulfonyl (PFOS) derivatives. *J. Environ. Sci. Health Part A-Toxic/Hazard. Subst. Environ. Eng.* **2007,** *42*, (3), 249-255.

47. Boutonnet, J. C.; Bingham, P.; Calamari, D.; de Rooij, C.; Franklin, J.; Kawano, T.; Libre, J. M.; McCulloch, A.; Malinverno, G.; Odom, J. M.; Rusch, G. M.; Smythe, K.; Sobolev, I.; Thompson, R.; Tiedje, J. M., Environmental risk assessment of trifluoroacetic acid. *Hum. Ecol. Risk Assess.* **1999,** *5*, (1), 59-124.

48. Natarajan, R.; Azerad, R.; Badet, B.; Copin, E., Microbial cleavage of C-F bond. *Journal of Fluorine Chemistry* **2005,** *126*, (4), 425-436.

CHAPTER 5

Biomimetic Reductive Defluorination of Pefluorooctane Sulfonate (PFOS) with Vitamin B_{12}

5.1 Abstract

Perfluorooctane sulfonate (PFOS) is under increased scrutiny as environmental pollutant due to recent reports of its world-wide distribution, environmental persistence and bioaccumulation potential. The chemical reductive dehalogenation of technical PFOS and branched PFOS isomers with vitamin B_{12} as catalyst and Ti(III)-citrate as bulk reductant in anoxic aqueous solution was reported in this study. The treatment conditions of the reductive dehalogenation were optimized by studying the effect of various parameters on the kinetics of the reaction. The optimal conditions of those tested in this study were found to be 260 µM vitamin B_{12}, 36 mM Ti(III) citrate, temperature of 70°C and solution pH 9. Defluorination was confirmed by fluoride release measurements 18% in technical PFOS, equivalent to the removal 3 mol F^- per mol PFOS, and 71% in PFOS branched isomers equivalent to the removal of 12 mol F^- per mol PFOS. Degradation of PFOS was further confirmed by monitoring the disappearance of PFOS compounds with reaction time by HPLC-suppressed conductivity detection, LC-MS/MS and ^{19}F NMR studies.

These studies showed that PFOS compounds differed in their susceptibility to reductive degradation by vitamin B_{12}/Ti(III) citrate. Chromatographic peaks corresponding to branched PFOS isomers disappeared whereas the peak corresponding to linear PFOS was stable. The reaction mechanism of the degradation of PFOS was investigated by EPR spectroscopy. These studies demonstrated the formation of a vitamin B_{12} carbon-center radical which presumably initiates the chemical reduction. To our knowledge this is the first report of reductive dehalogenation of PFOS catalyzed by a biomolecule.

Key words: PFOS, branched isomers, reductive dehalogenation and vitamin B_{12}.

A modified version of this chapter has been published as a paper entitled: *"Reductive Defluorination of Perfluorooctane Sulfonate"*, Environ. Sci. Technol., 2008, **42**:p. 3260-3264.

5.2 Introduction

Perfluorooctane sulfonate (PFOS, $C_8F_{17}SO_3H$) and related derivatives have been used for decades in a wide variety of industrial and consumer-based products ranging from surfactants, firefighting foams to coatings [1]. PFOS has been detected globally in human blood samples and wildlife tissues, including biota from pristine areas [2-5]. Recent evidence indicates that PFOS is a persistent toxic pollutant that accumulates in higher organisms and could pose a potential human health risk [6, 7]. These findings have prompted environmental regulatory agencies and the industry to initiate the phase out of PFOS [8].

Currently, little is known about the environmental sources and pathways of exposure of perfluoroalkyl compounds (PFAS). The widespread of PFOS and related substances in the environment could be attributed to the release during manufacture, use and disposal of industrial and consumer products as well as degradation of their precursors [9]. Studies suggested that the presence of PFAS in the remote regions is likely to occur through atmospheric or ocean water transport of their volatile PFAS precursors, fluorotelomer and fluorosulfoamido alcohols, which are ultimately transformed to stable perfluorooctanoic acid (PFOA) and PFOS, respectively [9, 10]. A study evaluating the fate of 171 perfluorinated substances using the biodegradation prediction software engine (CATABOL) estimated that 27% and 17% of the perfluorinated sulfonic acid and carboxylic acid containing compounds would be degraded to PFOS and PFOA, respectively [11]. Dispersion of PFOS and derivatives in

the environment could also occur via surface water, indoor air, and dust and adsorption to sediments and sludge [7].

The development of treatment techniques to decompose PFOS is crucial to reduce and eliminate the environmental release of this ubiquitous pollutant. To date neither biodegradation nor chemical degradation of PFOS under ambient conditions has been observed. PFOS displays an outstanding resistance against chemical and biological degradation. The recalcitrance has been attributed to the stability of the strong covalent carbon-fluorine bonds and the resistance to electrophilic or nucleophilic attack due to the shielding effect of the fluorine atoms [12].

Published data on the degradation of perfluorooctane sulfonate and related compounds is very limited. Moriwaki and coworkers [13] investigated the breakdown of PFOS by ultrasonic irradiation under argon and oxygen atmospheres. Perfluorinated carboxylic compounds were the products of the reaction. The decomposition of PFOS using zero-valent iron in 350ºC sub-critical water was examined by Hori *et al.* [14]. PFOS was partially degraded; however, no organic degradation products were detected. The photodegradation of PFOS by UV-light in water and alkaline 2-propanol solution was studied by Yamamoto and coworkers [15]. Short-chain fluorinated compounds were formed during the UV irradiation. A recent study conducted by Carter and coworkers [16] investigated the oxidative destruction of PFOS at boron-doped diamond film electrodes. Although, significant PFOS degradation was obtained, the formation of oxidation products was not reported.

In the quest for PFOS biotransformation, microbial reductive dehalogenation should be considered. Reductive dehalogenation is an important mechanism contributing to the biodegradation of highly chlorinated compounds such as trichloroethylene (TCE) and perchloroethylene (PCE) [17-19]. Anaerobic microorganisms, halorespiring bacteria, gain energy by reductively cleaving the carbon-halogen bond of the pollutants. Reductive dehalogenases are the key catalyst in the respiratory chain of these microorganisms. Most known reductive dehalogenases are dependent on the corrinoid cofactor, vitamin B_{12} (cyanocobalamin). This cobalt-containing cofactor catalyzes reductive dehalogenation in vitro when supplied with an appropriate reducing agent such as titanium (III) [20]. The direct catalysis of dehalogenation by enzyme cofactors is known as *biomimetic dehalogenation*, because it mimics reactions expected in microorganisms.

Several studies have reported the dehalogenation of polychlorinated and polybrominated compounds catalyzed by vitamin B_{12} [21-27]. Moreover, vitamin B_{12} and Ti(III) citrate have been used as an in situ remediation technique for treating groundwater contaminated with chlorinated solvent [28-30]. Here we report the defluorination of technical PFOS and branched PFOS isomers by biomimetic reduction with vitamin B_{12} as catalyst and Ti(III)-citrate as bulk reductant in anoxic aqueous solutions (Figure 5.1)

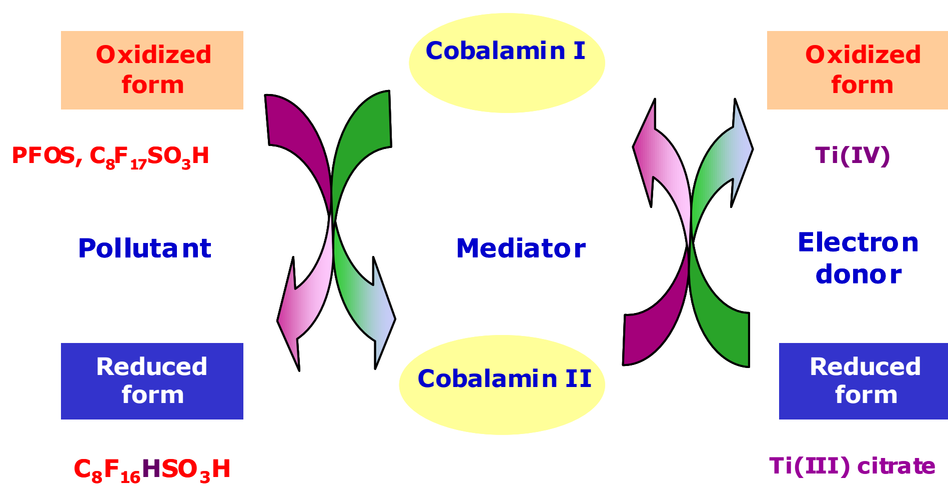


Figure 5.1 Proposed mechanism of the biomimetic reductive dehalogenation of PFOS with vitamin B_{12} and Ti(III) citrate.

5.3 Materials and Methods

5.3.1 Chemicals

Perfluorooctanesulfonic acid potassium salt, PFOS (98% purity) was purchased from SynQuest Laboratories (Alachua, FL). Titanium (III) chloride (15% solution in HCl), vitamin B_{12} (99%), perfluorooctanoic acid, PFOA (96%), 5,5'-dimethlylpyrroline-N-oxide, DMPO (97%), chromium acetylacetonate, $Cr(acac)_3$ (97%) and sodium fluoride (99%) were supplied by Sigma-Aldrich (St. Louis, MO). Methanol-D4 (99%) was purchased from Cambridge Isotope Laboratories (Andover, MA). 4'-(trifluoromethoxy)-acetanilide, 4-TFMeAc (97% purity) was obtained from Matrix Scientific (Columbia, SC). Granular activated carbon (GAC) Filtrasorb 400 was obtained from Calgon Carbon Corp. (Pittsburgh, PA). Zeolite NaY80 (Si/Al 80, product CBV 780) was purchased from Zeolyst Int. (Valley Forge, PA). HPLC-grade acetonitrile, methanol, sulfuric acid and boric acid (99.5%) were purchased from Mallinckrodt Chemicals (Phillispsburg, NJ). All chemicals were used as received.

5.3.2 Identification and quantification of structural PFOS isomers

Linear and branched structural isomers of technical perfluorooctane sulfonate acid potassium salt were identified based on tandem mass spectrometry (MS/MS) spectral data obtained by high-performance liquid chromatography coupled to MS/MS as suggested by Langlois and coworkers [31]. The composition of structural perfluorooctane sulfonate (PFOS, $C_8F_{17}SO_3H$) isomers in the technical salt was determined by HPLC-suppressed conductivity detection and LC-MS/MS. The amount of the branched PFOS isomers was calculated by relating the areas of the branched PFOS isomers peaks to the total area of all PFOS peaks, and it was found to be 24.6%. This observation was further confirmed by first-principle calculations performed in models of the linear and branched structural isomers of the compound. The chemical structure of the identified PFOS isomers and their abundance in the technical PFOS is depicted in Figure 5.2 and Table 5.1, respectively. Linear PFOS and 1-perfluoromethlyl-PFOS (1-CF_3-PFOS) were chromatographically separated. 3-perfluoromethyl- and 4-perfluoromethyl-PFOS (3- and 4-CF_3-PFOS), as well as 5-perfluoromethyl- and 6-perfluoroisopropyl-PFOS (5- and 6-CF_3-PFOS) coeluted as two different peaks.

5.3.3 Purification of PFOS isomers and linear PFOS

A solution containing technical PFOS (0.5 mM) and carbonate buffer (85 mM) was centrifuged (10,000 rpm, 10 min). The linear PFOS compound precipitates under these conditions while the branched PFOS isomers remain in solution. The supernatant consisting of branched PFOS isomers (94.2%) and traces of linear PFOS was employed in further experiments.

5.3.4 Reductive defluorination

Reductive defluorination of technical PFOS (332 μM) and the purified mixture of branched PFOS isomers (54 μM) by vitamin B_{12} and Ti(III)-citrate in a buffer solution was examined in batch assays at pH values ranging from 7.0 to 9.0. The concentrations of vitamin B_{12} and Ti(III) citrate were 260 μM and 36 mM, unless otherwise indicated. A carbonate buffer (85 mM) was utilized, unless otherwise specified. The Ti(III)-citrate solution was prepared as described by Hollinger *et al.* [26]. The pH of this solution was adjusted with concentrated NaOH to pH values ranging from 8.0 to 9.0. All experiments were set up in triplicate in an anaerobic box, utilizing 37 mL serum bottles sealed with viton stoppers and aluminum crimp caps and wrapped with aluminum foil to prevent decomposition of vitamin B_{12} by light. The flasks were incubated at 30ºC, 50ºC or 70ºC under static conditions. After various time intervals, samples of the liquid medium were

analyzed for fluoride by a VWR SympHony fluoride-selective combination electrode using a background matrix of TISAB (Total Ionic Strength Adjustment Buffer). Several control flasks lacking either vitamin B_{12} and/or Ti(III)-citrate or supplied with cobalt(II) in lieu of vitamin B_{12} were run in parallel. If evidence of fluoride release was obtained, selected samples were analyzed by HPLC-suppressed conductivity detection, ^{19}F NMR and/or LC-MS/MS. Solid phase extraction (SPE) procedures were conducted to remove interferences and concentrate analytes when required.

5.3.5 Clean-up and concentration of samples

SPE cartridges (3 mL, 500 mg ODS-C_{18}, Agilent Technologies, DE) mounted on a vacuum manifold were conditioned with 6 mL MeOH, followed by 6 mL of deionized water. The desired volume of sample was loaded at 1 mL min^{-1}. SPE cartridges were rinsed with 4 mL of deionized water and then centrifuged (10,000 rpm, 10 min) prior elution. Analytes were eluted with 4 mL methanol and collected in clean Nalgene flasks. Solutions for chromatographic analysis were spiked with known concentrations of internal standard (PFOA, 31 μM) to determine recovery efficiencies. Recovery efficiencies of 104.0 $\pm$ 14.1 % for triplicate samples were obtained.

5.3.6 ^{19}F NMR

Analysis of technical PFOS and fluoride ion by ^{19}F NMR was performed by a method adapted from Moody *et al.* [32]. Samples were dissolved in 90% H_2O/10% CD_3OD or in 90% CH_3OH/10% CD_3OD (0.7 mL) containing chromium acetylacetonate ($Cr(acac)_3$) (4 mg mL^{-1}) as the relaxation agent and the internal standard, 4'-(trifluoromethoxy)-acetanilide (4-TFMeAc, 140 mg L^{-1}), and then analyzed in 5-mm tubes. ^{19}F NMR spectra were acquired at 22°C on a Varian Unity-300 spectrometer operating at a ^{19}F frequency of 282.208 MHz using a 5mm 4-nucleus (^{31}P, ^{13}C, ^{19}F, ^{1}H) probe. Acquisition involved a relaxation delay of 1.44 s followed by a 90° pulse (16.2 μs) and a Hahn echo with an echo delay of 100 μs, with 16,384 complex data points and a spectral width of 33333 Hz. The acquisition time for all samples was 32.5 min. A 10 Hz line broadening was applied before zero filling to 32,768 data points and Fourier transform. Baseline correction was performed using a fifth-order polynomial, and chemical shifts were referenced to the internal standard at -58.08 ppm [33].

5.3.7 LC-MS/MS

Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) analysis was conducted by a method described by Langlois *et al.* [31]. LC-MS/MS measurements were performed on a Magic 2002 (Michrom Biosciences, Inc., San Jose, CA) / Thermoelectron

LCQ Classic HPLC-MS system. Chromatographic separation was conducted on a MagicMS C_{18} microbore column (5 µm, 200Å, 1 x 150 mm). The mobile phase consisted of a buffer solution of 10mM ammonium acetate (A) and methanol (B). A gradient program with 5% B to 90% B in 35 min was used to elute the components of the samples with a flow rate of 50 µL min^{-1}, temperature 40 ºC and injection volume 25 µL. Negative ionization was employed to detect fluorinated sulfonates and acids. Tandem MS/MS was also applied to get structural information on selected ions (e.g., on m/z 499, $CF_3(CF_2)_7SO_3^-$). Helium was used as a collision gas and a 35% relative collision energy was applied in the MS/MS experiments. MS/MS spectra were recorded within a mass range of m/z ranging from 75 to 1000 using a scan time of 0.2 s.

5.3.8 HPLC-suppressed conductivity detection

PFOS compounds were quantified by a HPCL system with suppressed conductivity detector (ICS-3000 detection system, DIONEX, Sunnyvale, CA). The chromatograph was equipped with an autosampler (injection volume 100 µL), a pump, a degasser, a guard column (Acclaim Polar Advantage II C_{18}, 4.3 mm i.d., 1 cm length) and a separation column (Acclaim Polar Advantage II, C_{18}, 4.6 mm i.d., 25 cm length) operating at 35 ºC. A mixture of 20 mM boric acid (pH 8.0) and 95% acetonitrile was used as the mobile phase at a flow rate of 1mL min^{-1}. The ratio of boric acid to acetonitrile varied with linear gradient program 0 min: 75:25 to 13.2 min: 45:55 (v/v).

The detection limit of branched PFOS isomers and linear PFOS was 1 mg L^{-1} based on a signal-to-noise ratio of 3. Since purified PFOS isomers are not available, the analytical sensitivity (peak height related to the concentration) was assumed to be the same for the different PFOS isomers.

5.3.9 EPR spectroscopy

Electron paramagnetic resonance (EPR) spectroscopy studies were conducted to elucidate the mechanism of the chemical degradation of PFOS catalyzed by vitamin B_{12}. The spin trap, 5,5'-dimethlylpyrroline-N-oxide (DMPO) (27 mM) was added to the reaction mixture to study the formation of radical adducts. The EPR spectra were acquired on a continuous wave (CW) Bruker ESP-300E EPR spectrometer (Bruker, Billerica, MA) at 77 K. The CW EPR operated at a microwave frequency of 9.650 GHz, microwave power of 2 mW at modulation amplitude of 1 G.

5.3.10 Ab initio calculations

Ab initio DFT calculations were performed to determine the relative stability of the various PFOS isomers. The calculations were conducted at the B3LYP/6-31+G(d,p) level as implemented in the GAUSSIAN03 computational package [34]. Cluster models were

employed to simulate the structures of the linear and branched PFOS isomers in the gas phase (i.e. isolated molecules), which, in a first stage, were fully optimized. On the optimal structures, vibrational frequencies were computed within the harmonic approximation. Thermodynamic predictions were calculated at standard conditions of temperature and pressure employing the harmonic Zero Point Energy (ZPE) obtained from the vibrational frequencies, and thermal effects computed with the standard expressions for an ideal gas in the canonical ensemble [35].

Table 5.1 Percent concentration of technical PFOS based on LC-MS/MS studies.

PFOS*	**Composition (%)**
1-CF_3-PFOS	8.7
3 and 4-CF_3-PFOS	2.7
5 and 6-CF_3-PFOS	13.2
Linear PFOS	75.4

* assuming equal response factors for branched and linear isomers.

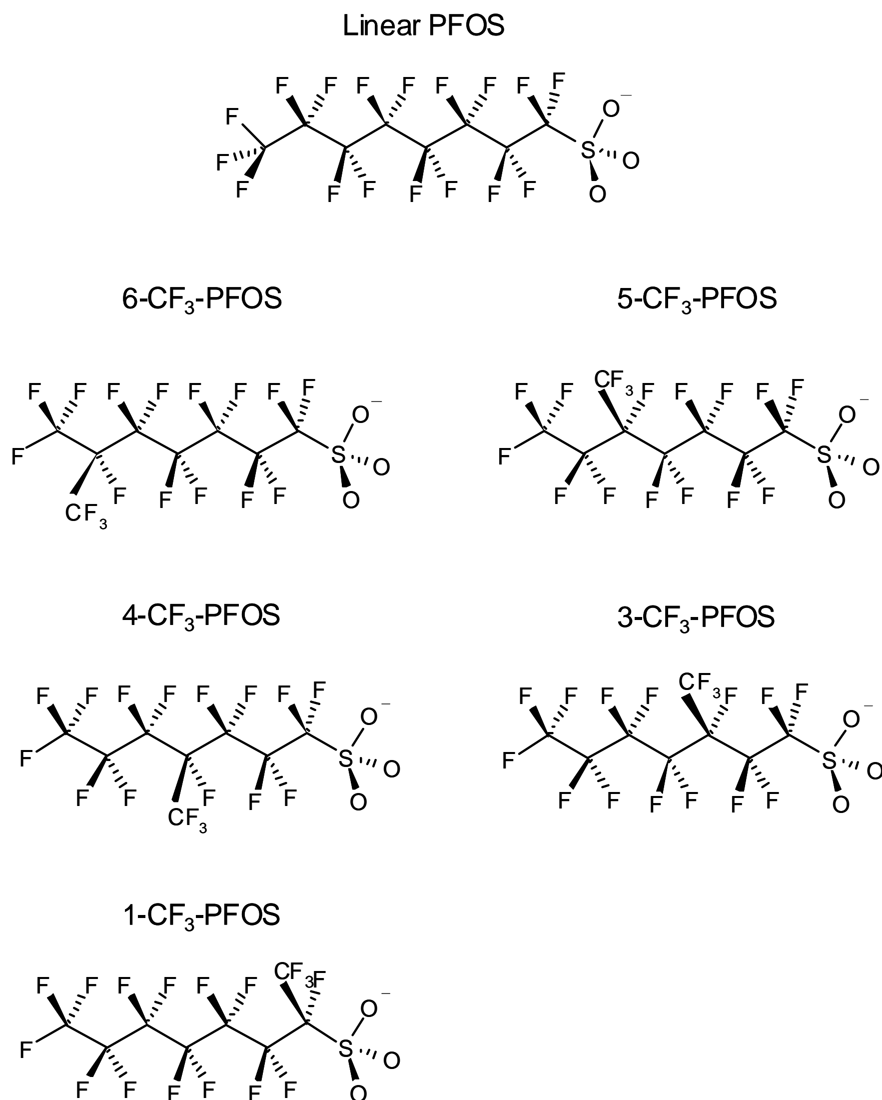


Figure 5.2 Chemical structure of linear PFOS and branched isomers, perfluoroisopropyl (6-CF_3-PFOS), 5-perfluoromethyl (5-CF_3-PFOS), 4-perfluoromethyl (4-CF_3-PFOS), 3-perfluoromethyl (3-CF_3-PFOS) and 1-perfluoromethyl (1-CF_3-PFOS).

5.4 Results and Discussion

The biomimetic reductive dehalogenation of technical PFOS was evaluated in this study. PFOS was shown to be chemically dehalogenated by vitamin B_{12} in a buffer Ti(III) citrate solution as demonstrated by the release of fluoride ions to solution where they were analyzed and quantified by a fluoride-selective combination electrode and ^{19}F NMR spectroscopy as discussed below.

The rate of reductive dehalogenation of PFOS was optimized by testing different temperatures, catalysts concentrations, Ti(III) citrate dosages, solution pH and buffers. The effect of temperature was the first parameter assessed to determine the best treatment conditions of the reaction. Figure 5.3 shows the fluoride release results obtained in experiments conducted at different temperature values. As much as, 0.5 mg F^{-} L^{-1} were released on day 36 at 30ºC, 3.0 mg F^{-} L^{-1} on day 15 at 50ºC and finally, 18.0 mg F^{-} L^{-1} were obtained on day 7 at 70ºC. The rate of PFOS degradation increased considerable with increasing temperature. In fact, an increase in temperature from 30°C to 70°C resulted in a 37-fold increase in the reaction rate. This finding is consistent with previous reports on the dependence of the rate of dehalogenation on the incubation temperature [21, 36, 37]. For example, Krone and coworkers [36] observed an approximate 2-fold increase in the rate of carbon tetrachloride transformation by raising the temperature from 35 to 65ºC.

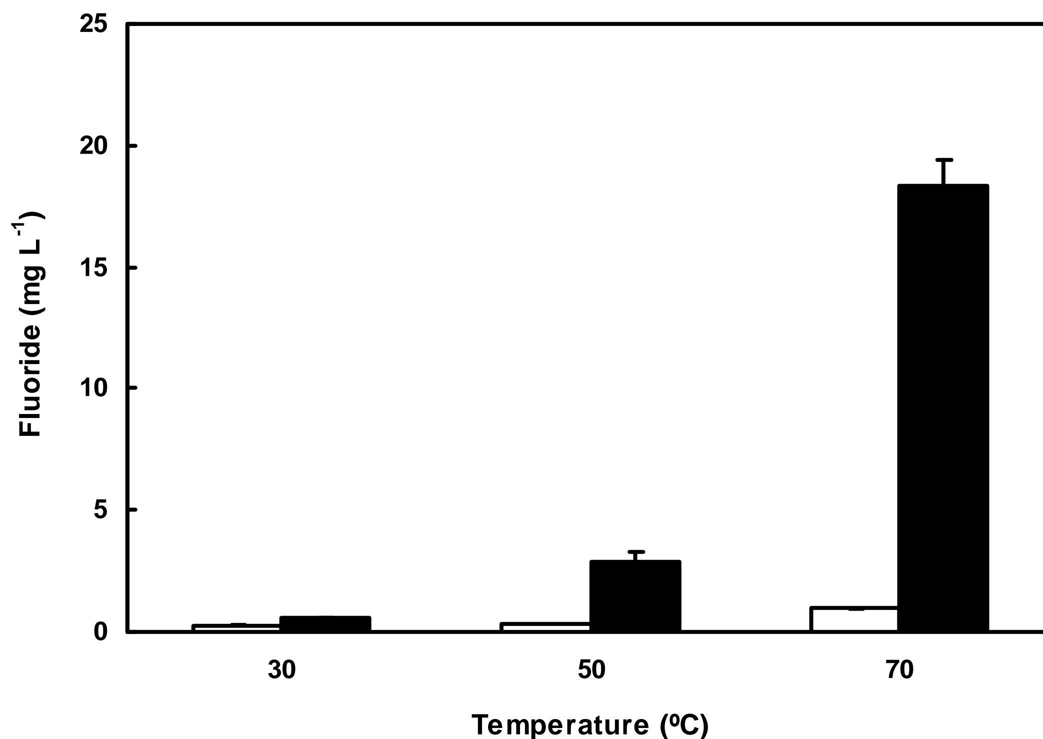


Figure 5.3 Effect of temperature on the biomimetic reductive dehalogenation of technical PFOS (332 µM) with vitamin B_{12} (260 µM) and Ti(III) citrate (36 mM) and pH 9.0 on day 36, 12 and 7 at 30, 50 and 70°C, respectively. Legend: control samples (PFOS + Ti(III) citrate) (white bars) and treatment samples (PFOS + Ti(III) citrate + vitamin B_{12}) (black bars). Error bars (shown if larger that the symbols) represent standard deviations of triplicate assays.

The rate constant of fluoride release during the reductive defluorination of PFOS measured at the three different temperature values was employed to calculate the activation energy (Ea) of the reaction based on Arrhenius equation. An experimental activation barrier of 105 kJ mol^{-1} was obtained as shown in Figure 5.4. This value is lower than the activation barriers calculated for the reaction of hydroxyl radicals with the PFOS molecule by means of DFT simulations reported by Carter *et al.* [16]. Activation energies ranging from 122 to 241 kJ mol^{-1} were obtained. These results suggest that the degradation of PFOS does not involve hydroxyl radical intermediates; instead it is related to the formation of carbon-center radicals of vitamin B_{12} as discussed later.

It is well established that reduced vitamin B_{12} (Co^{1+} cobalamin), one of the most powerful nucleophiles, is involved in the biomimetic dehalogenation of chlorinated compounds [20, 38]. Since vitamin B_{12} is reduced from Co^{3+} to Co^{1+} with Ti (III) citrate. The impact of vitamin B_{12} concentrations, Ti(III) citrate dosages and solution pH were assessed at 30°C and 70°C for comparison purposes.

Previous studies have reported vitamin B_{12}/halogenated molar ratios ranging from 0.02-0.05 for chloroethylenes that are highly susceptible to reductive dehalogenation to 1.5-2 molar ratios for recalcitrant hexachlorobenzene [21, 23, 39]. In this study, molar ratios varying from 0.07 to 3.2 equivalent to vitamin B_{12} concentrations in the range of 20 to 1000 µM were tested to find the best conditions as depicted in Figure 5.5. A vitamin B_{12} concentration of 260 µM (vitamin B_{12}/PFOS molar ratio of 0.8) was found to be the optimum catalyst concentration at low and high temperature values investigated in this

research. This result suggests that the rate of transformation of PFOS does not dependent on the concentration of vitamin B_{12}.

The concentrations of Ti(III) citrate dosages were also evaluated in this study as depicted in Figure 5.6. Increasing Ti(III) citrate concentrations while keeping the concentration of vitamin B_{12} constant had a positive effect in the rate of PFOS degradation. The concentrations were expressed as Ti(III)/vitamin B_{12} molar ratio. An increase of molar ratio from 11 to 110 lead to a 10-fold increase in the kinetics of PFOS degradation at 30°C, and a 6–fold increase at 70°C. As expected, raising the reaction pH had also a positive impact on the rate of PFOS defluorination at the temperature values evaluated in this study. Figure 5.7 illustrates the effect of the solution pH based on fluoride release measurements. An increase in pH from 6.5 to 9.0 translated into a 2.9 and 1.7–fold increase in the reaction rate at 30ºC and 70ºC, respectively. These results are in agreement with literature studies on the effect of pH and reducing agent dosages on the biomimetic dehalogenation of chlorinated compounds [26, 40-42]. Chiu *et al.* [41] reported that the rate of degradation of carbon tetrachloride strongly depends on the reducing power and concentrations of Ti(III). For instance, the yield of the degradation products increased by 1.7-fold when the solution pH increased from 7.3 to pH 10.3. Since the reducing power of titanium citrate (III) increases with pH values [43], complete reduction of vitamin B_{12} with Ti(III) citrate is achieved at alkaline pH. In the same terms, the slower rates of PFOS transformation at lower Ti(III) citrate concentrations are likely due to the incomplete reduction of vitamin B_{12} by Ti(III).

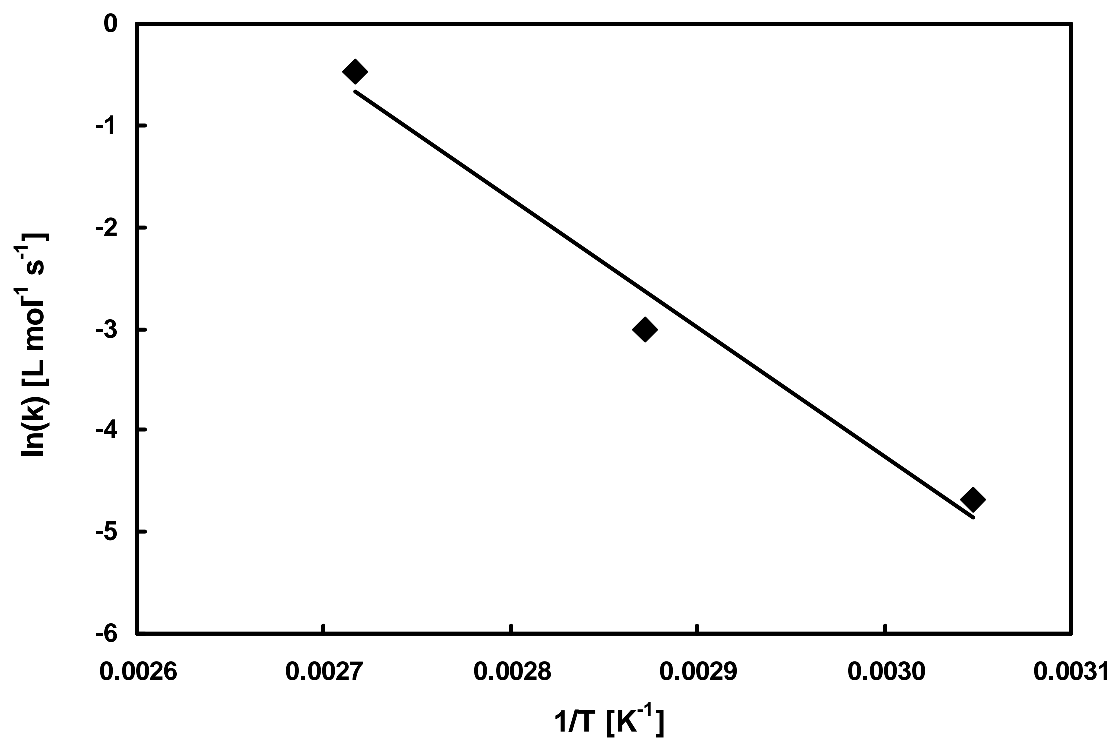


Figure 5.4 Arrhenius plot of the rate constant of fluoride release during the biomimetic reductive dehalogenation of technical PFOS (332 μM) with vitamin B_{12} and Ti(III) citrate (36 mM) at pH 9.0 at different temperatures.

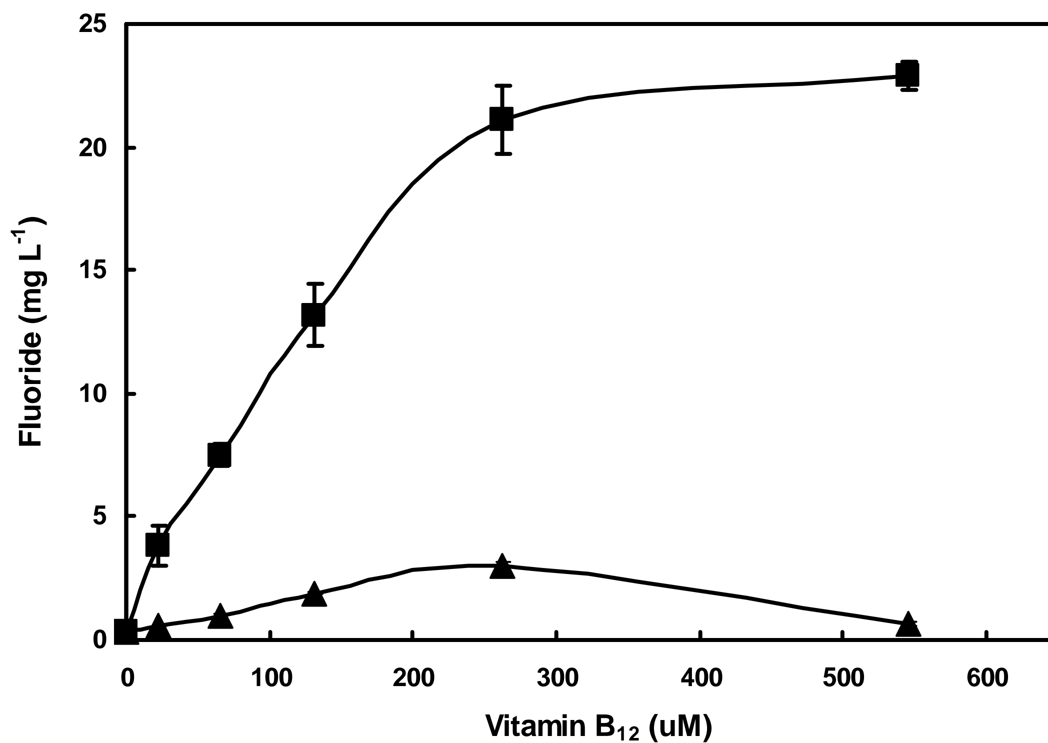


Figure 5.5 Effect of vitamin B_{12} concentration on the biomimetic reductive dehalogenation of technical PFOS (332 µM) with vitamin B_{12} and Ti(III) citrate (36 mM) at pH 9.0 in samples treated for 36 days at 30ºC (▲) and 7 days at 70ºC (■). Error bars (shown if larger that the symbols) represent standard deviations of triplicate assays.

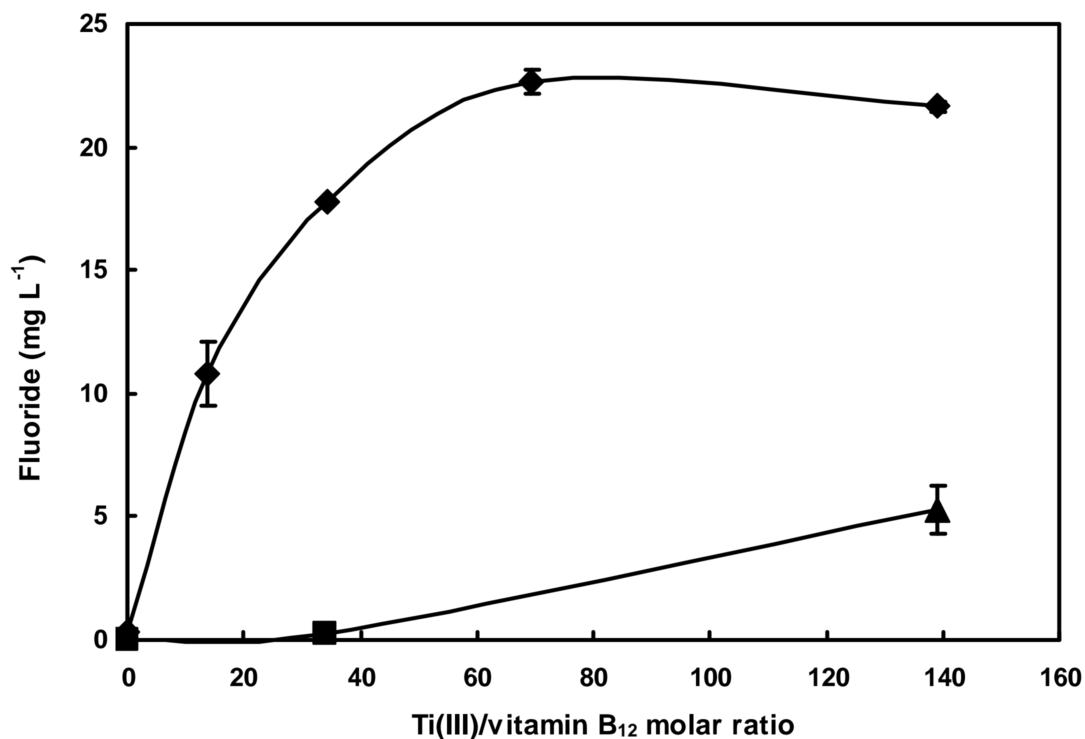


Figure 5.6 Effect of Ti(III) citrate dosages on the biomimetic reductive dehalogenation of technical PFOS (332 μM) with vitamin B_{12} (260 μM) and Ti(III) citrate at pH 9.0 in samples treated for 36 days at 30ºC (▲) and 7 days at 70ºC (■). Error bars (shown if larger that the symbols) represent standard deviations of triplicate assays.

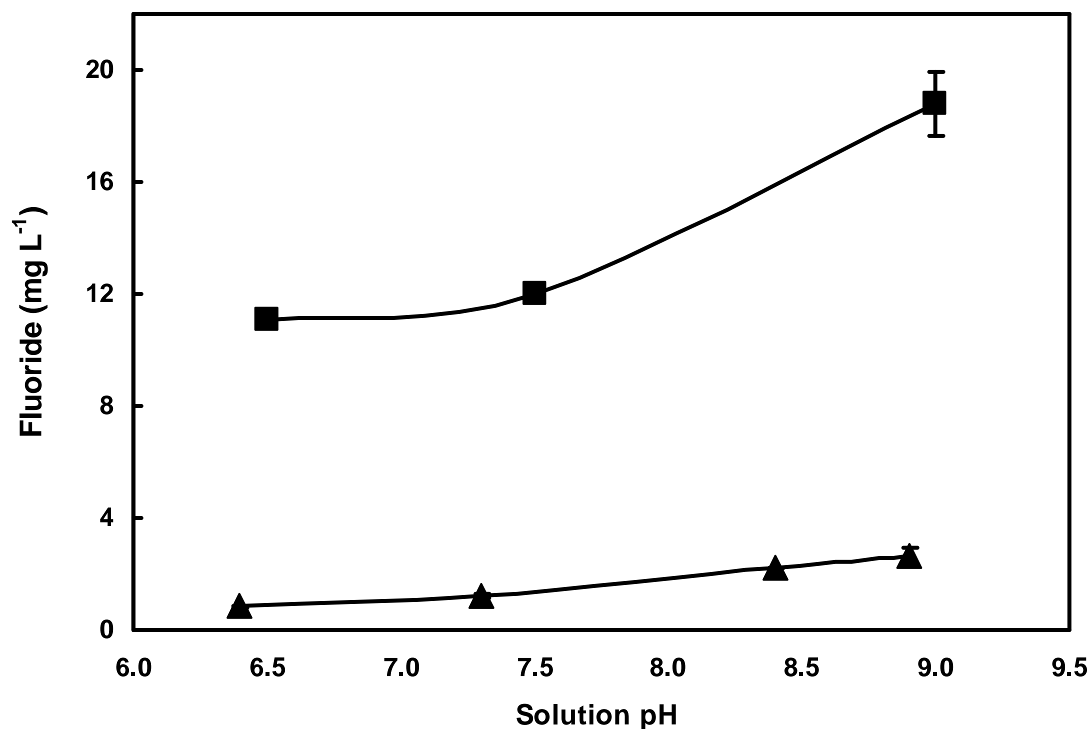


Figure 5.7 Effect of solution pH on the biomimetic reductive dehalogenation of technical PFOS (332 µM) with vitamin B_{12} (260 µM) and Ti(III) citrate (36 mM) in samples treated for 36 days at 30ºC (▲) and 7 days at 70ºC (■). Error bars (shown if larger that the symbols) represent standard deviations of triplicate assays.

Buffer solutions of carbonate (pH 9.0), Tris-HCl (pH 7.2) and borate (pH 8.2) at concentrations ranging from 70 to 85 mM were also studied at 70°C. As illustrated in Figure 5.8, the chemical degradation of PFOS in the presence of the carbonate buffer was 1.5-fold faster as compared to the degradation with tris-HCl and borate buffers. The former two buffers have been widely used in biomimetic studies of highly halogenated compounds at concentrations higher by one order of magnitude than those evaluated in this study [23, 36, 39]. In this report, the buffer concentration was carefully chosen to avoid precipitation of PFOS. The solubility of PFOS decreases from 500 mg L^{-1} in pure water to 12.4 mg L^{-1} in sea water [44]. Table 5.2 summarizes the optimal treatment conditions of those tested for the degradation of perfluorooctane sulfonate by the action of Ti(III) citrate and vitamin B_{12}.

Table 5.2 Optimal treatment conditions of those tested for the reductive dehalogenation of PFOS by Ti(III) citrate and vitamin B_{12}.

Reaction parameter	**Values**
Temperature	70°C
Solution pH	7
Vitamin B_{12}	260 uM
Ti(III)/PFOS molar ratio	110
Carbonate buffer	85 mM

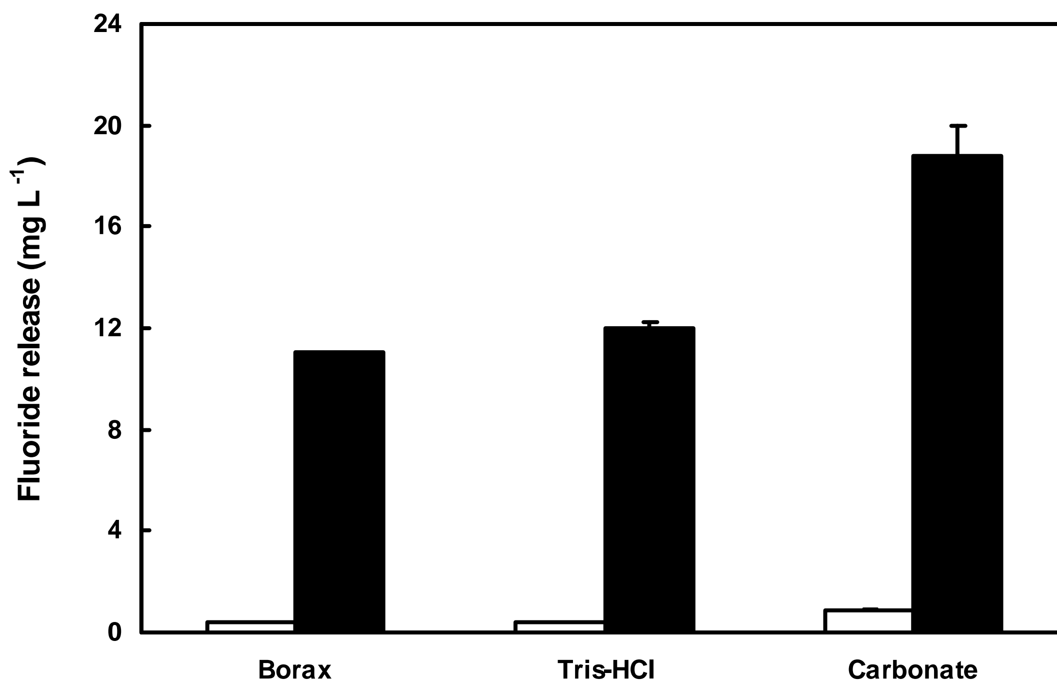


Figure 5.8 Effect of buffers on the biomimetic reductive dehalogenation of technical PFOS (332 μM) with vitamin B_{12} (260 μM) and Ti(III) citrate (36 mM) in samples treated for 7 days at 70ºC. Legend: control samples (PFOS + Ti(III) citrate) (white bars) and treatment samples (PFOS + Ti(III) citrate + vitamin B_{12}) (black bars). Error bars (shown if larger that the symbols) represent standard deviations of triplicate assays.

The effectiveness of vitamin B_{12} and Ti(III) citrate during the reduction of technical PFOS was evaluated under the best treatment conditions of temperature and solution pH, 70ºC and 9.0. Vitamin B_{12} is a cobalt-containing molecule, cobalt (II) concentrations up to 20-fold greater than those present in the assays amended with vitamin B_{12} were tested. As represented in Figure 5.9, no significant reduction of PFOS was observed in treatment samples containing cobalt (II) in lieu of vitamin B_{12}. In the case of the reducing agent, no PFOS degradation took place in the absence of Ti(III) citrate, or in controls with Ti(IV)- in lieu of Ti(III)-citrate. These results indeed suggest that both the biomolecule and the reducing agent play a key role in the dehalogenation of perfluorooctane sulfonate.

Immobilization of vitamin B_{12} on solid supports was also investigated to facilitate continuous treatment of technical PFOS. Granular activated carbon, GAC (F400, Calgon Carbon Corporation, PA) and faujasite zeolite (NaY80, Zeolyst International, PA) were added to the reaction mixture of the reductive dehalogenation of technical PFOS at 70ºC and solution pH 9.0 (data not shown). The addition of 0.1 g of zeolite did not affect the rate of PFOS transformation. On the other hand, in the presence of 0.1 g of GAC, the reaction slowed down by a factor of 1.5. If the decrease in kinetics of PFOS degradation could be attributed to the adsorption of the surfactant, similar behavior will be expected in the presence of NaY80 zeolyte. Recently, we reported that PFOS adsorbs moderately to GAC and NaY zeolite [45]. The reduction in the rate of transformation with GAC cannot be explained at this point and further studies should be conducted to understand this behavior.

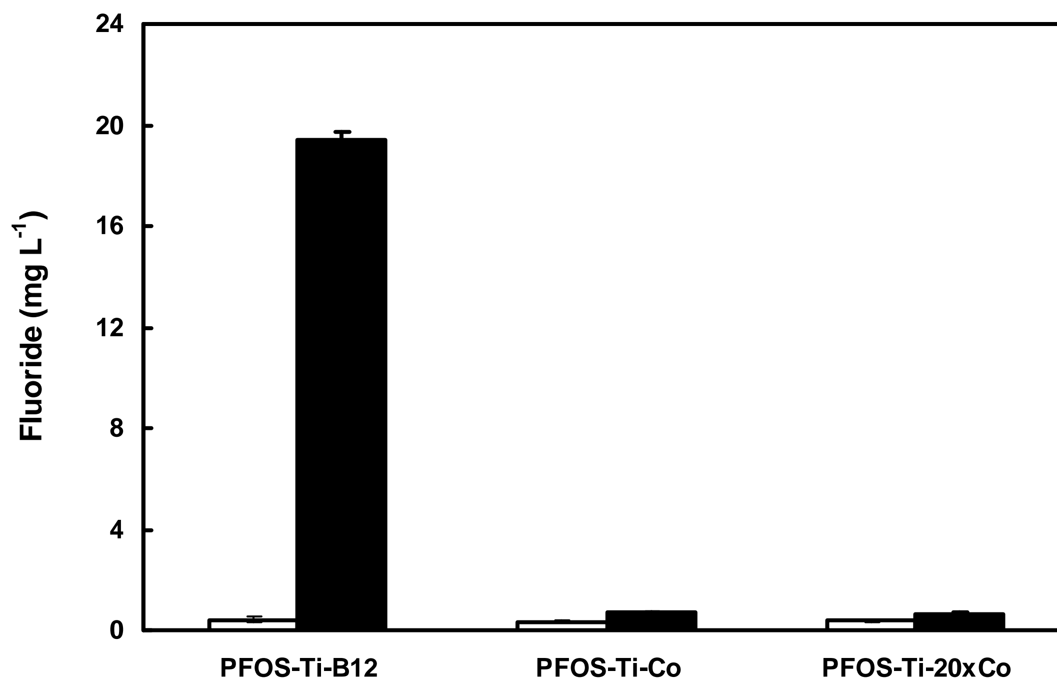


Figure 5.9 Effect of catalyst on the biomimetic reductive dehalogenation of technical PFOS (332 µM) with vitamin B_{12} (260 µM) and Ti(III) citrate (36 mM) at 70°C and pH 9.0 on day 7. The concentrations of cobalt (II) in treatment samples were 260 uM (PFOS-Ti-Co) and 5,236 uM (PFOS-Ti-20xCo). Legend: control samples (PFOS + Ti(III) citrate) (white bars) and treatment samples (PFOS + Ti(III) citrate + catalyst) (black bars). Error bars (shown if larger that the symbols) represent standard deviations of triplicate assays.

Figure 5.10 shows the time course of fluoride release of technical PFOS during the chemical reductive dehalogenation under optimal treatment conditions of those tested: vitamin B_{12} (260 μM) and Ti(III) citrate (36 mM) at 70°C and solution pH 9.0. After seven days, 18% PFOS defluorination was observed, equivalent to the removal of three fluorine atoms per mol of PFOS. ^{19}F NMR of the aqueous phase confirmed the presence of inorganic fluoride ions (Figure 5.11). The signal corresponding to fluoride ion appeared at –120.72 ppm. All chemical shifts were relative to the internal standard 4-TFMeAc (-58.08 ppm).

Monitoring the degradation of technical PFOS by suppressed conductivity detection, LC-MS/MS and ^{19}F NMR studies revealed that PFOS compounds differed in their susceptibility to reductive degradation by vitamin B_{12}/Ti(III) citrate. Chromatographic peaks corresponding to branched PFOS isomers disappeared whereas the peak corresponding to linear PFOS was stable. These findings suggest that branched PFOS isomers might behave different in the environment. In fact, branched isomers of perfluorooctanoic acid were reported to have faster elimination rates than the linear compound [46, 47]. In order to gain insights into the chemical and environmental behaviour of the branched PFOS isomers, degradation studies on these compounds were also conducted in the presence of vitamin B_{12} and Ti(III) citrate.

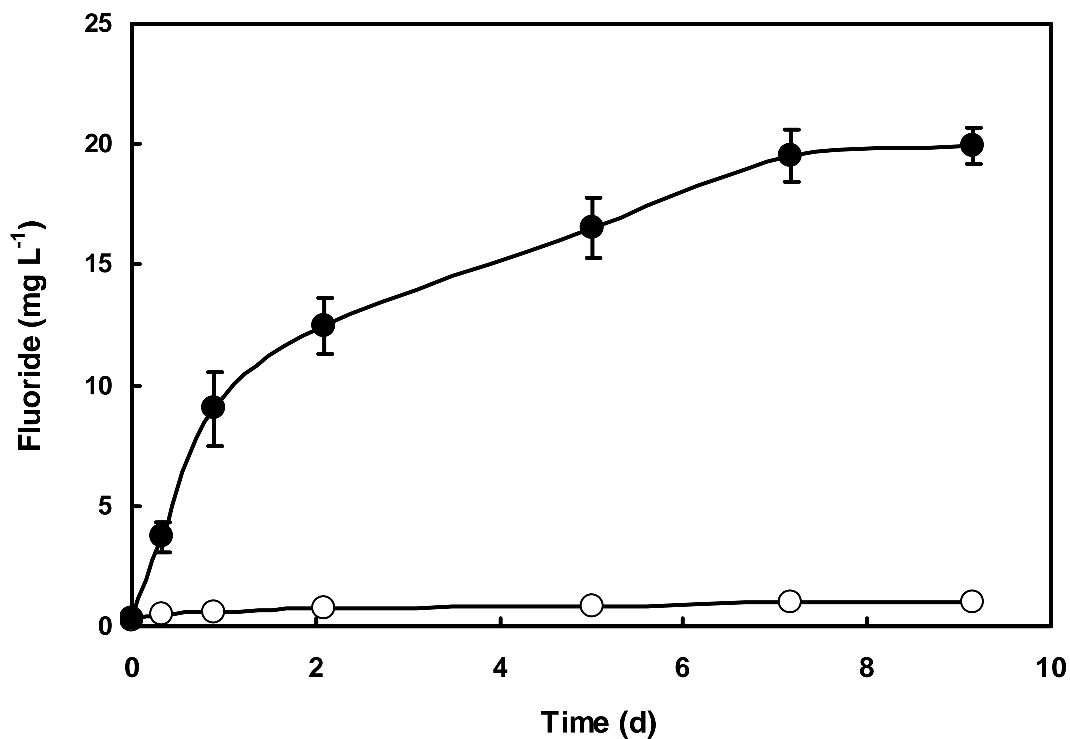


Figure 5.10 Time course of fluoride release of technical PFOS (332 µM) during the biomimetic reductive dehalogenation with vitamin B_{12} (260 µM) and Ti(III) citrate (36 mM) at 70ºC and solution pH 9.0 monitored by fluoride-selective electrode. Legend: control samples (PFOS + Ti(III) citrate) (○) and treatment samples (PFOS + Ti(III) citrate + vitamin B_{12}) (●). Error bars (shown if larger that the symbols) represent standard deviations of triplicate assays.

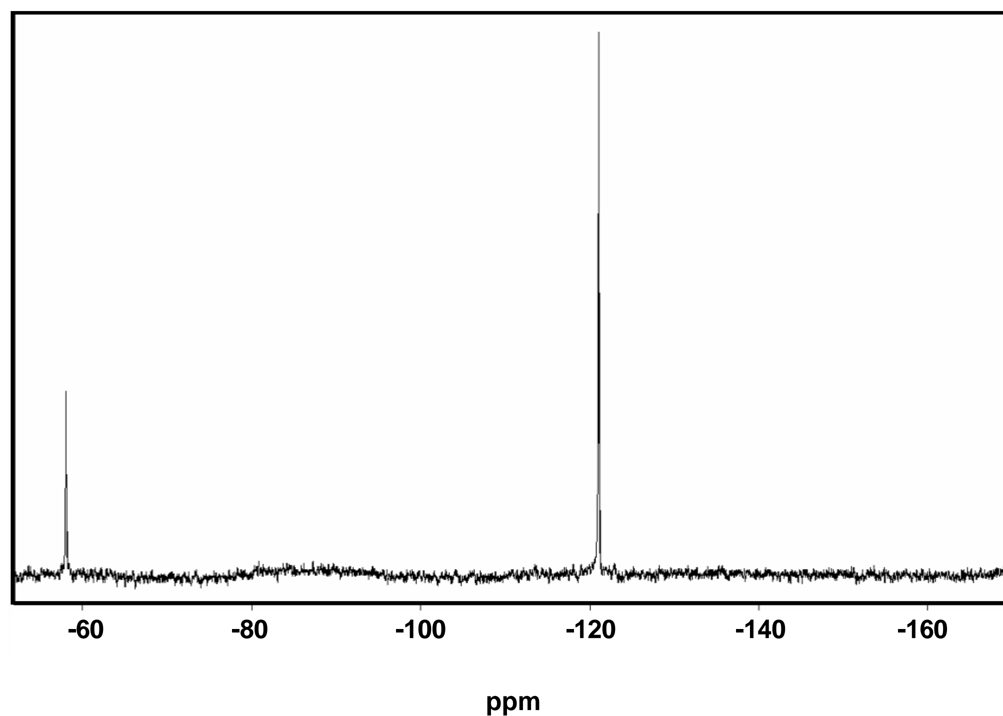


Figure 5.11 ^{19}F NMR of spectrum of the aqueous phase of technical PFOS (332 μM) after the biomimetic reductive dehalogenation with vitamin B_{12} (260 μM) and Ti(III) citrate (36 mM) at 70ºC and pH 9.0 in samples treated for 7 days. Chemical shifts are referenced to the internal standard, 4-TFMeAc, at -58.08 ppm [33].

The PFOS-based substances are commercially synthesized by electrochemical fluorination processes (EFC) or telomerization processes. In the electrochemical fluorination perfluoroalkyl isomers with linear and branched arrangements are formed, as well as homologues with different chain lengths [12]. In fact, technical PFOS used by industry is often a mixture of linear and branched structural isomers, with the latter making 20 to 30% of the total mass [48-50]. ^{19}F NMR and LC-MS/MS studies revealed that the PFOS material used in our study contained 24.6% branched isomers, consisting chiefly of the following: perfluoromonomethyl and perfluoroisopropyl isomers: 3-CF_3-PFOS, 4-CF_3-PFOS (peak I in LC-MS/MS trace, Figure 5.14), 5-CF_3-PFOS, 6-CF_3-PFOS (peak II) and 1-CF_3-PFOS (peak III).

Ab initio DFT calculations were performed to determine the relative stability of the various isomers as described in the *Materials and Methods* section. The relative stability of linear PFOS and its branched isomers was determined by comparing their Gibbs free energy, which was obtained by adding the zero point energy and the thermal effects to the electronic energy (Table 5.3). It was found that the linear structure is the most stable conformation of PFOS followed by 1-CF_3-PFOS and the 6-CF_3-PFOS with a difference in energy of ~0.3 and ~0.5 kcal/mol, respectively. The other branched isomers; namely, 3-CF_3-PFOS, 4-CF_3-PFOS and 5-CF_3-PFOS, resulted the less stable structures. Calculations established that the latter isomers have a similar Gibbs free energy, which differs from the most stable structure by about ~3 kcal/mol. These theoretical calculations are in agreement with experimental data obtained on the abundance of branched PFOS isomers in technical PFOS by LC-MS/MS studies (Table 5.1).

Table 5.3 Ab initio quantum mechanical calculations of the Gibbs free energy for the linear and branched isomers structures of PFOS. The differences in energy are referred to the most stable structure (*i.e.,* linear molecule). Values were obtained at standard conditions of temperature and pressure (298.15 K and 1 atm).

System	**G (H)**	**Δ (kcal mol^{-1})**
Linear	-2626.50612	0.00
6-CF_3-PFOS	-2626.50532	0.50
5-CF_3-PFOS	-2626.50085	3.31
4-CF_3-PFOS	-2626.50078	3.35
3-CF_3-PFOS	-2626.50125	3.05
2-CF_3-PFOS	-2626.50080	3.34
1-CF_3-PFOS	-2626.50558	0.34

The susceptibility of PFOS isomers to biomimetic reductive dehalogenation with vitamin B_{12} and Ti(III) citrate was also evaluated at the best operations conditions of temperature and solution pH, 70°C and 9.0, respectively. The branched PFOS isomeric fraction employed in the degradation studies was purified according to the protocol described in the *Materials and Methods* section. The dehalogenation of the branched PFOS isomers was confirmed by fluoride release measurements, ^{19}F NMR, HPLC-suppressed conductivity detection, and LC-MS/MS studies. Fluoride electrode measurements and ^{19}F NMR studies revealed that the release of fluoride was significantly higher in branched PFOS isomers as compared to that in technical PFOS (Figure 5.10). After five days, 71% of the initial fluorine was released, equivalent to the removal of 12 mol of fluorine atoms per mol of branched PFOS, compared to only 3 mol of fluorine atoms per mol of technical PFOS. Figure 5.12 shows the time course disappearance of branched PFOS isomers during chemical dehalogenation based on suppressed conductivity detection analysis.

The degradation of the branched PFOS isomers followed pseudo-first-order kinetics with a rate constant (K_1) of 0.0204 h^{-1} (Figure 5.13). Our results are in agreement with those reported for biomimetic dehalogenation of highly halogenated compounds [21-23]. For instance, the reductive dehalogenation of chlorinated ethylenes and polybrominated diphenyl ethers also followed pseudo-first order kinetics. However, the rate constant of PFOS is several orders of magnitude lower than the K_1 values commonly reported for easily degraded carbon tetrachloride and tetrachloroethylene [21, 27, 39]. Nonetheless, the observed rate of PFOS degradation is faster compared to other persistent

chlorinated pollutants. As an example, a K_1 value of 0.0017 h^{-1} has been reported for the reductive dehalogenation of *cis*-DCE by vitamin B_{12} [21]; and K_1 values of 0.0026 and 0.0001 h^{-1} were observed for hexachlorobenzene and polychlorinated benzene congener, respectively [23].

The LC-MS/MS chromatograms indicate removal of the isomers, 5- and 6-CF_3-PFOS by 80$\pm$1%, 3- and 4-CF_3-PFOS by 48$\pm$1%, and 1-CF_3-PFOS by 44$\pm$2 (Figure 5.14). Isomer degradation was confirmed by the nearly complete disappearance of the signal corresponding to the branched CF_3 group and other organic fluorine signatures distinctive of the branched PFOS structures in ^{19}F NMR spectra (Figure 5.15). These spectroscopic signals have been characterized and assigned for the individual branched PFOS isomers elsewhere [51].

Studies conducted on the identification and characterization the products of the reductive dehalogenation of PFOS with vitamin B_{12} and Ti(III) citrate will be discussed in Chapter 6.

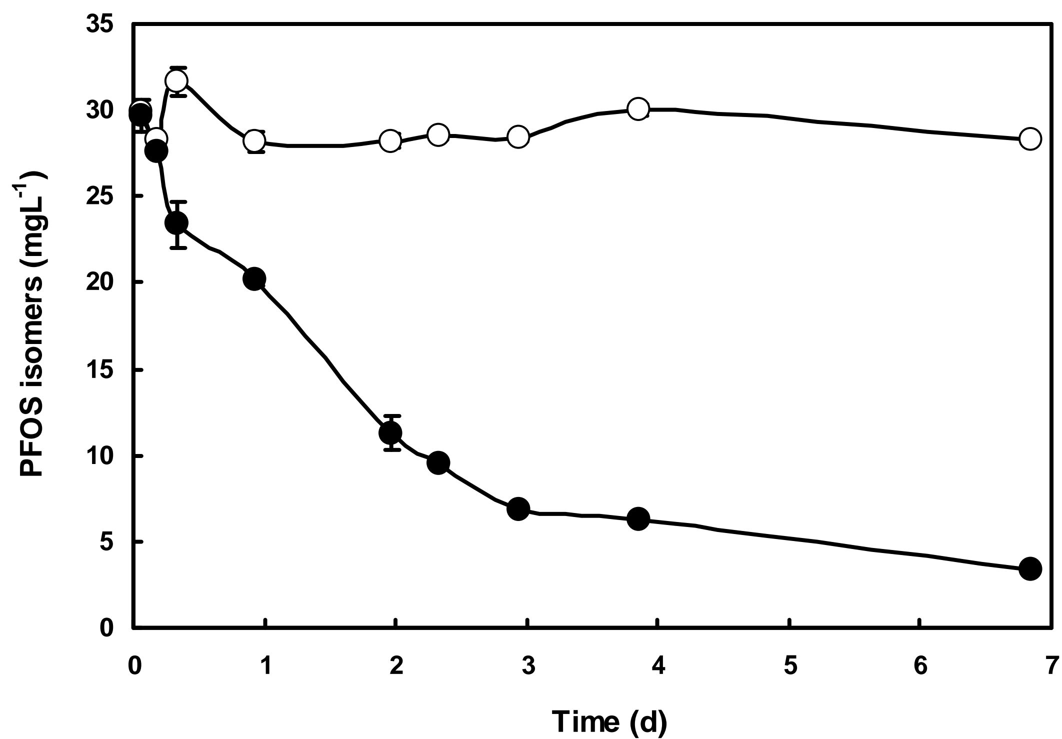


Figure 5.12 Time course disappearance of branched PFOS isomers (54 μm) in the chemical reductive dehalogenation with vitamin B_{12} (260 μM) and Ti(III) citrate (36 mM) at 70ºC and pH 9.0 based on HPLC-suppressed conductivity detection analysis. Control samples (PFOS + Ti(III) citrate) (○) and treatment samples (PFOS + Ti(III) citrate + vitamin B_{12}) (●). Error bars (shown if larger that the symbols) represent standard deviations of triplicate assays.

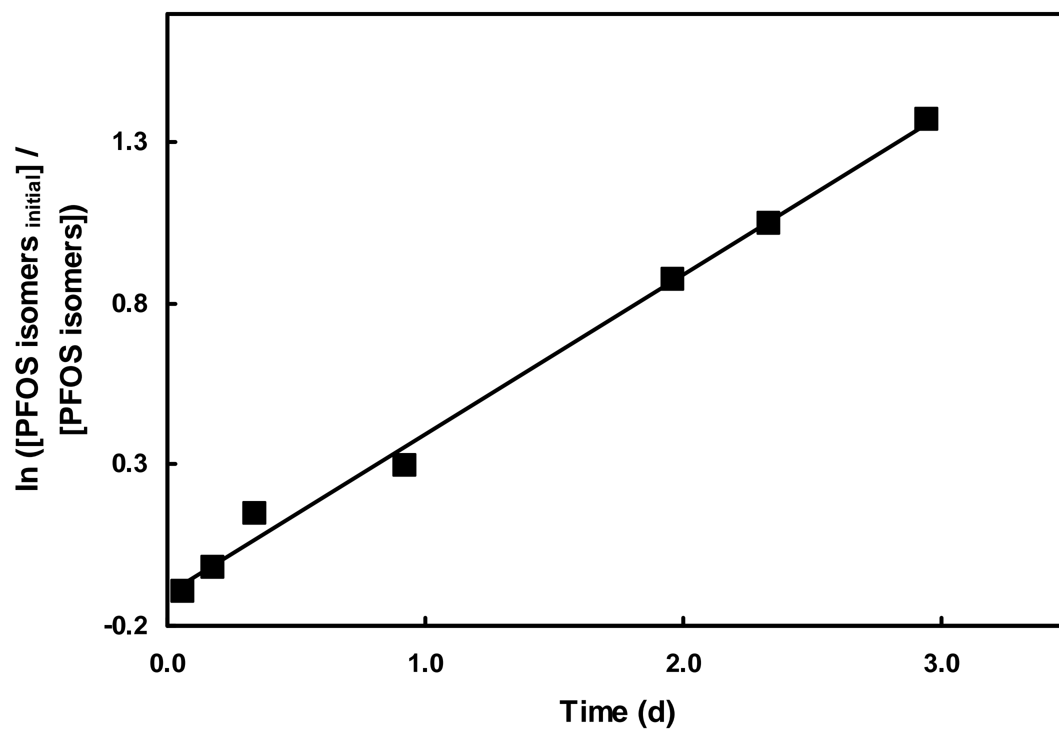


Figure 5.13 Pseudo-first order plot for the degradation of branched PFOS isomers (54 μM) with reaction time. Samples were incubated vitamin B_{12} (260 μM) and Ti(III) citrate (36 mM) at 70ºC and pH 9.0 for 7 days. Error bars (shown if larger that the symbols) represent standard deviations of triplicate assays.

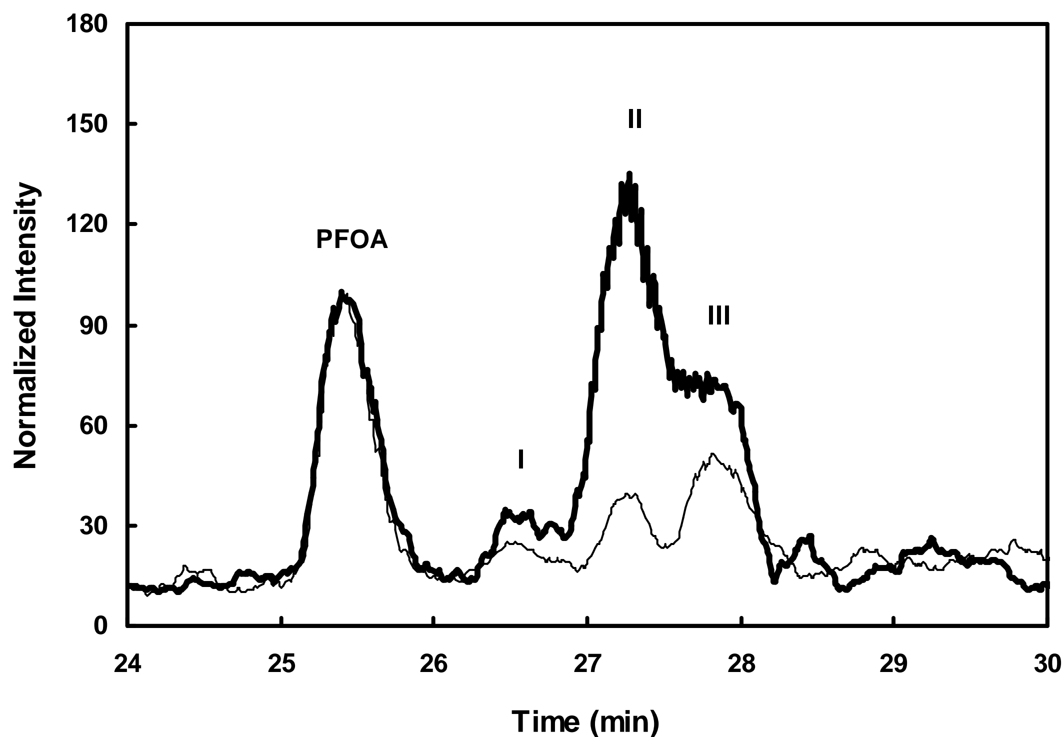


Figure 5.14 LC-MS/MS chromatograms of branched PFOS isomers in control samples (PFOS + Ti(III) citrate) (thick line) and treatment samples (PFOS + Ti(III) citrate + vitamin B_{12}) (thin line) during the biomimetic reductive dehalogenation of branched PFOS isomers (54 μM) with vitamin B_{12} (260 μM) and Ti(III) citrate (36 mM) at 70ºC and pH 9.0 on day 7. Samples were pre-concentrated by SPE procedures.

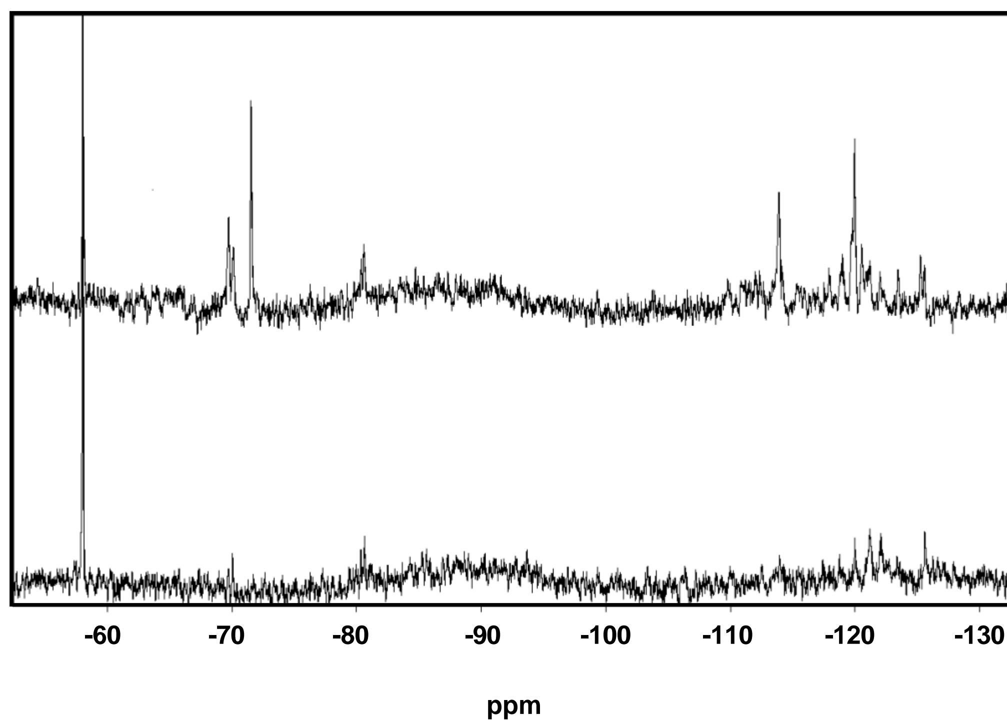


Figure 5.15 ^{19}F NMR spectra of branched PFOS isomers in control (upper panel) and treatment samples (lower panel) after the biomimetic reductive dehalogenation of branched PFOS isomers (54 μM) with vitamin B_{12} (260 μM) and Ti(III) citrate (36 mM) at 70ºC and pH 9.0 on day 7. Samples were pre-concentrated by SPE procedures. Chemical shifts are referenced to the internal standard, 4-TFMeAc, at -58.08 ppm [33].

As described previously, approaches involving cometabolic reductive dehalogenation are potentially promising for the degradation of perfluorinated surfactants. The reaction mechanism of vitamin B_{12}-catalyzed reductive dehalogenation is poorly understood. The most commonly accepted models hypothesize that the reductive attack of chlorinated compounds involves radical intermediates [20, 52]. The mechanisms of chemical and biological reactions involving radical intermediates have been extensively investigated by EPR spectroscopy [53]. In this study, EPR measurements were conducted to elucidate the mechanism of PFOS degradation. Figure 5.16 shows the EPR spectra of treatment and control samples following addition of the spin trap DMPO. As expected, no radical intermediates were formed in the control samples (Figure 5.16C). Radical adducts with DMPO were observed in sample containing Ti(III) citrate and vitamin B_{12} in the presence (Figure 16A) and absence of the perfluorinated surfactant (Figure 16B). These results clearly demonstrate the formation of a vitamin B_{12} carbon-center radical in the presence of a strong electron donor such as Ti(III) citrate. This vitamin B_{12} radical intermediate presumably initiates the reductive dehalogenation of PFOS. The existence of PFOS-radicals could not be confirmed due to concentration issues. The concentration of vitamin B_{12} in solution exceeded that of PFOS by a factor of 3; therefore, the signal of any PFOS radical will be suppressed by those of vitamin B_{12} radicals.

The enhanced susceptibility of branched PFOS isomers as compared to linear PFOS to reductive dehalogenation may be related to the stabilizing effect of branched structures on radical intermediates resulting from the reductive attack. Tertiary radicals

are widely known to be more stable than primary or secondary radicals [54]. Recently, Douvris *et al.* [55] reported that tertiary fluorocarbons were more susceptible to catalytic conversion as compared to aromatic fluorocarbons. Moreover, steric hindrance caused by $–CF_3$ groups decreases the strength of the C-C bond in branched perfluoroalkanes [56, 57]. So presumably the reaction mechanism of reductive dehalogenation of branched PFOS isomers involves the cleavage of the most substituted C–C bond followed by rearrangements of intermediate radicals. Additional research needs to be conducted to understand the reaction mechanism of the reductive dehalogenation of PFOS catalyzed by vitamin B_{12}/Ti(III) citrate.

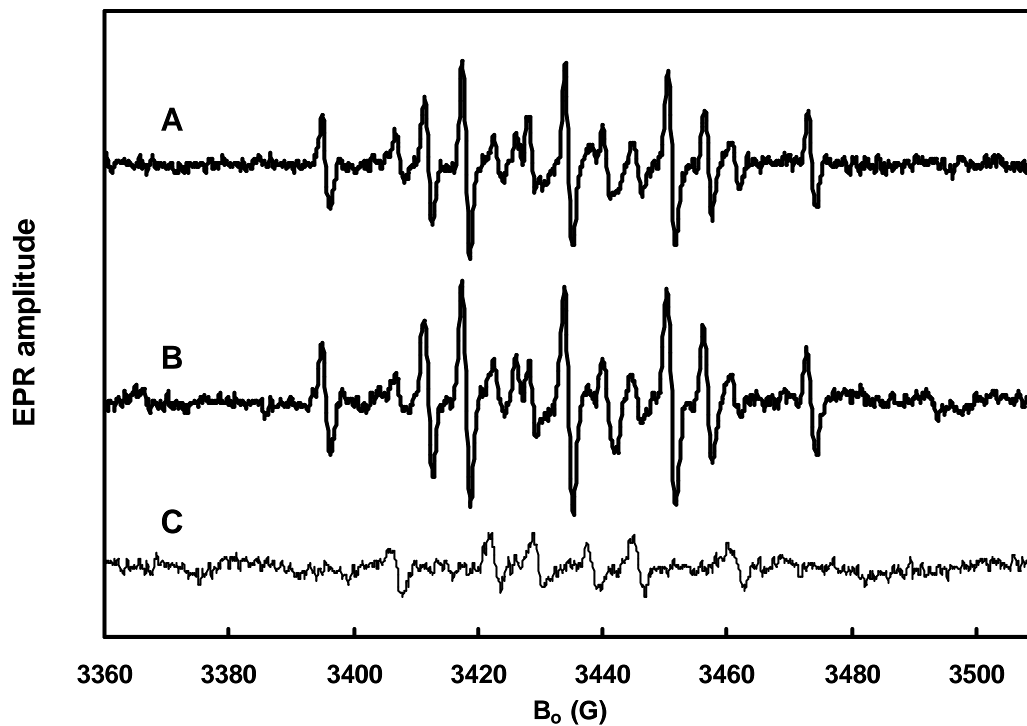


Figure 5.16 EPR spectra of the chemical reductive dehalogenation of technical PFOS (332 µM) with vitamin B_{12} (260 µM) and Ti(III) citrate (36 mM) at 70ºC and pH 9.0 on day 7 in the presence of the spin trap DMPO (27 mM). Legend: (A) PFOS + Ti(III) citrate + B_{12} + DMPO, (B) Ti(III) citrate + B_{12} + DMPO, and (C) PFOS + Ti(III) citrate + DMPO.

5.5 Conclusions

The biomimetic reductive dehalogenation of PFOS with vitamin B_{12} and Ti(III) citrate was successfully conducted in this study. The optimal treatment conditions of those tested were found to be 260 µM vitamin B_{12}, 36 mM Ti(III) citrate, 70°C and solution pH 9.0. To our knowledge this is the first report of reductive dehalogenation of PFOS catalyzed by a biomolecule. These results suggest that microbial transformation of some PFOS isomers might be possible in anaerobic environments. The reductive dehalogenation of PFOS catalyzed by vitamin B_{12} involves a radical intermediate; however the reaction mechanism needs to be elucidated. Furthermore, the observation that branched PFOS isomers are more prone to attack than linear PFOS provides clues for the design of more biodegradable perfluorinated chemicals.

5.6 Acknowledgments

This study was conducted with financial support from the Semiconductor Research Corporation/Sematech Engineering Research Center for Environmentally Benign Semiconductor Manufacturing. We are thankful to Dr. Javier Torres from the Department of Chemical and Environmental Engineering, University of Arizona, for his aid in ab initio quantum mechanical calculations.

5.7 References

1. Schultz, M. M.; Barofsky, D. F.; Field, J. A., Fluorinated alkyl surfactants. *Environ. Eng. Sci.* **2003,** *20*, (5), 487-501.
2. Kannan, K.; Corsolini, S.; Falandysz, J.; Fillmann, G.; Kumar, K. S.; Loganathan, B. G.; Mohd, M. A.; Olivero, J.; Van Wouwe, N.; Yang, J. H.; Aldous, K. M., Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. *Environ. Sci. Technol.* **2004,** *38*, (17), 4489-4495.
3. Giesy, J. P.; Kannan, K., Global distribution of perfluorooctane sulfonate in wildlife. *Environ. Sci. Technol.* **2001,** *35*, (7), 1339-1342.
4. Senthilkumar, K.; Ohi, E.; Sajwan, K.; Takasuga, T.; Kannan, K., Perfluorinated compounds in river water, river sediment, market fish, and wildlife samples from Japan. *Bull. Environ. Contam. Toxicol.* **2007,** *79*, (4), 427-431.
5. Houde, M.; Martin, J. W.; Letcher, R. J.; Solomon, K. R.; Muir, D. C. G., Biological monitoring of polyfluoroalkyl substances: A review. *Environ. Sci. Technol.* **2006,** *40*, (11), 3463-3473.
6. Kannan, K.; Koistinen, J.; Beckmen, K.; Evans, T.; Gorzelany, J. F.; Hansen, K. J.; Jones, P. D.; Helle, E.; Nyman, M.; Giesy, J. P., Accumulation of perfluorooctane sulfonate in marine mammals. *Environ. Sci. Technol.* **2001,** *35*, (8), 1593-1598.

7. Butenhoff, J. L.; Olsen, G. W.; Pfahles-Hutchens, A., The applicability of biomonitoring data for perfluorooctanesulfonate to the environmental public health continuum. *Environ. Health Perspect.* **2006,** *114*, (11), 1776-1782.

8. Martin, J. W.; Kannan, K.; Berger, U.; De Voogt, P.; Field, J.; Franklin, J.; Giesy, J. P.; Harner, T.; Muir, D. C. G.; Scott, B.; Kaiser, M.; Jarnberg, U.; Jones, K. C.; Mabury, S. A.; Schroeder, H.; Simcik, M.; Sottani, C.; Van Bavel, B.; Karrman, A.; Lindstrom, G.; Van Leeuwen, S., Analytical challenges hamper perfluoroalkyl research. *Environ. Sci. Technol.* **2004,** *38*, (13), 248A-255A.

9. Prevedouros, K.; Cousins, I. T.; Buck, R. C.; Korzeniowski, S. H., Sources, fate and transport of perfluorocarboxylates. *Environ. Sci. Technol.* **2006,** *40*, (1), 32-44.

10. Richardson, S. D., Water analysis: Emerging contaminants and current issues. *Anal. Chem.* **2007,** *79*, (12), 4295-4323.

11. Dimitrov, S.; Kamenska, V.; Walker, J. D.; Windle, W.; Purdy, R.; Lewis, M.; Mekenyan, O., Predicting the biodegradation products of perfluorinated chemicals using CATABOL. *Sar and Qsar in Environmental Research* **2004,** *15*, (1), 69-82.

12. Kissa, E., *Fluorinated Surfactants and Repellants*. Marcel Dekker, Inc.: New York, 2001.

13. Moriwaki, H.; Takagi, Y.; Tanaka, M.; Tsuruho, K.; Okitsu, K.; Maeda, Y., Sonochemical decomposition of perfluorooctane sulfonate and perfluorooctanoic acid. *Environ. Sci. Technol.* **2005,** *39*, (9), 3388-3392.

14. Hori, H.; Nagaoka, Y.; Yamamoto, A.; Sano, T.; Yamashita, N.; Taniyasu, S.; Kutsuna, S.; Osaka, I.; Arakawa, R., Efficient decomposition of environmentally persistent perfluorooctanesulfonate and related fluorochemicals using zerovalent iron in subcritical water. *Environ. Sci. Technol.* **2006,** *40*, (3), 1049-1054.

15. Yamamoto, T.; Noma, Y.; Sakai, S. I.; Shibata, Y., Photodegradation of perfluorooctane sulfonate by UV irradiation in water and alkaline 2-propanol. *Environ. Sci. Technol.* **2007,** *41*, (16), 5660-5665.

16. Carter, K. E.; Farrell, J., Oxidative destruction of perfluorooctane sulfonate using boron-doped diamond film electrodes. *Environ. Sci. Technol.* **2008,** *42*, (16), 6111-6115.

17. Smidt, H.; de Vos, W. M., Anaerobic microbial dehalogenation. *Annu. Rev. Microbiol.* **2004,** *58*, 43-73.

18. Bhatt, P.; Kumar, M. S.; Mudliar, S.; Chakrabarti, T., Biodegradation of chlorinated compounds - A review. *Crit. Rev. Environ. Sci. Technol.* **2007,** *37*, (2), 165-198.

19. Field, J. A.; Sierra-Alvarez, R., Biodegradability of chlorinated solvents and related chlorinated aliphatic compounds. *Reviews in Environmental Science & Bio/Techonology* **2004,** *3*.

20. Banerjee, R.; Ragsdale, S. W., The many faces of vitamin B-12: Catalysis by cobalamin-dependent enzymes. *Annu. Rev. Biochem.* **2003,** *72*, 209-247.

21. Gantzer, C. J.; Wackett, L. P., Reductive Dechlorination Catalyzed by Bacterial Transition-Metal Coenzymes. *Environ. Sci. Technol.* **1991,** *25*, (4), 715-722.

22. Tokarz, J. A.; Ahn, M. Y.; Leng, J.; Filley, T. R.; Nies, L., Reductive debromination of polybrominated diphenyl ethers in anaerobic sediment and a biomimetic system. *Environ. Sci. Technol.* **2008,** *42*, (4), 1157-1164.

23. Assafanid, N.; Nies, L.; Vogel, T. M., Reductive Dechlorination of a Polychlorinated Biphenyl Congener and Hexachlorobenzene by Vitamin-B12. *Appl. Environ. Microbiol.* **1992,** *58*, (3), 1057-1060.

24. Rodriguez-Garrido, B.; Arbestain, M. C.; Monterroso, M. C.; Macias, F., Reductive dechlorination of alpha-, beta-, delta-, and gamma-hexaxhlorocyclohexane isomers by hydrocobalamin in the presence of either dithiothreitol or titanium(III) citrate as reducing agents. *Environ. Sci. Technol.* **2004,** *38*, (19), 5046-5052.

25. Arguello, J. E.; Costentin, C.; Griveau, S.; Saveant, J. M., Role of protonation and of axial ligands in the reductive dechlorination of alkyl chlorides by vitamin B-12 complexes. Reductive cleavage of chloroacetonitrile by Co(I) cobalamins and cobinamides. *Journal of the American Chemical Society* **2005,** *127*, (14), 5049-5055.

26. Holliger, C.; Schraa, G.; Stupperich, E.; Stams, A. J. M.; Zehnder, A. J. B., Evidence for the Involvement of Corrinoids and Factor-F430 in the Reductive Dechlorination of 1,2-Dichloroethane by Methanosarcina-Barkeri. *J. Bacteriol.* **1992,** *174*, (13), 4427-4434.

27. Krone, U. E.; Thauer, R. K.; Hogenkamp, H. P. C.; Steinbach, K., Reductive Formation of Carbon-Monoxide from Ccl4 and Freon-11, Freon-12, and Freon-13 Catalyzed by Corrinoids. *Biochemistry* **1991,** *30*, (10), 2713-2719.

28. Lesage, S.; Brown, S.; Millar, K., Vitamin B-12-catalyzed dechlorination of perchloroethylene present as residual DNAPL. *Ground Water Monit. Remediat.* **1996,** *16*, (4), 76-85.

29. Sorel, D.; Lesage, S.; Brown, S.; Millar, K., Vitamin B-12 and reduced titanium for remediation of residual chlorinated solvents: Field experiment. *Ground Water Monit. Remediat.* **2001,** *21*, (4), 140-148.

30. Lesage, S.; Brown, S.; Millar, K.; Steer, H., Simulation of a ground water recirculation well with a dual-column laboratory setup. *Ground Water Monit. Remediat.* **2003,** *23*, (2), 102-110.

31. Langlois, I.; Oehme, M., Structural identification of isomers present in technical perfluorooctane sulfonate by tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2006,** *20*, (5), 844-850.

32. Moody, C. A.; Kwan, W. C.; Martin, J. W.; Muir, D. C. G.; Mabury, S. A., Determination of perfluorinated surfactants in surface water samples by two independent analytical techniques: Liquid chromatography/tandem mass spectrometry and F-19 NMR. *Anal. Chem.* **2001,** *73*, (10), 2200-2206.

33. Tugnait, M.; Lenz, E. M.; Phillips, P.; Hofmann, M.; Spraul, M.; Lindon, J. C.; Nicholson, J. K.; Wilson, I. D., The metabolism of 4-trifluoromethoxyaniline and [C-13]-4-trifluoromethoxyacetanilide in the rat: detection and identification of

metabolites excreted in the urine by NMR and HPLC-NMR. *J. Pharm. Biomed. Anal.* **2002,** *28*, (5), 875-885.

34. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramill, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Lui, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A., *Gaussian 03, Revision C.02*. Gaussian, Inc: Wallingford CT, 2004.

35. McQuarrie, D. A., *Statistical Mechanis*. University Science Books: Sausalito, CA, 2000.

36. Krone, U. E.; Thauer, R. K.; Hogenkamp, H. P. C., Reductive Dehalogenation of Chlorinated C1-Hydrocarbons Mediated by Corrinoids. *Biochemistry* **1989,** *28*, (11), 4908-4914.

37. Koons, B. W.; Baeseman, J. L.; Novak, P. J., Investigation of cell exudates active in carbon tetrachloride and chloroform degradation. *Biotechnol. Bioeng.* **2001,** *74*, (1), 12-17.

38. Liptak, M. D.; Brunold, T. C., Spectroscopic and computational studies of Co1+ cobalamin: Spectral and electronic properties of the "superreduced" B-12 cofactor. *Journal of the American Chemical Society* **2006,** *128*, (28), 9144-9156.

39. Burris, D. R.; Delcomyn, C. A.; Smith, M. H.; Roberts, A. L., Reductive dechlorination of tetrachloroethylene and trichloroethylene catalyzed by vitamin B-12 in homogeneous and heterogeneous systems. *Environ. Sci. Technol.* **1996,** *30*, (10), 3047-3052.

40. Assafanid, N.; Hayes, K. F.; Vogel, T. M., Reductive Dechlorination of Carbon-Tetrachloride by Cobalamin(Ii) in the Presence of Dithiothreitol - Mechanistic Study, Effect of Redox Potential and Ph. *Environ. Sci. Technol.* **1994,** *28*, (2), 246-252.

41. Chiu, P. C.; Reinhard, M., Metallocoenzyme-Mediated Reductive Transformation of Carbon-Tetrachloride in Titanium(Iii) Citrate Aqueous-Solution. *Environ. Sci. Technol.* **1995,** *29*, (3), 595-603.

42. Krone, U. E.; Laufer, K.; Thauer, R. K.; Hogenkamp, H. P. C., Coenzyme-F430 as a Possible Catalyst for the Reductive Dehalogenation of Chlorinated-C1

Hydrocarbons in Methanogenic Bacteria. *Biochemistry* **1989,** *28*, (26), 10061-10065.

43. Zehnder, A. J. B.; Wuhrmann, K., Titanium(Iii) Citrate as a Nontoxic Oxidation-Reduction Buffering System for Culture of Obligate Anaerobes. *Science* **1976,** *194*, (4270), 1165-1166.

44. Brooke, D.; Footitt, A.; Nwaogu, T. A., Environmental risk evaluation report: Perfluorooctanesulphonate (PFOS). *Environment Agency, Chemicals Assessment Section, Wallingford, UK* **2004**.

45. Ochoa-Herrera, V.; Sierra-Alvarez, R., Removal of prefluorinated surfactants by sorption onto granular activated carbon, zeolite and sludge. *Chemosphere* **2008,** *72*, 1588 - 1593.

46. Loveless, S. E.; Finlay, C.; Everds, N. E.; Frame, S. R.; Gillies, P. J.; O'Connor, J. C.; Powley, C. R.; Kennedy, G. L., Comparative responses of rats and mice exposed to linear/branched, linear, or branched ammonium perfluorooctanoate (APFO). *Toxicology* **2006,** *220*, (2-3), 203-217.

47. Conder, J. M.; Hoke, R. A.; De Wolf, W.; Russell, M. H.; Buck, R. C., Are PFCAs bioaccumulative? A critical review and comparison with regulatory lipophilic compounds. *Environ. Sci. Technol.* **2008,** *42*, (4), 995-1003.

48. *3M Company. Fluorochemical Isomer Distribution by 19F-NMR Spectroscopy*; U.S. Evironmental Protection Agency Public Docket AR226-0564. : 1997.

49. De Silva, A. O.; Mabury, S. A., Isolating isomers of perfluorocarboxylates in polar bears (Ursus maritimus) from two geographical locations. *Environ. Sci. Technol.* **2004,** *38*, (24), 6538-6545.

50. Vyas, S. M.; Kania-Korwel, I.; Lehmler, H. J., Differences in the isomer composition of perfluoroctanesulfonyl (PFOS) derivatives. *J. Environ. Sci. Health Part A-Toxic/Hazard. Subst. Environ. Eng.* **2007,** *42*, (3), 249-255.

51. Arsenault, G.; McCrindle, R.; Chittim, B.; McAlees, A., Separation and fluorine nuclear magnetic resonance spectroscopy (^{19}F-NMR) analysis of the individual branched isomers present in technical perfluorooctanesulfonic acid (PFOS). *Organohalogen Compounds* **2005,** *67*, 818 - 822.

52. Schmitz, R. P. H.; Wolf, J.; Habel, A.; Neumann, A.; Ploss, K.; Svatos, A.; Boland, W.; Diekertt, G., Evidence for a radical mechanism of the dechlorination of chlorinated propenes mediated by the tetrachloroethene reductive dehalogenase of Sulfurospirillum multivorans. *Environ. Sci. Technol.* **2007,** *41*, (21), 7370-7375.

53. Maillard, P.; Giannotti, C., Photolysis of Alkylcobaloximes, methyl-salen, cobalamines and coenzyme-B12 in protic solvents - ESR and spin-trapping technique study. *Journal of Organometallic Chemistry* **1979,** *182*, (2), 225-237.

54. McMurry, J., *Organic Chemistry*. 6th ed.; Brooks/Cole - Thomson Learning: USA, 2004.

55. Douvris, C.; Ozerov, O. V., Hydrodefluorination of perfluoroalkyl groups using silylium-carborane catalysts. *Science* **2008,** *321*, (5893), 1188-1190.

56. Giannetti, E., Semi-crystalline fluorinated polymers. *Polymer International* **2001,** *50*, (1), 10-26.

57. Sandford, G., Perfluoroalkanes. *Tetrahedron* **2003,** *59*, (4), 437-454.

CHAPTER 6

Characterization of Degradation Products from the Reductive Dehalogenation of Perfluorooctane Sulfonate (PFOS)

6.1 Abstract

Perfluorooctane sulfonate (PFOS) is an environmental contaminant of special public concern due to its global distribution, persistence and bioaccumulation properties. Recently, we reported for the first time the reductive dehalogenation of PFOS by reaction with vitamin B_{12} and Ti(III) citrate under anoxic conditions. Fluoride was the only degradation product detected from this reaction. The identification and characterization of other degradation products of the chemical reductive defluorination of PFOS were investigated in this study. Branched PFOS isomers employed in the degradation studies were concentrated and purified by means of SPE and preparative HPLC procedures. LC-MS/MS, HPLC-suppressed conductivity detection, solid and liquid ^{19}F NMR and GC/MS studies were conducted to detect PFOS-derivative metabolites and/or organic chemicals. Carbon dioxide was found to be the main degradation product of the reductive dehalogenation of PFOS. GC-MS measurements of the headspace of the reaction flasks demonstrated that 14.7% of total carbon present in the PFOS molecule was converted to

CO_2 after seven days of treatment. Interestingly, small amounts of volatile fluorinated compounds, representing less than 0.1% of the PFOS degraded, were also detected in the headspace. Those compounds included perfluorinated as well as partially fluorinated hydrocarbons. No PFOS degradation products were detected in the reaction solution with the techniques employed. LC-MS and ^{19}F NMR analysis of the insoluble material extracted with different organic solvents revealed only the presence of linear PFOS.

Key words: PFOS, branched isomers, reductive dehalogenation, degradation and products.

6.2 Introduction

Perfluorooctane sulfonate (PFOS) is an emerging contaminant that has been found in many environmental, human and wildlife samples worldwide. PFOS and related compounds have been in the market for more than 50 years in well-known industrial and consumer products such as the surface protector, Scotchgard® from 3M [1, 2]. Unfortunately, there is increasing evidence indicating that these chemicals are toxic, persistent and bioaccumulative [3-7].

In 2000, 3M, the major producer of PFOS in the US, announced the phase out of this chemical and derivatives from its production lines [8]. Following this announcement, the US Environmental Protection Agency (EPA) proposed a "significant new use rule" (SNUR) to limit and restrict the use of 88 PFOS-related compounds [1]. However, PFOS is currently used as a polymer additive in the photolithography operations of the semiconductor manufacturing [9].

Wastewaters are considered to be an important conduit for the introduction of PFOS in the environment [10-12]. High PFOS concentrations have been detected in effluents from manufacturing companies that utilize perfluorinated chemicals in various industrialized countries [13-18]. For instance, here in the U.S., PFOS concentrations as high as 1650 mg L^{-1} have been reported in wastewaters from semiconductor manufacturing [15]. Hence, feasible methods for the removal of PFOS and derivatives from industrial effluents are needed to minimize the environmental release of these pollutants. Literature studies on the chemical degradation of PFOS have been reported in

the last three years. These strategies include degradation of PFOS by sonochemical irradiation [19], oxidation in subcritical water [20] and at boron-doped diamond film electrodes [21], and photochemical degradation [22]. Recently, we have also demonstrated that PFOS can be defluorinated by biomimetic reduction with vitamin B_{12} as catalyst and Ti(III)-citrate as bulk reductant in anoxic aqueous solution at 70°C and pH 9.0 [23].

The products of the chemical degradation of PFOS vary depending on the treatment technique employed. Moriwaki and coworkers [19] reported that sonochemical decomposition of PFOS resulted in PFOA and short-chain perfluorinated carboxylic compounds. In the decomposition of PFOS using zero-valent iron in sub-critical water examined by Hori *et al.* [20], the authors suggested that PFOS adsorbed on the iron surface was thermally decomposed, and F^- ion was slowly released. No organic degradation products were detected. In the same manner, no degradation products were identified in the oxidation of PFOS using boron-doped diamond electrodes reported by Carter *et al.* [21]. The authors claimed that PFOS underwent oxidation, however, no evidence of organic oxidation products such as carbon dioxide was provided. The photodegradation of PFOS by UV irradiation in water and alkaline 2-propanol solution studied by Yamamoto and coworkers [22] resulted into two major PFOS degradation compounds, C_7HF_{15} and $C_7F_{15}OH$. Formation of short-chain fluorocarbons including, CF_4, C_2F_6 and C_3F_8, was also observed. We reported that PFOS is efficiently decomposed to F^- by means of chemical reduction involving Ti(III) and vitamin B_{12}. In the latter

study, no evidence of degradation products or PFOS-derivatives in the reaction mixture was obtained by LC-MS and ^{19}F NMR analysis [23].

The goal of this study is to identify the degradation products other than fluoride of the biomimetic reductive defluorination of branched PFOS isomers. The reaction solution, the soluble/colloidal materials and the headspace of the chemical degradation was evaluated to assess the presence of organic compounds and/or partially fluorinated PFOS-derivates. The feasibility of isolating branched PFOS isomers via concentration by solid phase extraction (SPE) procedures and purification by preparative HPLC was also investigated.

6.3 Materials and Methods

6.3.1 Chemicals

Perfluorooctane sulfonic acid potassium salt, PFOS (98% purity), linear perfluorooctane sulfonic acid potassium salt, L-PFOS (99%) and 1H-perfluorohexane (99%) were purchased from SynQuest Laboratories (Alachua, FL). Titanium(III) chloride (15% solution in HCl), vitamin B_{12} (99.0%), chromium acetylacetonate, $Cr(acac)_3$ (97%), sodium fluoride (99%), and sodium hydroxide solution (50-52% in H_2O) were supplied by Sigma-Aldrich (St. Louis, MO). CO_2 (> 99.99%) was obtained from Scotty Specialty Gases (San Bernardino, CA). Methanol-D4 (99%) was purchased from Cambridge Isotope Laboratories (Andover, MA). 4'-(trifluoromethoxy)-acetanilide, 4-TFMeAc (97%) was obtained from Matrix Scientific (Columbia, SC). HPLC-grade acetonitrile, methanol, sulfuric acid, and boric acid (99.5%) were purchased from Mallinckrodt Chemicals (Phillispsburg, NJ). Purified branched PFOS isomers were employed in the reductive dehalogenation experiments. Purification procedures are described in the next sections. All other chemicals were used as received.

6.3.2 Purification of PFOS isomers and linear PFOS

Branched PFOS isomers have a higher aqueous solubility that the linear PFOS isomer [24]. A solution containing technical PFOS (0.5 mM) (75% linear and 25% isomer compounds) and carbonate buffer (85 mM) was centrifuged (10,000 rpm, 10 min). The supernatant consisting of branched PFOS isomers and traces of linear PFOS was concentrated by solid phase extraction (SPE) as described below and then subjected to preparative HPLC.

6.3.3 Clean-up and concentration of samples

SPE cartridges (3 mL, 500 mg ODS-C_{18}, Agilent Technologies, DE) mounted on a vacuum manifold were conditioned with 6 mL MeOH, followed by 6 mL of deionized water. The desired volume of sample was loaded at 1 mL min^{-1}. SPE cartridges were rinsed with 4 mL of deionized water and then centrifuged (10,000 rpm, 10 min) prior elution. Analytes were eluted with 4 mL methanol and collected in clean Nalgene flasks. Solutions were spiked with known concentrations of PFOS (100 mg L^{-1}) to determine recovery efficiencies. Recovery efficiencies of 103.0 $\pm$ 8.2 % for triplicate samples were obtained.

6.3.4 Purification of PFOS isomers by preparative HPLC

PFOS isomers concentrated by SPE procedures were further purified by preparative high performance liquid chromatography (HPLC). Chromatographic separation was conducted on a home-made ODS-C_{18} column (Agilent Technologies, DE) packed under vacuum (1 cm i.d. and 25 cm length). The mobile phase consisted of a methanol-water solution with a concentration gradient varying from 55 to 75% (v/v). Fractions were collected every 2 min and, subsequently, they were analyzed by HPLC-suppressed conductivity detection and LC-MS/MS.

6.3.5 Reductive defluorination

Reductive defluorination of PFOS was conducted following the same procedure described in our previous publication with minor modifications [23]. Briefly, purified branched PFOS isomers (343 µM) were reductively dehalogenated under optimized treatment conditions, vitamin B_{12} (260 µM), Ti(III)-citrate (36 mM), solution pH 9.0 and temperature of 70ºC. A carbonate buffer (85 mM) and/or phosphate buffer (20 mM) were employed to assess the degradation products of the chemical reduction. Ti(III)-citrate solutions as well as Ti(III) solutions ($TiCl_3$) were employed in this study. The pH of these solutions was adjusted to 9.0 with a high purity NaOH solution. All experiments were set up in triplicate in an anaerobic box, utilizing 37 mL serum bottles sealed with viton

stoppers and aluminum crimp caps and wrapped with aluminum foil. Several control flasks lacking either vitamin B_{12} and/or Ti(III) were run in parallel. The headspace was then flushed with helium to maintain anaerobic conditions.

Standards and samples were analyzed by VWR SympHony fluoride-selective combination electrode using a background matrix of TISAB (Total Ionic Strength Adjustment Buffer), HPLC-suppressed conductivity detection, ^{19}F NMR, LC-MS/MS and GC-MS.

6.3.6 ^{19}F NMR

Analysis of branched PFOS isomers and degradation products of reductive dehalogenation by ^{19}F NMR was performed as described elsewhere [23]. Briefly, samples were dissolved in 90% H_2O/10% CD_3OD containing chromium acetylacetonate ($Cr(acac)_3$) (4 mg mL^{-1}) as the relaxation agent and the internal standard, 4'-(trifluoromethoxy)-acetanilide (4-TFMeAc, 140 mg L^{-1}). ^{19}F NMR spectra were acquired at 22°C on a Varian Unity-300 spectrometer 4-nucleus (^{31}P, ^{13}C, ^{19}F, ^{1}H) probe. The chemical shifts were referenced to the internal standard at -58.08 ppm [25].

6.3.7 HPLC-suppressed conductivity detection

A high performance liquid chromatography (HPLC) system with suppressed conductivity detector (ICS-3000 ion chromatography system, DIONEX, Sunnyvale, CA) was utilized to detect and quantify PFOS compounds. The system consisted of an autosampler (injection volume 100 μL), a pump, a degasser, a guard column and (Acclaim Polar Advantage II, C_{18}, 4.3 mm i.d., 1 cm length) and a separation column (Acclaim Polar Advantage II, C_{18}, 4.6 mm i.d., 25 cm length) operating at 35ºC. The mobile phase consisted of boric acid (20 mM, pH 8.0) and acetonitrile (95%) at a flow rate of 1 mL min^{-1}. The ratio of boric acid to acetonitrile varied with linear gradient program 0 min: 75:25 to 13.2 min: 45:55 (v/v). The detection limit of branched PFOS isomers was 1 mg L^{-1} based on a signal-to-noise ratio of 3. The analytical sensitivity of branched PFOS isomers was assumed to be the same for the different PFOS isomers. Arsenault and coworkers [26] recently demonstrated that the response factor of branched PFOS isomers was equivalent to that of the linear compound.

6.3.8 LC-MS/MS

Liquid chromatography couple to tandem mass spectrometry (LC-MS/MS) analysis of PFOS isomers was conducted by a method adapted from Arsenault *et al.* [26]. LC-MS/MS measurements were performed on a Waters Acquity Ultra Performance Liquid

Chromatography (UPLC) Quattro Premier XE tandem mass spectrometer (Waters, Milford, MA). Chromatographic separation was conducted on an Acquity UPLC BEH based on bridged ethylsiloxane/silica hybrid (BEH) particles - C_{18} column (1.7 µm, 2.1 x 50 mm) (Waters, Milford, MA). The mobile phase consisted of 10 mM ammonium acetate buffer (A) and methanol (B). A gradient program with 47% B for 6 minutes followed by an increase to 49% B in 23 minutes was used to elute PFOS isomers at a flow rate of 0.35 mL min^{-1}, column temperature of 36ºC, and injection volume of 5 µL. PFOS was detected using negative mode electrospray ionization with a capillary voltage of 2.6 kV, a cone voltage of 60 V, and a N_2 gas flow of 650 L hr^{-1}. Selective Ion Monitoring (SIM) at m/z 499 was employed to elute different PFOS isomers, and a daughter ion scan experiment (MS/MS) was used to observe fragmentation. Linear calibration curves ($r^2 > 0.99$) using known concentrations of linear PFOS standard varying from 0.5 to 500 ug L^{-1} were employed for quantification purposes. The detection limit of linear PFOS ranged between 0.1 to 0.5 µg L^{-1}.

6.3.9 GC-MS

The presence of volatile fluorinated metabolites and/or carbon dioxide in the gas phase was assessed using a Micromass gas chromatogram time of flight mass spectrometer (GC-TOF/MS) (*Micromass* Ltd., Manchester, UK). The system consisted of a gas chromatogram (Agilent 6890N) with a DB-1MS column (0.25mm i.d., 30 m length, J&W

Scientific, Folsom, CA) and a time of flight (TOF) mass spectrometer. Helium was used as the carrier gas and the electron impact (EI) source was operated at 70eV. The initial column temperature was 40ºC for 5 minutes followed by a temperature ramp of 15ºC min^{-1} for 10 minutes. The injection volume applied by an airtight syringe varied from 5 to 100 µL depending on the target gas phase compound. MS spectra were recorded within a mass range of m/z 20–800 using a scan time of 0.5 s. Selective Ion Monitoring (SIM) for ions m/z 44, 51, 69 and 131 was utilized for identification and quantification purposes. Linear calibration curves ($r^2 > 0.98$) using known CO_2 concentrations ranging from 0.2 to 2.0% (v/v) were employed to quantify the amount of CO_2 in the gas phase. The detection limit of CO_2 was 0.1%. Liquid standards of 1H-perfluorohexane in the range of 39 to 190 mg L^{-1} were prepared and allowed to reach equilibrium for 3 hours prior GC-MS analysis of the headspace. The amount of volatile fluorinated compounds measure in the gas phase was related to that present in the liquid phase by employing linear calibration curves ($r^2 > 0.99$). Since the Henry's constant for 1H-perfluorohexane is not available, the amount of the standard calculated only represents the concentration dissolved in the liquid phase. Because the compounds were present in concentration relatively low, samples of the gas phase were analyzed by injecting a volume 5-fold higher than that of carbon dioxide standards and 10-fold higher than of 1H-perfluorohexane.

6.4 Results and Discussion

6.4.1 Purification of branched PFOS isomers

Electrochemical fluorination (EFC) is the main synthesis process of the production of fluorinated alkyl compounds. In this process, HF is used to replace all C-H bonds by C-F bonds. EFC manufacturing yields approximately 70% linear carbon chains and 30% branched derivate organic compounds [27, 28]. The PFOS salt employed in this study contains 24.6% of branched PFOS isomers based on ^{19}F NMR and LC-MS/MS studies [23]. The chemical structures of the isomers present in the technical salt employed in our study are depicted in Figure 6.1. Interestingly, these branched isomers are more prone to reductive dehalogenation as compared to the linear compound [23]. Because purified PFOS isomers employed in this study are not commercially available, the compounds were purified by SPE procedures and preparative HPLC as described in the *Materials and Methods* section.

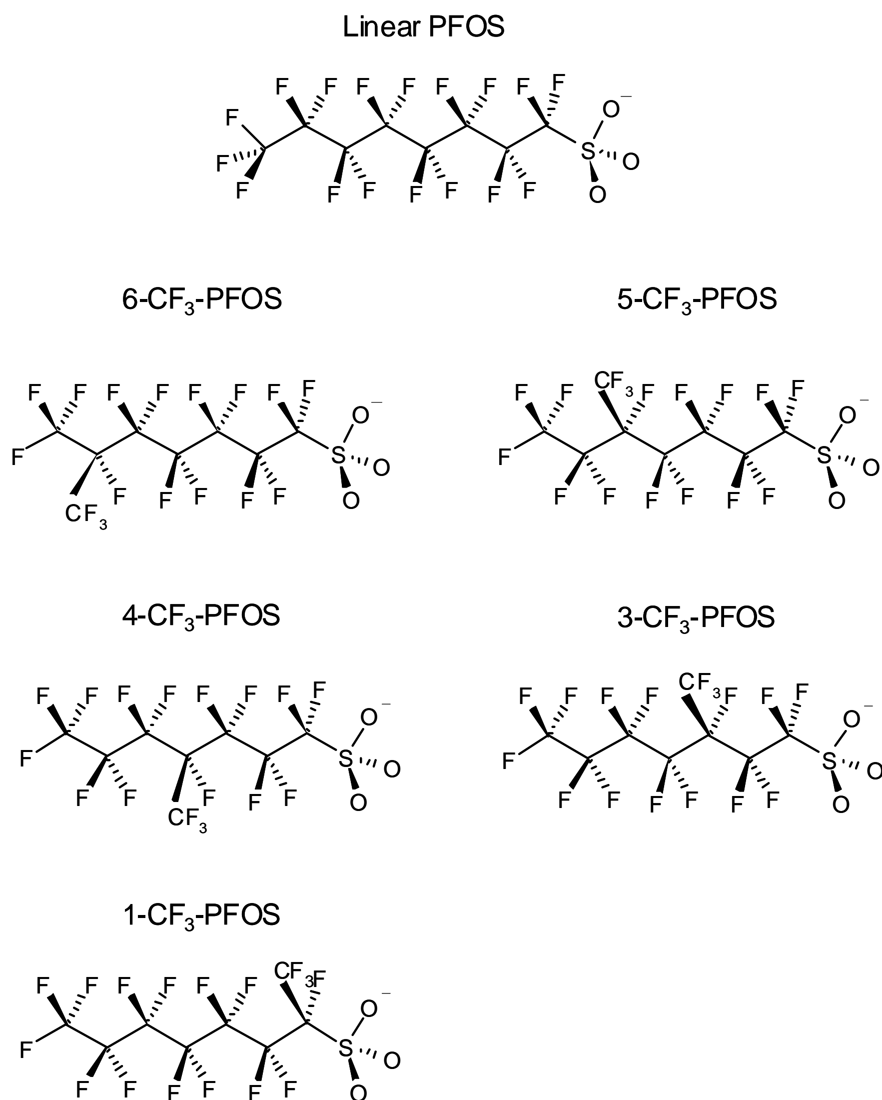


Figure 6.1 Chemical structure of linear PFOS and branched isomers, perfluoroisopropyl (6-CF_3-PFOS), 5-perfluoromethyl (5-CF_3-PFOS), 4-perfluoromethyl (4-CF_3-PFOS), 3-perfluoromethyl (3-CF_3-PFOS) and 1-perfluoromethyl (1-CF_3-PFOS).

Preparative HPLC is one the most powerful and versatile methods for compound purification [29]. In this study a home-made C_{18} column was employed to purified branched PFOS isomers following SPE concentration. Figure 6.2 illustrates the preparative HPLC-suppressed conductivity chromatogram of PFOS compounds as a function of the fraction number. The methanol gradient is also depicted in the same figure. The fractioning revealed that seven PFOS isomers are present in the technical PFOS salt. In order to identify and characterize these isomer compounds, two purified fractions were analyzed by LC-MS/MS and SIM based on the PFOS anion (m/z 499). Fraction A corresponds to fraction number 52, and fraction B corresponds to fractions number 55 to 66.

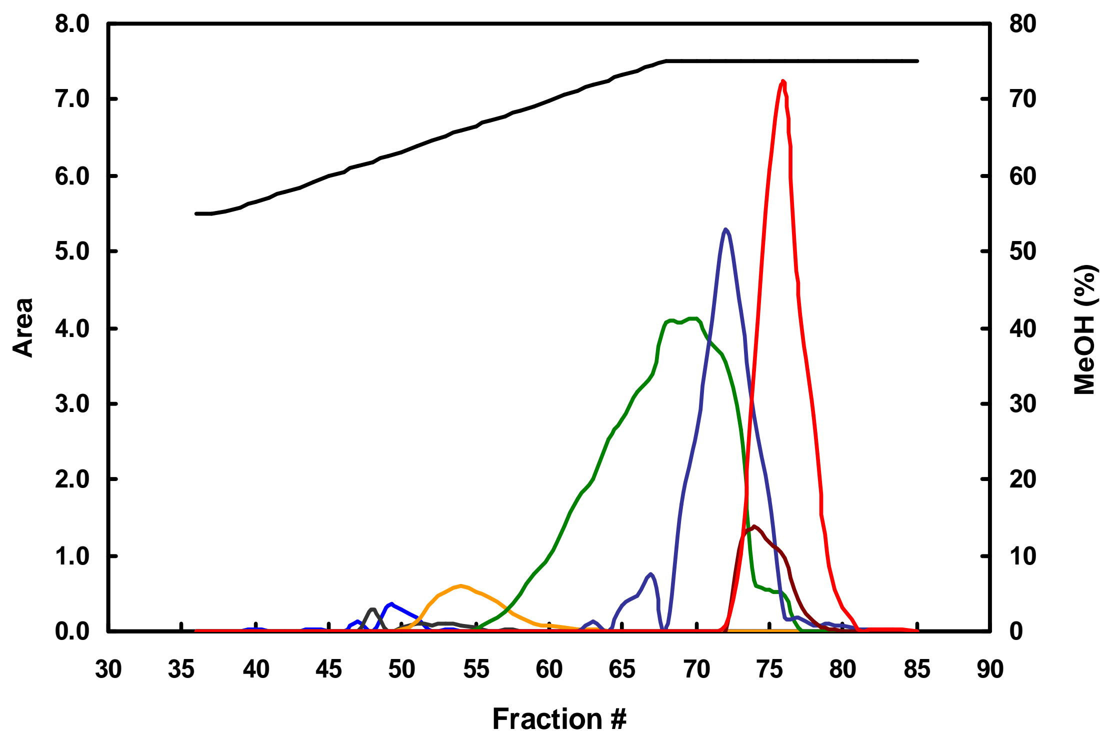


Figure 6.2 HPLC-suppressed conductivity chromatogram of PFOS isomers concentrated by SPE during the preparative HPLC fractionation. Legend: Branched *f* (blue), branched *s* (black), branched *0* (orange), branched 1 (green), branched 2 (navy blue), branched 3 (brown) and linear PFOS (red). The methanol concentration is displayed in black in the secondary Y axis.

The LC-MS/MS chromatograms of fractions A and B, and that of the technical mixture obtained in SIM mode (m/z 499) are shown in Figure 6.3. Fraction A elutes as one peak at 16.9 min while fraction B elutes as two peaks 16.8 and 18.3 min. In the case of the standard mixture, 5 distinctive peaks are observed which eluted at 13.1, 16.9, 18.0, 18.4 and 23.0 min, respectively. The PFOS isomers were identified based on the fragmentation pattern as described by Langlois and coworkers [30]. The peak at 23 min was identified as the linear PFOS isomer. Fraction A corresponds to isomers 3-CF_3- and 4-CF_3-PFOS (16.8 min). Fraction B is a mixture of mainly 3-CF_3- and 4-CF_3-PFOS that eluted as the first peak (16.8 min) and 5-CF_3 and 6-CF_3-PFOS coeluted as the second peak (18.3 min). These assignments are in agreement with those reported for the separation of branched PFOS isomers by means of LC-MS/MS employing C_{18}-based columns. In our earlier publication, the structural monomethyl isomers were separated with a C_{18} column, 3-CF_3- and 4-CF_3-PFOS as well as 5-CF_3 and 6-CF_3-PFOS coeluted as two different peaks [23]. Arsenault *et al.* [26] reported the separation of branched PFOS isomers using a modified C_{18} column, Shield RP_{18} column in which a carbamate group is incorporated into the bonded phase ligand. Although, these four isomers eluted in the same order as the one described in this study, mono- and multibranched isomers were chromatographically separated and identified. Table 6.1 summarizes the tentative identification of branched PFOS isomers purified by preparative HPLC and analyzed by HPLC-suppressed conductivity detection and LC-MS/MS. Since commercial standard of branched PFOS isomers are not commercially available, an accurate assignment of the branched PFOS isomers cannot be conducted.

Table 6.1 Tentative assignments of branched PFOS isomers purified by means of preparative HPLC.

Compound	**Branched PFOS isomer**
f	Not identified
s	Not identified
0	3-CF_3-PFOS
1	4,5-CF_3-PFOS
2	6-CF_3-PFOS
3	1-CF_3-PFOS
Linear PFOS	L-PFOS

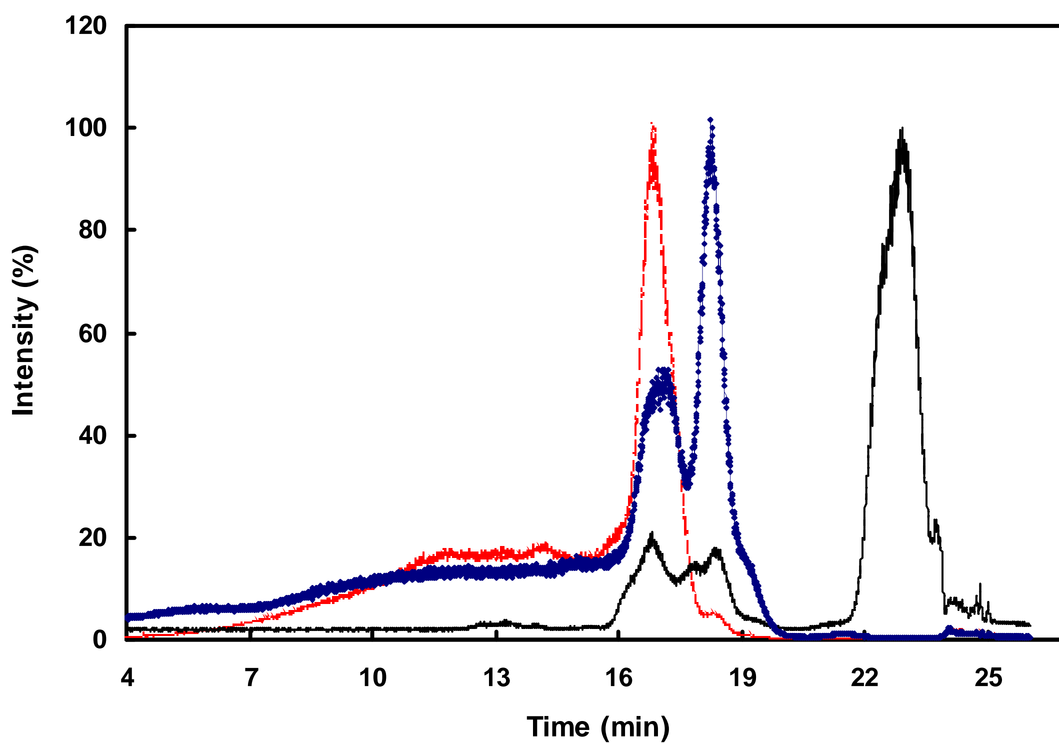


Figure 6.3 LC-MS/MS chromatograms of technical PFOS and branched PFOS isomers fractions purified by preparative HPLC. Legend: technical PFOS (black), branched *A* (red) and fraction *B* (blue).

The purified PFOS solution, which contained mainly branched isomers (95.6%) and traces of linear PFOS, was employed in the biomimetic reductive dehalogenation experiments (Figure 6.4). The identification of the branched PFOS isomers is summarized in Table 6.1. The ^{19}F NMR spectrum of the mentioned branched PFOS isomers sample and that of technical PFOS are depicted in Figure 6.5. A comparison of the ^{19}F NMR spectra reveals significant differences between the purified branched isomers and the technical salt. In the spectrum of the branched PFOS isomers, the typical signals of the branched CF_3 (-73 ppm) of the different isomer compounds [31, 32] are the predominant and distinctive features. Whereas, the signal corresponding to the terminal CF_3 (-81 ppm) [31, 33], which is characteristic of the linear PFOS compound, decreases significantly as compared to that in the technical salt. The fluorine signatures in the region ranging from –110 to –130 ppm have been reported to correspond to intermediate CF_2 moieties of the PFOS structure [31, 32].

Taking into consideration that six different methyl PFOS isomers and linear PFOS are present in this solution, it is very challenging to assign those signals to a specific compound. Besides, the lack of branched PFOS isomers standards compromises the characterization process.

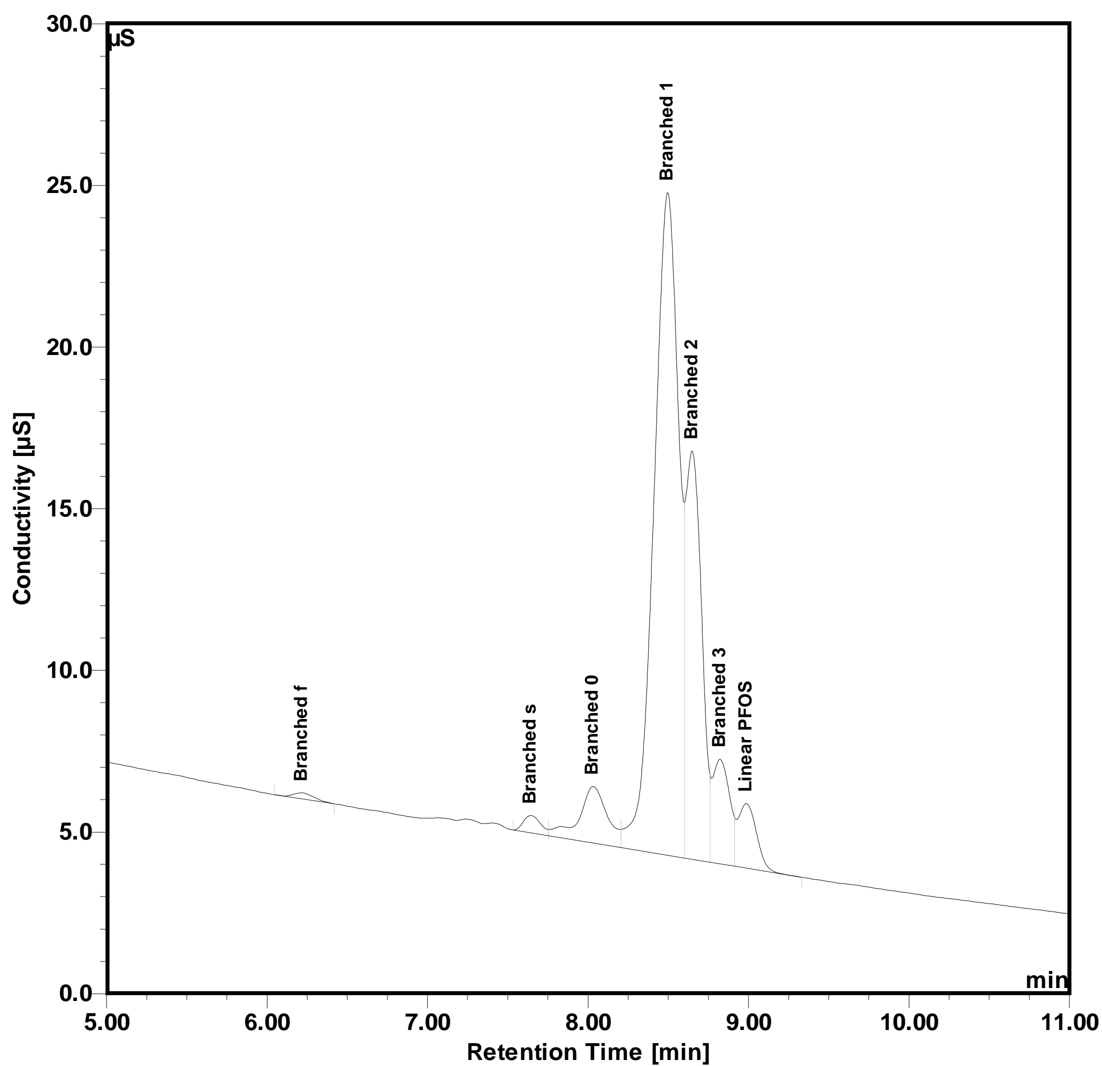


Figure 6.4 HPLC-suppressed conductivity detection chromatogram of purified branched PFOS isomers (343 μM) employed in the biomimetic reductive dehalogenation experiments.

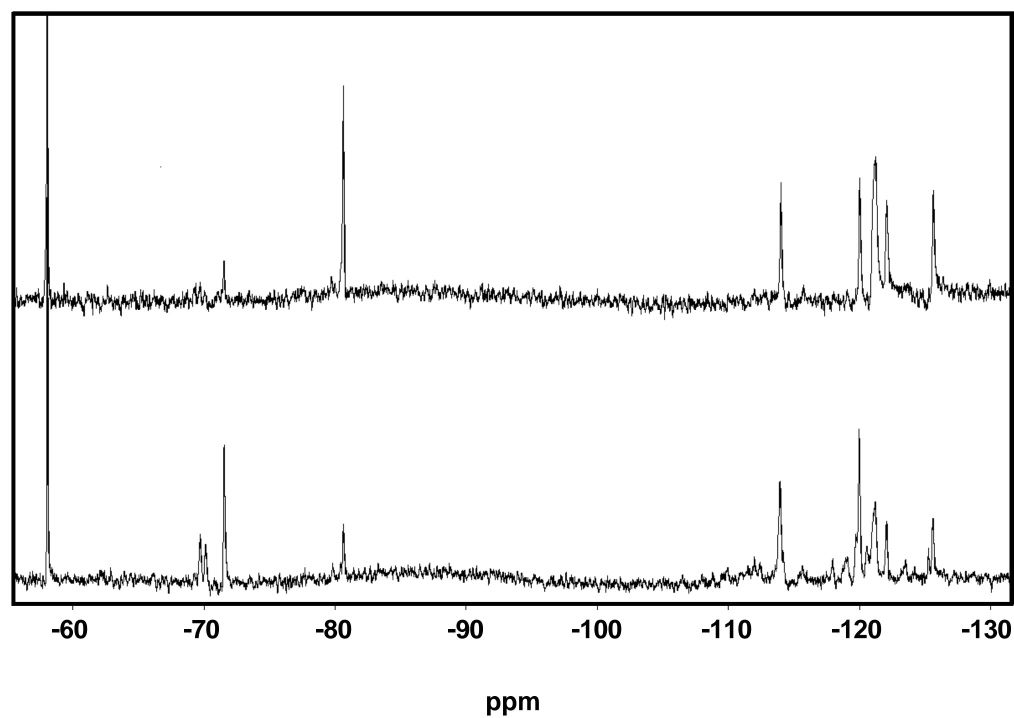


Figure 6.5 ^{19}F NMR spectra of technical PFOS (332 μM) (upper panel) and branched PFOS isomers (343 μM) concentrated and purified by SPE followed by preparative HPLC (lower panel).

6.4.2 Characterization of degradation products from the biomimetic reductive defluorination of branched PFOS isomers

In a previous publication, our research group reported the reductive dehalogenation of PFOS by Ti(III) citrate and vitamin B_{12}, a cobalt-based macromolecule [23]. After seven days, 18% of the total fluorine content in technical PFOS was released as fluoride ions, which is equivalent to the removal of 3 mol of fluoride per mol of PFOS. On the other hand, reductive treatment of the purified branched PFOS isomers for the same time period led to the release of 71% of the total fluorine in the organic molecules as fluoride, which is equivalent to the removal of 12 mol of fluoride per mol of PFOS. We also demonstrated that the susceptibility of PFOS to chemical reductive dehalogenation differed among isomer compounds. Chromatographic peaks corresponding to branched PFOS isomers disappeared whereas the peak corresponding to linear PFOS was stable.

The objective of this study is to characterize other degradation products aside of fluoride of the biomimetic reductive dehalogenation of PFOS isomers employing the purified isomers solution described above. HPLC-suppressed conductivity detection, LC-MS/MS and ^{19}F NMR spectroscopy of the liquid phase were carried out to assess the presence of products from PFOS degradation. In agreement with our previous results, branched PFOS isomers were the only fluorinated compounds detected in the reaction solution after treatment with vitamin B_{12} and Ti(III) citrate [23].

In the quest for PFOS degradation products, the insoluble materials present in the reaction mixture were also analyzed. After seven days of treatment a blue precipitate was

detected in the reaction flask. This precipitate is assumed to be Ti(III) hydroxide [34]. Solid-liquid extractions of the mentioned precipitate were performed with common organic solvents of variable polarity (Table 6.2). The ^{19}F NMR spectra of the precipitated extracted with methanol (polar protic solvent) and diethyl ether (non-polar solvent) are depicted in Figure 6.6. Linear PFOS was the only fluorinated compound present in the extract; no signs of PFOS-derivatives or other fluorinated chemicals were obtained either by liquid ^{19}F NMR. These findings were further confirmed by LC-MS/MS analysis and solid ^{19}F NMR (data not shown).

Table 6.2 Organic solvents employed in the solid-liquid extraction of the precipitate grouped into polar protic, polar aprotic and non-polar solvents. The polarity is expressed as dielectric constant [35].

Solvent	Dielectric constant
Polar protic	
Methanol	33
Ethanol	24
Polar aprotic	
Dimethyl sulfoxide (DMSO)	47
Acetonitrile	37
Acetone	21
Non-polar	
Diethyl ether	4.3
Hexane	2.0

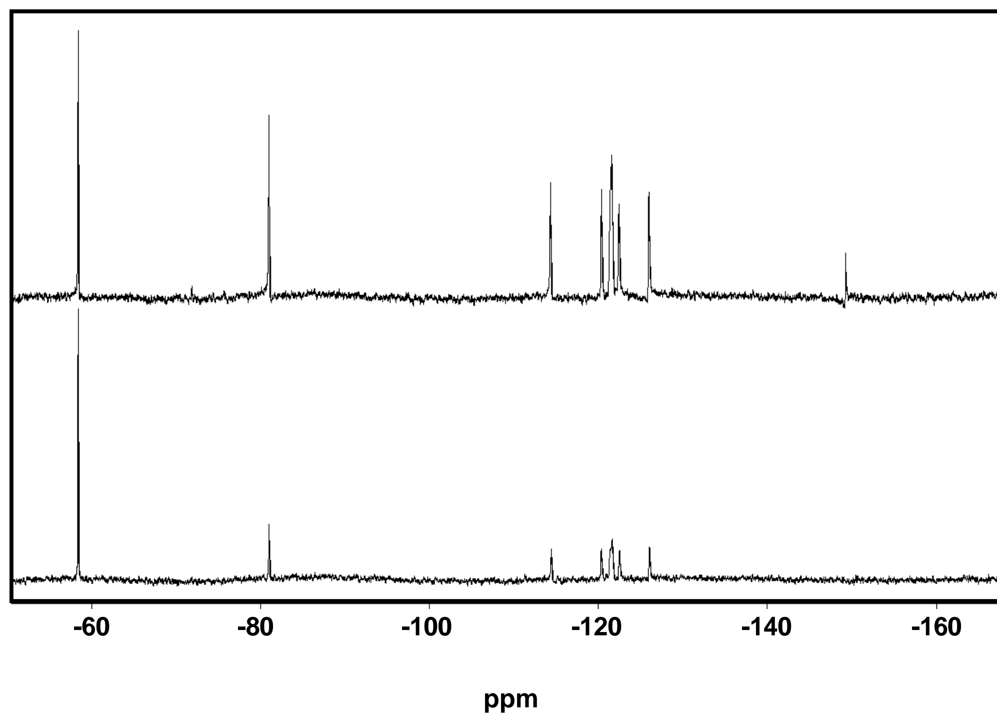


Figure 6.6 ^{19}F NMR spectra of the precipitate in treatment samples (PFOS + Ti(III) citrate + vitamin B_{12}) during the chemical reductive dehalogenation of branched PFOS isomers (343 μM) with vitamin B_{12} (260 μM) and Ti(III) citrate (36 mM) at 70ºC and pH 9.0 on day 7. Precipitate extracted with methanol (upper panel) and diethyl ether (lower panel).

Samples of the headspace of the reaction flasks were analyzed by GC-MS to examine the possible formation of volatile fluorinated metabolites as well as mineralized products such as carbon dioxide. For this purpose, the reductive defluorination of the technical salt and branched PFOS isomers was conducted using a Ti(III) solution ($TiCl_3$) instead of Ti(III) citrate. Degradation experiments were carried out with a phosphate buffer in lieu of the bicarbonate buffer to eliminate carbon background interferences. Sodium hydroxide is well known to absorb atmospheric carbon dioxide; therefore, a high purity NaOH solution was employed to overcome this problem. Helium was used to maintain anaerobic conditions; the response factor of molecular nitrogen in the GC analysis is quite high, so its presence was reduced to eliminate possible signal suppressions of other volatile compounds.

Figure 6.7 illustrates the time course of the reductive dehalogenation of branched PFOS isomers with bicarbonate buffer and Ti(III) citrate and phosphate buffer and Ti(III) ($TiCl_3$). In control samples, lacking vitamin B_{12}, PFOS degradation corresponding to less than 1% of fluoride release was observed as depicted in Figure 6.6. This degradation can be attributed to the strong reducing power of Ti(III). In contrast, 71% defluorination in the case of bicarbonate buffer and Ti(III) citrate and 24% in phosphate buffer and Ti(III) ($TiCl_3$) were obtained in the treatment samples containing vitamin B_{12}. These data confirm the key role of vitamin B_{12} in the dehalogenation of perfluorooctane sulfonate [23].

The rate of the reductive dehalogenation of the branched PFOS isomers with vitamin B_{12} and Ti(III) ($TiCl_3$) in phosphate buffer solution was reduced by 3-fold as

depicted in Figure 6.7. The same reducing effect in the rate of PFOS transformation was observed in the biomimetic reductive experiments conducted with the typical bicarbonate buffer and Ti(III) ($TiCl_3$) (data not shown). This decrease in the rate of PFOS dehalogenation can be attributed to an incomplete reduction of vitamin B_{12} by the action of Ti(III) [36-38]. In the absence of citrate, the reducing power of Ti(III) could be reduced since it precipitates as Ti(III) hydroxide [34].

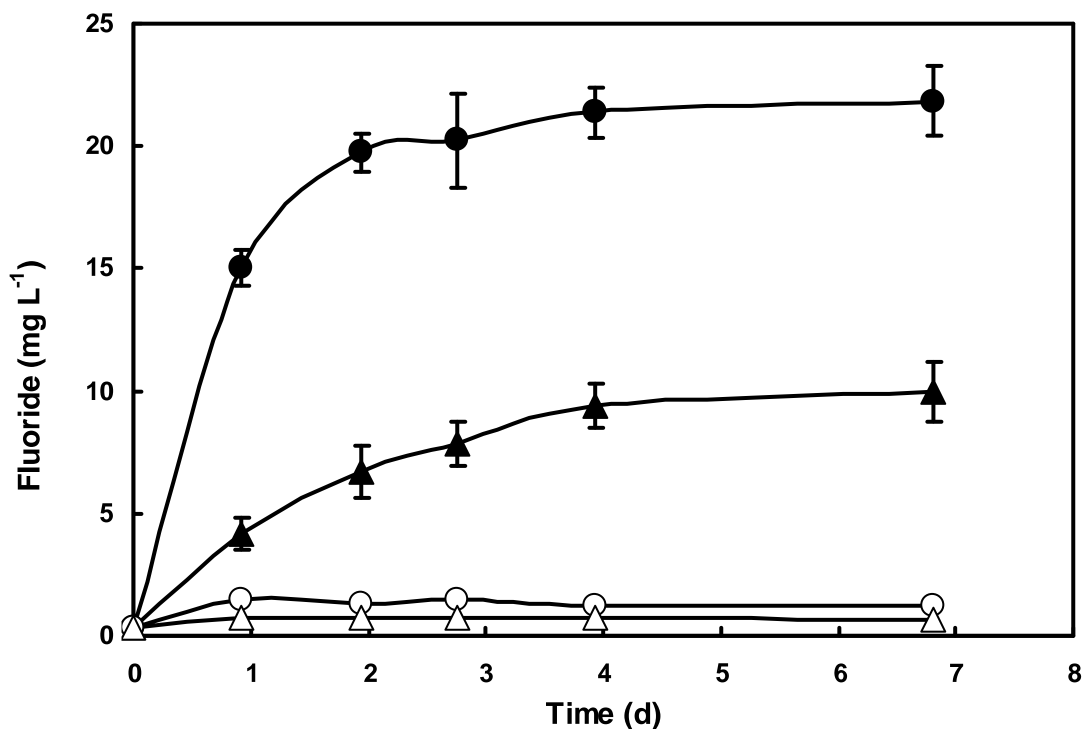
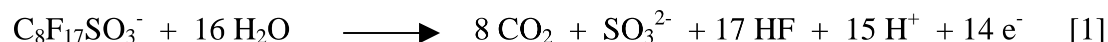


Figure 6.7 Time course of fluoride release of purified branched PFOS isomers (343 μM) with vitamin B_{12} (260 μM) and Ti(III) citrate and Ti(III) ($TiCl_3$) (36 mM) at 70ºC and pH on day 7 in treated samples by fluoride-selective electrode. Legend: bicarbonate buffer: control samples (PFOS + Ti(III) citrate) (○) and treatment samples (PFOS + Ti(III) citrate + vitamin B_{12}) (●) and phosphate buffer: control samples (PFOS + Ti(III)) (Δ) and treatment samples (PFOS + Ti(III) + vitamin B_{12}) (▲). Error bars (shown if larger that the symbols) represent standard deviations of triplicate assays.

Figure 6.8 shows the SIM GC-MS chromatograms for carbon dioxide, m/z 44 fragment, of control and treatment samples during the biomimetic reductive dehalogenation of branched PFOS isomers. Some carbon dioxide background was observed in the control samples presumably due to the degradation of branched PFOS isomers by the reducing action of Ti(III). Therefore, the amount of CO_2 formed as a result of the reductive degradation of PFOS catalyzed by vitamin B_{12} was calculated by subtracting background CO_2. Since the purified PFOS solution employed in this study is a mixture of seven isomer compounds (Figure 6.4), the total quantity of CO_2 produced (gas phase and liquid phase) was expressed as a function of all carbon atoms in the PFOS chemicals based on the following redox reaction [1].



$$C_8F_{17}SO_3^- + 16\ H_2O \longrightarrow 8\ CO_2 + SO_3^{2-} + 17\ HF + 15\ H^+ + 14\ e^- \quad [1]$$

Our results indicate that 14.7% of total carbon present in the PFOS molecule was converted to CO_2. These findings confirm the mineralization of PFOS to carbon dioxide and HF in the presence of Ti(III) and vitamin B_{12}. Sulfate measurements by detection were also conducted to confirm the mineralization of PFOS. Unfortunately, no accurate sulfate readings were obtained because of matrix interferences and the high salt content of the samples.

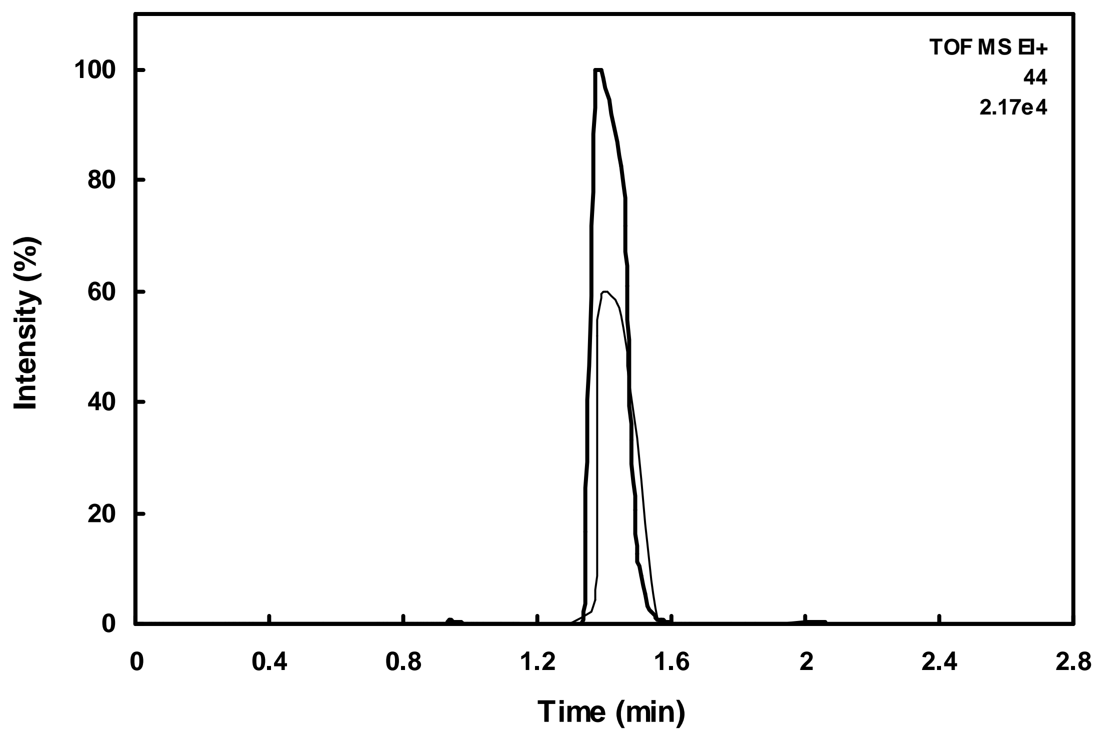


Figure 6.8 SIM (m/z 44) GC-TOF-MS chromatograms of the headspace of the biomimetic reduction of branched PFOS isomers (343 µM) with vitamin B_{12} (260 µM) and Ti(III) ($TiCl_3$) (36 mM) at 70℃ and pH 9.0 on day 7. Legend: control samples (PFOS + Ti(III)) (thin line) and treatment samples (PFOS + Ti(III) + vitamin B_{12}) (thick line).

Small quantities of fluorinated hydrocarbons were also detected in the gas phase. GC-MS chromatograms of the treatment sample indicated the formation of two volatile products as a result of the degradation of branched PFOS isomers. These products were also found in the control sample but in concentrations significantly lower that those reported in the biomimetic treatment as shown in Figure 6.9. The EI/MS spectrum of the volatile degradation products shows fragments at m/z of 51, 69, 101, 119, 131, 151 and 169 (Figure 6.10). This fragmentation pattern appears to correspond to that of fluorinated compounds. 1H-perfluorohexane was used as the standard to quantify and confirm the possible identity of one of the degradation products of the chemical reduction of PFOS. Figure 6.11 illustrates the EI/MS spectrum of 1H-perfluorohexane. The spectrum of the standard is pretty similar to that observed in headspace samples of the biomimetic treatments with the only difference being the intensity of the fragments. The anion m/z 69 is the predominant fragment in the MS spectrum of the samples whereas the fragment at m/z 51 is the main feature in the spectrum of the 1H-pefluorohexane standard. The m/z 69 corresponds to CF_3 which is a typical fragment anion from perfluorinated compounds [22]. Based on these results we conclude that the degradation products could be derivatives of 1H-perfluorohexane and other perfluorinated or partially fluorinated hydrocarbons. The structures of the possible volatile PFOS degradation products are depicted in Figure 6.12.

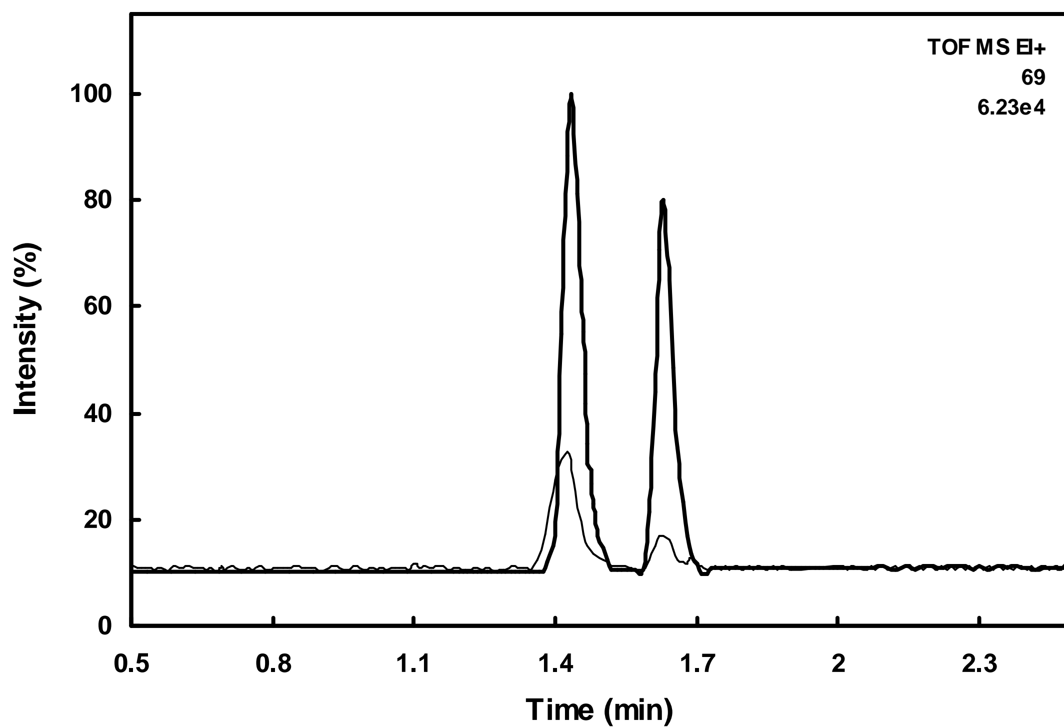


Figure 6.9 SIM (m/z 69) GC-TOF-MS chromatograms of the headspace of the biomimetic reduction of branched PFOS isomers (343 µM) with vitamin B_{12} (260 µM) and Ti(III) ($TiCl_3$) (36 mM) at 70ºC and pH 9.0 on day 7. Legend: control samples (PFOS + Ti(III)) (thin line) and treatment samples (PFOS + Ti(III) + vitamin B_{12}) (thick line).

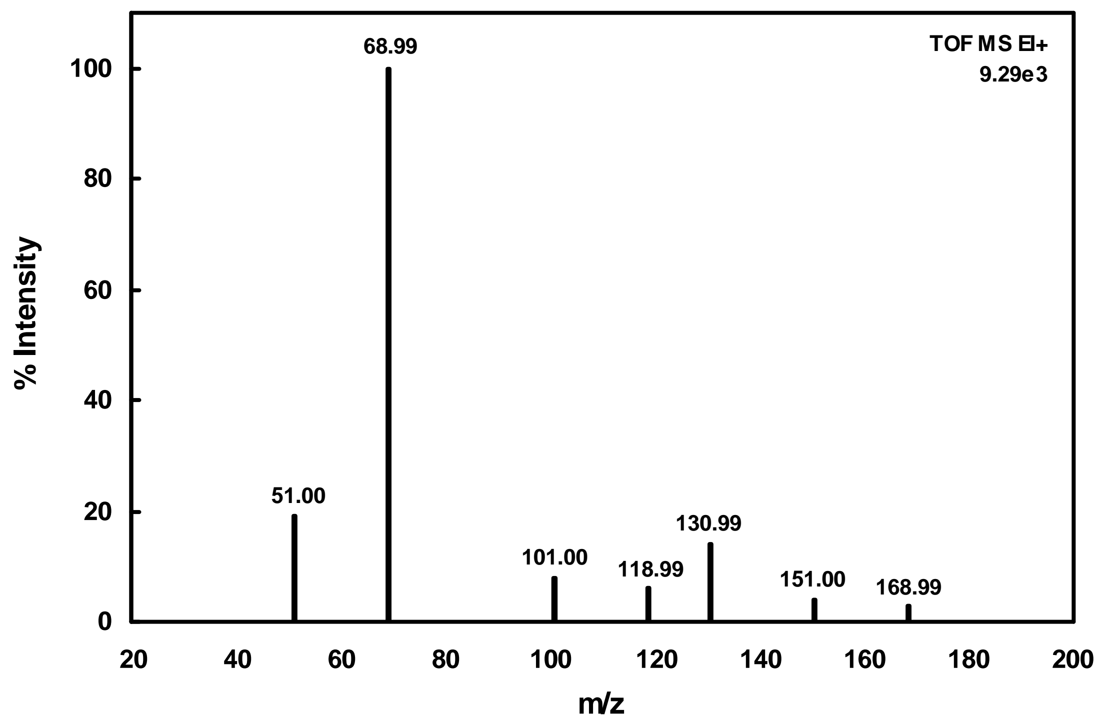


Figure 6.10 GC/MS spectrum of the treatment sample (PFOS + Ti(III) + vitamin B_{12}) of the reductive dehalogenation of branched PFOS isomers (343 µM) with vitamin B_{12} (260 µM) and Ti(III) (36 mM) at 70ºC and pH 9 on day 7.

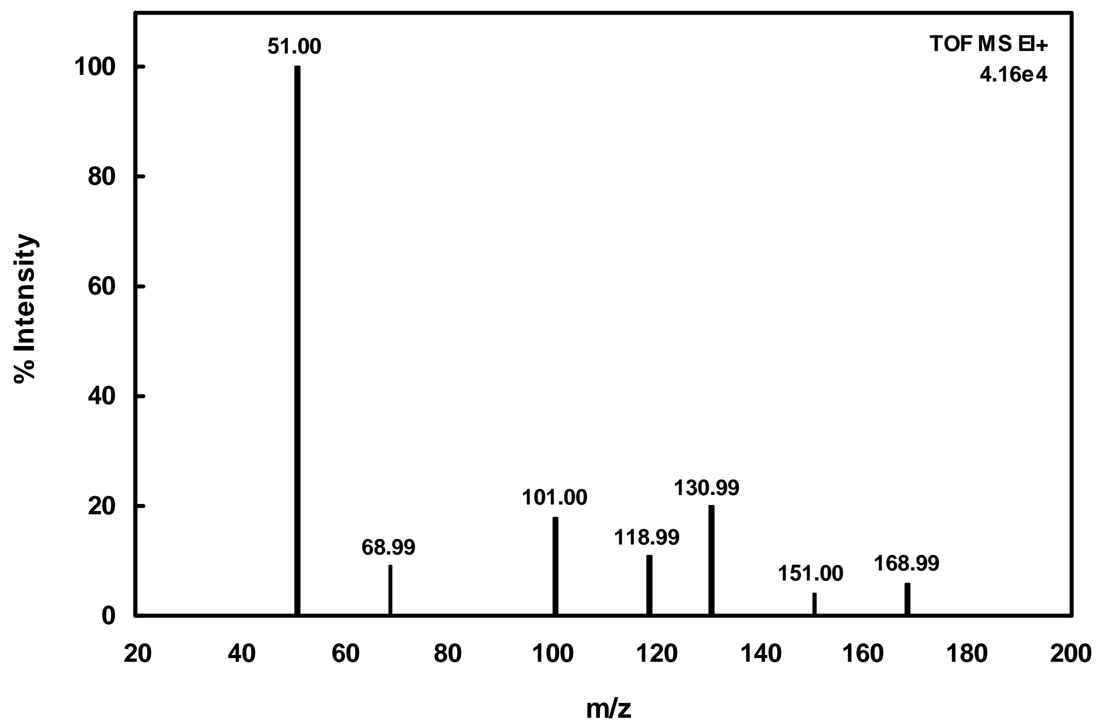
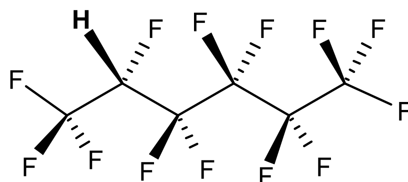
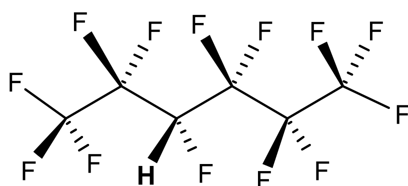


Figure 6.11 GC/MS spectrum of the headspace of aqueous 1H-perfluorohexane standard (76 mg L^{-1}).

A)



B)



C)

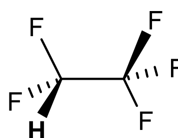


Figure 6.12 Tentative chemical structure of the volatile fluorinated compounds detected in the headspace of reaction flasks following reductive dehalogenation of branched PFOS isomers (343 μM) with vitamin B_{12} (260 μM) and Ti(III) (36 mM) at 70ºC and pH 9.0 on day 7. Legend: A) 2H-perfluorohexane, B) 3H-perfluorohexane, and C) 1H-perfluoroethane.

The lack of commercial standards limits the accurate characterization of the volatile fluorinated chemicals; however, quantification efforts based on 1H-perfluorohexane revealed that these compounds are present in very small amounts (0.2 mg L^{-1}) equivalent to less than 0.1% of the total PFOS degraded. These results indicate that although the PFOS molecule is highly defluorinated by the biomimetic treatment (12 mol F^- released per mol branched PFOS in 7 days), small fractions of PFOS appear to undergo cleavage of the sulfonic group resulting in the formation of volatile degradation products. Yamamoto and coworkers [22] also reported the formation of short-chain fluorinated compounds such as C_7HF_{15} during the photodegradation of PFOS by UV light.

Previous studies on the biomimetic reductive dehalogenation of PFOS suggested that the mechanism of chemical degradation involves the formation of radical intermediates [23]. The reaction mechanisms of chlorinated compounds highly susceptible to reductive dehalogenation with vitamin B_{12} and Ti(III) are still not well understood. Further research needs to be conducted to clarify the degradation mechanisms of perfluorooctane sulfonate by vitamin B_{12}.

6.5 Conclusions

Branched PFOS isomers were successfully purified by SPE procedures and preparative HPLC. The identification of the isomers was conducted by analyzing the fragmentation patterns obtained with LC-MS/MS operating in the SIM mode for the PFOS anion.

Reductive treatment of the purified branched PFOS isomers for seven days led to release of 71% of the total fluorine in the organic molecules as fluoride, which is equivalent to the removal of 12 mol of fluoride per mol of PFOS. Fluoride ions and CO_2 were the major degradation products detected in the liquid- and gas phase of the reaction system, respectively. Traces of volatile fluorinated compounds, representing less than 0.1% of the PFOS degraded, were also found in the headspace. These results suggest that perfluorooctane sulfonate was mineralized to fluoride ions and CO_2 by the action of Ti(III) and vitamin B_{12}, with minor accumulation of breakdown products.

6.6 Acknowledgments

We would like to thank the Semiconductor Research Corporation/Sematech Engineering Research Center for Environmentally Benign Semiconductor Manufacturing for financial support. We are grateful to Dr. Penn (Stephanie) Shu and Dr. Arpad Somogyi from the Department of Chemistry, University of Arizona, and Dr. Leif Abrell from the

Department of Soil, Water and Environmental Sciences, University of Arizona, for their invaluable support with the GC/MS and LC-MS/MS analyses.

6.7 References

1. EPA *Perfluorooctyl Sulfonates; Proposed Significant New Use Rule*; U.S. Environmental Protection Agency: Washington, DC, 2002; pp 62319 - 62333.
2. Schultz, M. M.; Barofsky, D. F.; Field, J. A., Fluorinated alkyl surfactants. *Environ. Eng. Sci.* **2003,** *20*, (5), 487-501.
3. Brooke, D.; Footitt, A.; Nwaogu, T. A., Environmental risk evaluation report: Perfluorooctanesulphonate (PFOS). *Environment Agency, Chemicals Assessment Section, Wallingford, UK* **2004**.
4. Houde, M.; Martin, J. W.; Letcher, R. J.; Solomon, K. R.; Muir, D. C. G., Biological monitoring of polyfluoroalkyl substances: A review. *Environ. Sci. Technol.* **2006,** *40*, (11), 3463-3473.
5. Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J., Perfluoroalkyl acids: A review of monitoring and toxicological findings. *Toxicol. Sci.* **2007,** *99*, (2), 366-394.
6. Conder, J. M.; Hoke, R. A.; De Wolf, W.; Russell, M. H.; Buck, R. C., Are PFCAs bioaccumulative? A critical review and comparison with regulatory lipophilic compounds. *Environ. Sci. Technol.* **2008,** *42*, (4), 995-1003.

7. Hansen, K. J.; Clemen, L. A.; Ellefson, M. E.; Johnson, H. O., Compound-specific, quantitative characterization of organic: Fluorochemicals in biological matrices. *Environ. Sci. Technol.* **2001,** *35*, (4), 766-770.

8. Weber, J., 3M's Big Cleanup: Why it decided to pull the plug on its best selling stain repellant. *BusinessWeek* 2000, pp 96 - 98.

9. Anonymous, Environmental Protection Agency tentatively OKs PFOS for semiconductor industry. *Solid State Technology* **2002,** *45*, (32).

10. Boulanger, B.; Vargo, J. D.; Schnoor, J. L.; Hornbuckle, K. C., Evaluation of perfluorooctane surfactants in a wastewater treatment system and in a commercial surface protection product. *Environ. Sci. Technol.* **2005,** *39*, (15), 5524-5530.

11. Higgins, C. P.; Field, J. A.; Criddle, C. S.; Luthy, R. G., Quantitative determination of perfluorochemicals in sediments and domestic sludge. *Environ. Sci. Technol.* **2005,** *39*, (11), 3946-3956.

12. Schultz, M. M.; Barofsky, D. F.; Field, J. A., Quantitative determination of fluorinated alkyl substances by large-volume-injection liquid chromatography tandem mass spectrometry - Characterization of municipal wastewaters. *Environ. Sci. Technol.* **2006,** *40*, (1), 289-295.

13. Anonymous, Ground-water sampling for PFOA near the Washington Co. landfill. *Minnesota Pollution Control Agency, Minnesota Department of Health. St. Paul, MN* **2004**.

14. Moody, C. A.; Hebert, G. N.; Strauss, S. H.; Field, J. A., Occurrence and persistence of perfluorooctanesulfonate and other perfluorinated surfactants in

groundwater at a fire-training area at Wurtsmith Air Force Base, Michigan, USA. *J. Environ. Monit.* **2003,** *5*, (2), 341-345.

15. Tang, C. Y. Y.; Fu, Q. S.; Robertson, A. P.; Criddle, C. S.; Leckie, J. O., Use of reverse osmosis membranes to remove perfluorooctane sulfonate (PFOS) from semiconductor wastewater. *Environ. Sci. Technol.* **2006,** *40*, (23), 7343-7349.

16. Skutlarek, D.; Exner, M.; Farber, H., Perfluorinated surfactants in surface and drinking water. *Environ. Sci. Pollut. Res.* **2006,** *13*, (5), 299-307.

17. Becker, A. M.; Gerstmann, S.; Frank, H., Perfluorooctane surfactants in waste waters, the major source of river pollution. *Chemosphere* **2008,** *72*, (1), 115-121.

18. Plumlee, M. H.; Larabee, J.; Reinhard, M., Perfluorochemicals in water reuse. *Chemosphere* **2008,** *72*, (10), 1541-1547.

19. Moriwaki, H.; Takagi, Y.; Tanaka, M.; Tsuruho, K.; Okitsu, K.; Maeda, Y., Sonochemical decomposition of perfluorooctane sulfonate and perfluorooctanoic acid. *Environ. Sci. Technol.* **2005,** *39*, (9), 3388-3392.

20. Hori, H.; Nagaoka, Y.; Yamamoto, A.; Sano, T.; Yamashita, N.; Taniyasu, S.; Kutsuna, S.; Osaka, I.; Arakawa, R., Efficient decomposition of environmentally persistent perfluorooctanesulfonate and related fluorochemicals using zerovalent iron in subcritical water. *Environ. Sci. Technol.* **2006,** *40*, (3), 1049-1054.

21. Carter, K. E.; Farrell, J., Oxidative destruction of perfluorooctane sulfonate using boron-doped diamond film electrodes. *Environ. Sci. Technol.* **2008,** *42*, (16), 6111-6115.

22. Yamamoto, T.; Noma, Y.; Sakai, S. I.; Shibata, Y., Photodegradation of perfluorooctane sulfonate by UV irradiation in water and alkaline 2-propanol. *Environ. Sci. Technol.* **2007,** *41*, (16), 5660-5665.

23. Ochoa-Herrera, V.; Sierra-Alvarez, R.; Somogyi, A.; Jacobsen, N. E.; Wysocki, V. H.; Field, J. A., Reductive defluorination of perfluorooctane sulfonate. *Environ. Sci. Technol.* **2008,** *42*, (9), 3260-3264.

24. Gramstad, T.; Haszeldine, R. N., Perfluoroalkyl Derivatives of Sulphur .6. Perfluoroalkanesulphonic Acids Cf3.[Cf2]N.So3h (N=1-7). *Journal of the Chemical Society* **1957**, (JUN), 2640-2645.

25. Tugnait, M.; Lenz, E. M.; Phillips, P.; Hofmann, M.; Spraul, M.; Lindon, J. C.; Nicholson, J. K.; Wilson, I. D., The metabolism of 4-trifluoromethoxyaniline and [C-13]-4-trifluoromethoxyacetanilide in the rat: detection and identification of metabolites excreted in the urine by NMR and HPLC-NMR. *J. Pharm. Biomed. Anal.* **2002,** *28*, (5), 875-885.

26. Arsenault, G.; Chittim, B.; McAlees, A.; McCrindle, R.; Riddell, N.; Yeo, B., Some issues relating to the use of perfluoro octane sulfonate (PFOS) samples as reference standards. *Chemosphere* **2008,** *70*, (4), 616-625.

27. 3M, The Science of organic fluorochemistry. *St Paul, MN* **1999**.

28. Kissa, E., *Fluorinated Surfactants and Repellants*. Marcel Dekker, Inc.: New York, 2001.

29. Brandt, A.; Kueppers, S., Practical aspects of preparative HPLC in pharmaceutical and development production. *LCGC Europe* 2002.

30. Langlois, I.; Oehme, M., Structural identification of isomers present in technical perfluorooctane sulfonate by tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2006,** *20*, (5), 844-850.

31. Vyas, S. M.; Kania-Korwel, I.; Lehmler, H. J., Differences in the isomer composition of perfluoroctanesulfonyl (PFOS) derivatives. *J. Environ. Sci. Health Part A-Toxic/Hazard. Subst. Environ. Eng.* **2007,** *42*, (3), 249-255.

32. Arsenault, G.; Chittim, B.; Gu, J.; McAlees, A.; McCrindle, R.; Robertson, V., Separation and fluorine nuclear magnetic resonance spectroscopic (F-19 NMR) analysis of individual branched isomers present in technical perfluorooctanesulfonic acid (PFOS). *Chemosphere* **2008,** *73*, (1), S53-S59.

33. Arsenault, G.; McCrindle, R.; Chittim, B.; McAlees, A., Separation and fluorine nuclear magnetic resonance spectroscopy (^{19}F-NMR) analysis of the individual branched isomers present in technical perfluorooctanesulfonic acid (PFOS). *Organohalogen Compounds* **2005,** *67*, 818 - 822.

34. Chiu, P. C.; Reinhard, M., Metallocoenzyme-Mediated Reductive Transformation of Carbon-Tetrachloride in Titanium(Iii) Citrate Aqueous-Solution. *Environ. Sci. Technol.* **1995,** *29*, (3), 595-603.

35. McMurry, J., *Organic Chemistry*. 6th ed.; Brooks/Cole - Thomson Learning: USA, 2004.

36. Krone, U. E.; Thauer, R. K.; Hogenkamp, H. P. C., Reductive Dehalogenation of Chlorinated C1-Hydrocarbons Mediated by Corrinoids. *Biochemistry* **1989,** *28*, (11), 4908-4914.

37. Holliger, C.; Schraa, G.; Stupperich, E.; Stams, A. J. M.; Zehnder, A. J. B., Evidence for the Involvement of Corrinoids and Factor-F430 in the Reductive Dechlorination of 1,2-Dichloroethane by Methanosarcina-Barkeri. *J. Bacteriol.* **1992,** *174*, (13), 4427-4434.

38. Assafanid, N.; Hayes, K. F.; Vogel, T. M., Reductive Dechlorination of Carbon-Tetrachloride by Cobalamin(Ii) in the Presence of Dithiothreitol - Mechanistic Study, Effect of Redox Potential and Ph. *Environ. Sci. Technol.* **1994,** *28*, (2), 246-252.

CHAPTER 7

Removal of Perfluorinated Surfactants by Sorption onto Granular Activated Carbon, Zeolite and Sludge

7.1 Abstract

Perfluorinated surfactants are emerging pollutants of increasing public health and environmental concern due to recent reports of their world-wide distribution, environmental persistence and bioaccumulation potential. Treatment methods for the removal of anionic perfluorochemical (PFC) surfactants from industrial effluents are needed to minimize the environmental release of these pollutants. Removal of PFC surfactants from aqueous solutions by sorption onto various types of granular activated carbon (GAC) was investigated. Three anionic PFC surfactants, *i.e.,* perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorobutane sulfonate (PFBS), were evaluated for the ability to adsorb onto activated carbon. Additionally, the sorptive capacity of zeolites and sludge for PFOS was compared to that of granular activated carbon. Adsorption isotherms were determined at constant ionic strength in a pH 7.2 phosphate buffer at 30°C. Sorption of PFOS onto activated carbon was stronger than PFOA and PFBS, suggesting that the length of the fluorocarbon chain and the nature of

the functional group influenced sorption of the anionic surfactants. Among all adsorbents evaluated in this study, activated carbon (Freundlich K_F values = 36.7-60.9) showed the highest affinity for PFOS at low aqueous equilibrium concentrations, followed by the hydrophobic, high-silica zeolite NaY (Si/Al 80, K_F = 31.8), and anaerobic sludge (K_F = 0.95-1.85). Activated carbon also displayed a superior sorptive capacity at high soluble concentrations of the surfactant (up to 80 mg L^{-1}). These findings indicate that activated carbon adsorption is a promising treatment technique for the removal of PFOS from dilute aqueous streams.

Key words: PFC, PFOS, PFOA, PFBS, adsorption, biosorption, GAC, zeolite and sludge.

This chapter has been published as a paper entitled "*Removal of perfluorinated surfactants by sorption onto granular activated carbon, zeolite and sludge*", Chemosphere, 2008, **72**:p. 1588-1593.

7.2 Introduction

Perfluorinated (PFC) surfactants are emerging pollutants that have been used for the last 50 years in a wide variety of industrial processes and consumer-based products, including polymer additives, lubricants, fire retardants and suppressants, pesticides, and surfactants [1]. Quantitatively, pefluorooctane sulfonate (PFOS, $C_8F_{17}SO_3H$) and perfluorooctanoic acid (PFOA, $C_7F_{15}COOH$) are the most important anionic PFC surfactants detected in the environment. Large quantities of PFOS (e.g., 3500 metric tons in 2000) and PFOA (1200 metric tons in 2004) have been produced and utilized worldwide during the last decades [2]. In addition, these two chemicals are the final degradation products of a variety of precursor perfluorinated chemicals including fluorotelomer alcohols and fluorinated polymers [3-7].

Long chain PFC surfactants including PFOS and PFOA are under increased scrutiny as global environmental pollutants due to recent reports of their global distribution, persistence, toxicity and bioaccumulation potential [2, 8]. In response to these concerns, regulatory agencies in numerous industrialized countries have initiated studies to quantify the use of perfluorinated chemicals, assess their potential risks, and consider regulations restricting or banning their use. More environmentally sound chemicals are also being developed to replace long chain PFC surfactants. Perfluorobutane sulfonate (PFBS, $C_4F_9SO_3H$) has been commercialized recently as an alternative to PFOS in a variety of applications [9]. PFBS is a homologous compound of

PFOS that has a four-carbon backbone. The shorter carbon chain makes PFBS less bioaccumulative compared to PFOS [10].

Ionic PFCs have been detected in municipal effluents, surface and ground water in various countries at concentrations ranging from below detection level to the ng L^{-1} range [11-14]. Elevated concentrations of PFCs have been detected near sites impacted by manufacturing sites or accidental emissions of PFCs [15, 16]. As an example, sum concentrations of PFCs as high as 43,348 ng L^{-1} have been detected in a river of a highly industrialized area in Germany, with the major components being PFOA (33,900 ng L^{-1}) and PFOS (3,160 ng L^{-1}) [11]. Considerably higher concentrations of PFCs can be found in effluents from chemical plants manufacturing fluorochemicals and from other industrial activities that utilize ionic PFCs. As an example, PFOS concentrations of 1650 mg L^{-1} have been reported in effluents generated from semiconductor manufacturing [17]. The semiconductor manufacturing industry has secured a temporary exemption to utilize PFOS in wafer photolithography [18].

The application of conventional treatments for removing anionic PFC surfactants from aqueous streams containing elevated concentrations of these contaminants is restricted by technical and/or economical constraints. PFOS and PFOA are not amenable to biological treatment due to their outstanding stability against microbial attack. Similarly to its longer chain homologues, PFBS appears to be highly persistent in the environment [19]. Literature data on the removal of PFOS and related compounds by physico-chemical treatment methods are limited. The strategies investigated include membrane processes [17], decomposition by chemical approaches involving

electrochemical treatments [20] and high-temperature conditions such as sonochemical treatment [21] and reduction with zero-valent iron in subcritical water [22]. These treatment technologies present some drawbacks and limitations, mainly due to their high energy demand and/or interference by other compounds present in the wastewater.

Adsorption of anionic PFC surfactants onto granular activated carbon (GAC) could offer a viable alternative for the removal from aqueous streams. Activated carbon adsorption is widely applied in the removal of organic contaminants in wastewaters due to the effectiveness, versatility, and relatively low cost of this approach. Although published data on the removal of PFC surfactants are lacking, there is some evidence that non-fluorinated alkyl sulfonated compounds can be adsorbed onto activated carbon [23, 24].

The objective of this study is to evaluate the adsorption of PFOS from aqueous solutions onto various commercial grades of granular activated carbon. The adsorption of two related PFC surfactants, PFOA and PFBS, was also evaluated on the best performing activated carbon. The capacity of GAC to adsorb PFOS was compared to that of alternative sorbents, including several types of zeolites and wastewater treatment sludges. Adsorption isotherms were determined at constant ionic strength in a pH 7.2 phosphate buffer at 30°C.

7.3 Materials and Methods

7.3.1 Chemicals

Perfluorooctane sulfonic acid potassium salt, PFOS (98%) was purchased from SynQuest Laboratories (Alachua, FL). Perfluorobutane sulfonic acid potassium salt, PFBS (98.2%) was kindly provided by the 3M Company (St. Paul, MN). Perfluorooctanoic acid, PFOA (96%) and sodium fluoride (99%) were obtained from Sigma-Aldrich (St. Louis, MO). HPLC chemicals, sulfuric acid (95-98% ACS grade) was purchased from Sigma-Aldrich (St. Louis, MO), acetonitrile (99.8% HPLC grade) and boric acid (99.5% ACS grade) were obtained from Mallinckrodt Chemicals (Phillipsburg, NJ). All chemicals were used as received.

7.3.2 Sorbents

The sorbents evaluated included different grades of granular activated carbon (GAC), *i.e.* Filtrasorb 300, Filtrasorb 400, abbreviated as F300 and F400, respectively, and URV-MOD 1 (Calgon Carbon Corp., Pittsburg, PA), and several zeolites (microporous crystalline hydrated aluminosilicates) differing in their molar Si/Al ratio, *i.e.*, zeolite 13X (Si/Al 2.8, Fluka product 96096, Sigma-Aldrich, St. Louis, MO), zeolite NaY (Si/Al 5.5,

product HS-320 from Wako Chemicals, Richmond, VA), and zeolite NaY80 (Si/Al 80, product CBV 780 from Zeolyst Int., Valley Forge, PA). The activated carbon utilized in the experiments was washed thoroughly with deionized water and dried at 70ºC to minimize interferences by soluble organic residues in the material.

Three types of wastewater treatment sludge were also evaluated for their effectiveness for PFOS biosorption. The sludges were obtained from different wastewater treatment plants and they included anaerobic granular sludge, return aerobic activated sludge (RAS) and anaerobically digested sewage sludge (ADS). The anaerobic granular sludge was obtained from an industrial anaerobic reactor treating alcohol distillery wastewater (Nedalco, The Netherlands). The samples of RAS and ADS were obtained from the Ina Road municipal wastewater treatment plant in Tucson, AZ. The content of total suspended solids (TSS) in the granular anaerobic sludge, RAS and ADS was 7.45, 1.64 and 17.20%, respectively. The organic matter content of the sludges was estimated by determination of their content in volatile suspended solid (VSS). The VSS content in the anaerobic granular sludge, RAS and ADS was 6.98, 1.04 and 11.76%.

7.3.3 Adsorption of perfluorinated surfactants

Sorption isotherm experiments with activated carbon and zeolites were conducted in duplicate using Nalgene flasks (200 to 500 mL) supplemented with the sorbent (0.1 g) and a known volume of 3 mM phosphate buffer (pH 7.2) spiked with the target

perfluoroalkyl compound. The solution volume was 100 mL and the final PFC concentrations were 15, 25, 50, 75, 100, 125 and 150 mg L^{-1}. Samples of activated carbon were also contacted with 200, 300 and 400 mL of 3 mM phosphate buffer spkiked with 150 mg PFC L^{-1} in order to test the feasibility of obtaining increasing loadings of PFC on the sorbent. Additional sorption experiments were conducted to evaluate the partitioning of PFOS in more diluted solutions onto activated carbon. In the latter experiments, the initial adsorbate concentrations were 0.05, 0.125, 0.25, 0.35 and 0.5 mg L^{-1}, and the solution volume was 130 mL.

Sorption isotherm experiments with wastewater treatment sludges (0.12 to 1.76 g VSS l^{-1}) were performed in duplicate using Nalgene flasks (200 mL) supplied with 100 mL of a 3 mM phosphate buffer (pH 7.2) spiked with known concentrations of PFOS (e.g. 15, 25, 50, 75, 100 and 150 mg L^{-1}). Sludges were also contacted with 150 mL of an aqueous solution spiked with 150 mg PFOS L^{-1} to obtain increasing loadings of the adsorbate on the sorbent.

Control flasks lacking sorbent were run in parallel to correct for possible removal of PFCs by other mechanisms than adsorption. Flasks were shaken in an orbital shaker (Innova 4300, New Brunswick Scientific, Edison, NJ) at 150 rpm for 2 d at 30ºC. Removal of PFCs from solution was determined using a Total Organic Carbon analyzer (Shimadzu TOC-V CSH/CSN system, Columbia, MD) and/or HPLC with suppressed conductivity detection following centrifugation and filtration (0.45 µm) of the samples to remove insoluble matter. All liquid samples were analyzed by HPLC with the exception of samples from adsorption experiments with the activated carbons Fitrasorb 300 and

URV-MOD 1 which were analyzed using the TOC analyzer. Liquid samples containing less than 0.5 mg PFOS L^{-1} were concentrated by solid phase separation prior to analysis.

7.3.4 Solid phase extraction

Solid phase extraction (SPE) was conducted to concentrate liquid samples containing less than 0.5 mg PFOS L^{-1}. SPE cartridges (3 mL, 500 mg ODS-C18, Agilent Technologies, New Castle, DE) were mounted in a vacuum manifold and conditioned with methanol (6 mL) and then deionized water (6 mL). Subsequently, the sample (125 mL) was loaded at a rate of 1-2 mL min^{-1}. SPE cartridges were rinsed with deionized water (6 mL) and then centrifuged at 4000 rpm for 25 min. Analytes were eluted with methanol (4 mL) and collected in Nalgene flasks. Phosphate buffer blanks spiked with known PFOS concentrations were extracted in parallel to determine recovery efficiencies (106 + 18 % for triplicate samples).

7.3.5 Analytical methods

HPLC analysis of PFCs in aqueous samples was conducted using a chromatographic system fitted with a suppressed conductivity detector (Dionex ICS-3000, Sunnyvale, CA, USA). An Acclaim Polar Advantage II C_{18}-guard column (4.3 mm i.d., 1 cm length) and

an Acclaim Polar Advantage II C_{18}-column (4.6mm i.d., 25 cm length), both from Dionex, were used for chromatographic separation and they were maintained at 35 ºC in a thermostated column compartment. A mixture of 20 mM boric acid (pH 8.0) and 95% acetonitrile was used as the mobile phase at a flow rate of 1mL min^{-1}. The ratio of boric acid to acetonitrile varied with linear gradient program was: 0 min: from 75:15 (v/v) to 45:55 (v/v) at 13.2 min. The volume of sample injected on the column was 100 µL. Blanks were continuously run to assure that the column was clean and traces of the analyte were not carried over between samples. The total concentration of PFCs in liquid samples was obtained by linear calibration curves ($r^2 > 0.99$) using known concentrations of the respective compounds in methanol or 3 mM phosphate buffer (pH 7.2), depending on the experiment, ranging from 0.5– 25 mg L^{-1}. The detection limit of PFOS, PFBS and PFOA was 0.5 mg L^{-1}.

Dissolved organic carbon analyses were performed using a total organic carbon analyzer (Shimadzu TOC-V CSH/CSN system, Columbia, MD). All liquid samples, including blanks and standards, were acidified with HCl to pH 2.20-2.50 prior to analysis. Instrument calibration was performed using potassium hydrogen phtalate standards ranging from 0.40 to 25 mg TOC L^{-1}. The coefficient of determination (r^2) for each calibration was > 0.99. Calibration lines obtained using potassium hydrogen phtalate and PFCs standards were nearly identical confirming the suitability of TOC measurements for PFC quantification.

7.4 Results and Discussion

7.4.1 PFOS adsorption onto activated carbon

The adsorption of PFOS from aqueous solutions onto three different types of granular activated carbon, Calgon F300, Calgon F400 and Calgon URV-MOD 1, was evaluated. These activated carbons are widely used for the treatment of surface and ground water sources for the production of drinking water. The physical properties of the various GAC tested are described in Table 7.1. The adsorption isotherms obtained for the removal of PFOS by the three GAC types are depicted in Figure 7.1.

The adsorptive capacity of the various activated carbons over a range of different concentrations was determined by fitting the experimental data to Langmuir and Freundlich models. The Langmuir isotherm is defined by:

$$C_s = \frac{a \cdot b \cdot C_e}{1 + b \cdot C_e} \quad (1)$$

Where C_s is the concentration of the solute in the solid phase (mg PFC g^{-1} sorbent), C_e is the equilibrium concentration of the solute in solution (mg PFC L^{-1}), a and b are Langmuir adsorption constants; a represents the maximum achievable surface concentration of the solute (mg PFC g $sorbent^{-1}$), and b is the equilibrium constant for the

sorption reaction (L mg PFC^{-1}). The Langmuir equation assumes that there is no interaction between the sorbate molecules and that the sorption is localized in a monolayer. It also assumes that once a sorbate molecule occupies a site, no further sorption can take place at that site. Theoretically, therefore, a saturation value is reached, beyond which no further sorption occurs.

The Freundlich equation is an empirical relationship describing the sorption of solutes from a liquid to a solid surface. The equation is widely used and has been found to describe adequately the adsorption process for many compounds in dilute solution. The Freundlich equation is defined by:

$$C_s = K_F \cdot C_e^{\ n} \qquad (2)$$

Where K_F [(mg PFC g^{-1} sorbent)(mg PFC L^{-1})$^{-n}$] is the Freundlich adsorption constant or capacity factor and n is the Freundlich exponent which provides a measure for the sorption intensity. For $n = 1$ the partition between the two phases is independent of the concentration and the isotherm becomes linear. The K_F value represents the carbon loading in mg compound per gram of carbon at an aqueous equilibrium concentration of 1.0 mg L^{-1} of the compound.

Table 7.1 Physical properties of GAC Calgon F300, Calgon F400 and Calgon URV-MOD1.

Property	F300	F400	URV-MOD 1
Total Pore Volume, mL/g	0.709*	0.767*	0.643
Micropore, mL/g	0.378-0.408*	0.312-0.391*	0.386
Mesopore, mL/g	0.063-0.378*	0.071-0.172*	n/a
Micropore average diameter, nm	0.841*	0.817*	n/a
Porosity	0.608*	0.627*	0.592
Particle diameter, mm	0.85 - 1.70	0.85 - 1.70	n/a
Iodine Number, mg/g (Min)	900	1000	1250
Effective Size, mm	0.8 - 1.0	0.55 - 0.75	1.0 - 1.18

Sources: Calgon F300 and F400 (Calgon Carbon Corporation and * [25]; Calgon URV-MOD 1 [26].

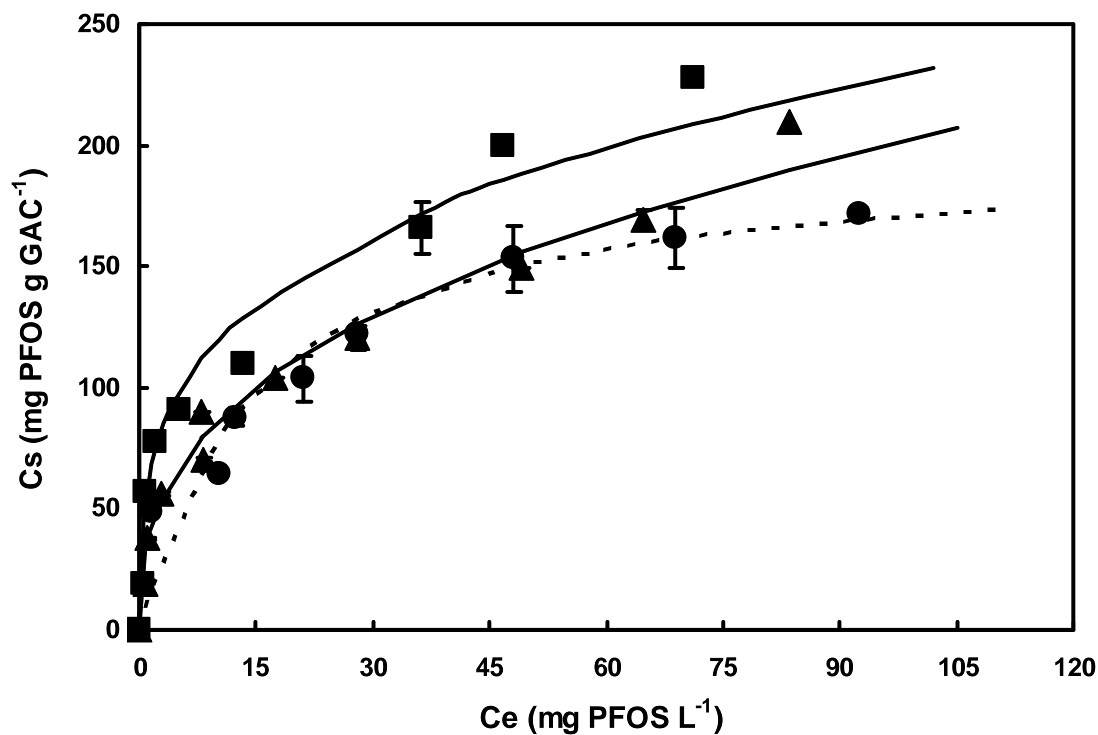


Figure 7.1 Adsorption isotherms of PFOS onto granular activated carbon at intermediate-high equilibrium concentrations. Legend: Calgon F400 (■), Calgon F300 (●), and Calgon URV-MOD 1 (▲). Experimental data fit to Langmuir model (dashed line); experimental data fit to Freundlich model (solid line). Error bars (shown if larger that the symbols) represent standard deviations of duplicate assays.

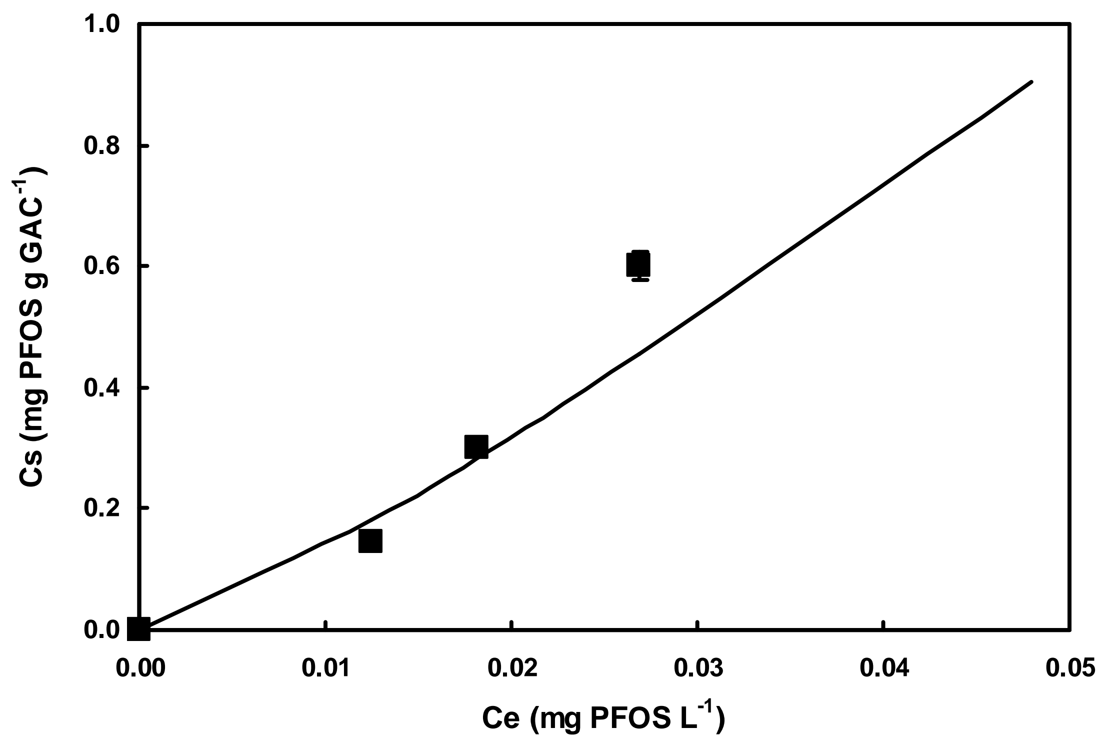


Figure 7.2 Adsorption isotherm of PFOS onto granular activated carbon Calgon F400 at low equilibrium concentrations. Experimental data fit to Freundlich model. Error bars (shown if larger that the symbols) represent standard deviations of duplicate assays.

The constants determined for the different Freundlich and Langmuir isotherms are listed in Table 7.2. The experimental data obtained at intermediate to high equilibrium concentrations of PFOS ($C_e > 0.5$ mg L^{-1}) fitted well to both isotherm models and showed a tendency to attain a maximum adsorption capacity (plateau) as the concentration of PFOS in solution increased (Figure 7.1). In contrast, adsorption data determined with F400 at low equilibrium concentrations of PFOS (12.4-290.0 µg L^{-1}) fitted best to a linear Freundlich model ($n \approx 1$) (Figure 7.2). Linear isotherms are often determined for dissolved species in dilute solutions that are present at concentrations significantly lower than their aqueous solubility.

The isotherms obtained show a strong adsorption of PFOS at low aqueous equilibrium concentrations (< 2 mg l^{-1}) followed by a low to moderate adsorption at higher PFOS concentrations, and they indicate that the affinity of GAC for PFOS is moderate when compared to chemical compounds traditionally considered as suitable for activated carbon treatment. For instance, the K_F determined for the adsorption of PFOS by F300 was 38.5, which is in the same order of magnitude as the Freundlich distribution coefficients reported with the same activated carbon for phenol (21 mg g^{-1}), trichloroethylene (28 mg g^{-1}) and 2-chlorophenol (51 mg g^{-1}) [27]. These K_F values are one order magnitude lower compared to those determined for highly hydrophobic compounds known to have a high affinity for GAC such as lindane (256 mg g^{-1}) or hexachlorobenzene (450 mg g^{-1}) [27].

Table 7.2 Langmuir isotherm constants *a* (mg PFC g sorbent^{-1}) and *b* (L mg PFC^{-1}) and Freundlich isotherm constants K_F [(mg PFC g sorbent^{-1})(mg PFC L^{-1})$^{-n}$] and *n* for the adsorption of PFCs onto various sorbent materials at 30ºC and solution pH 7.2

		Langmuir isotherm			Freundlich isotherm		
Contaminant	**Sorbent**	***a***	***b***	r^2	K_F	***n***	r^2
PFOS	GAC Calgon F300	196.2	0.068	0.977	38.5	0.332	0.939
PFOS	GAC Calgon URV-MOD1	211.6	0.080	0.947	36.7	0.371	0.979
PFOS	GAC Calgon F400	236.4	0.124	0.959	60.9	0.289	0.969
PFOS	GAC Calgon F400-low range	--	--	--	25.9	1.123	0.979
PFOA	GAC Calgon F400	112.1	0.038	0.968	11.8	0.443	0.955
PFBS	GAC Calgon F400	98.70	0.034	0.985	9.3	0.463	0.959
PFOS	NaY zeolite	--	--	--	0.01	1.577	1.000
PFOS	13X zeolite	12.0	0.018	0.993	0.73	0.507	0.976
PFOS	NaY80 zeolite	114.7	0.218	0.991	31.8	0.339	0.988
PFOS	Anaerobic granular sludge*	--	--	--	0.95	1.083	0.983
PFOS	ADS*	6.137	0.115	1.000	1.85	0.262	0.957

* Sludge concentrations expressed as gram dry solids (total suspended solids, TSS).

F400 was the most effective activated carbon as evidenced by the stronger adsorption of PFOS observed at all the concentrations tested (Figure 7.1). Nonetheless, at high aqueous concentrations the adsorption capacity of the three sorbents was relatively close. The superior performance of F400 at low equilibrium concentrations cannot be attributed to the variation in specific surface areas or pore size of the materials, since all three activated carbons have similar characteristics (Table 7.1). The stronger adsorption of PFOS might be accounted for by differences in the chemical composition of the surface layer of F400. Surface chemistry is known to have a great impact on the sorption behavior of activated carbon [28, 29].

7.4.2. PFOA and PFBS adsorption onto activated carbon

Sorption of PFOS by activated carbon F400 was compared to PFOA and PFBS, which are two other perfluoroalkyl surfactants of environmental concern. For the three surfactants, adsorption tended to reach a plateau as their aqueous equilibrium concentration exceeded about 40 to 50 mg L^{-1}, suggesting saturation of the available adsorption sites (Figure 7.3). Both Langmuir and Freundlich isotherms provide a good fit for the sorption of PFOA and PFBS onto F400 (Table 7.2).

PFOA and PFBS were effectively sorbed by GAC, although to a lower extent than PFOS. For instance, for an equilibrium concentration of the surfactant in the aqueous phase of 27 mg L^{-1}, the estimated concentrations of PFOA and PFBS sorbed onto

activated carbon are 57 mg g^{-1} GAC and 48 mg g^{-1} GAC, respectively, compared to 182 mg PFOS g^{-1} GAC. These results are in agreement with a recent study reporting stronger sorption onto sediments for sulfonates as compared to carboxylates of equal perfluorocarbon chain length [30], this observation was attributed to the larger size of the sulfonate substituent [30]. Adsorption of perfluoroalkyl sulfonates onto sediments has also been reported to increase with increasing chain length, with distribution coefficient (K_d) values increasing for each additional CF_2 moiety in a regular fashion [30].

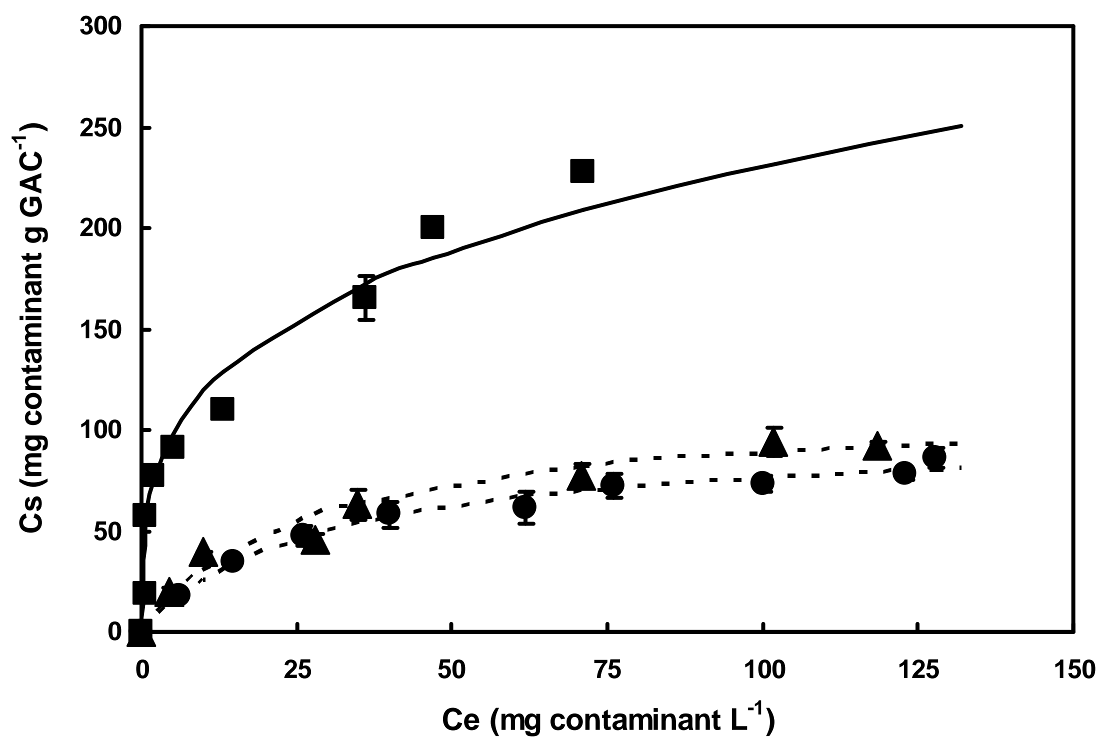


Figure 7.3 Adsorption isotherms of PFOS (■), PFOA (▲), and PFBS (●) PFBS onto activated carbon Calgon F400. Experimental data fit to Langmuir model (dashed line); experimental data fit to Freundlich model (solid line). Error bars (shown if larger that the symbols) represent standard deviations of duplicate assays.

7.4.3 Adsorption of PFOS onto faujasite zeolites

Zeolites are an important class of aluminosilicates widely used in catalysis as well as in the separation and purification fields due to their uniform, small pore size, high internal surface area, and controlled chemistry [31, 32]. Three faujasite zeolites with different Si/Al ratios, zeolite 13 X (Si/Al = 2.8), NaY (Si/Al = 5.5) and NaY80 (Si/Al = 80), were evaluated in this study for their ability to adsorb PFOS. The physical properties of the zeolites are summarized in Table 7.3. PFOS was found to adsorb strongly to the NaY80 (Si/Al 80) zeolite, but poorly to the 13X (Si/Al 2.8) and NaY (Si/Al 5.5) zeolites (Figure 7.4). As an example, the K_F value determined for NaY80 was 31.8, compared to only 0.73 and 0.01 for 13X and NaY, respectively (Table 7.2). It is interesting to note that the capacity of zeolite NaY80 to adsorb PFOS from aqueous solutions was of the same order of magnitude as that of the activated carbons evaluated in this study, which had K_F values ranging from 36.7 to 60.9.

Table 7.3 Physical properties of faujasite zeolites evaluated in this study.

Zeolite	**Pore apertures (nm)**	**SiO_2/Al_2O_3 (mol ratio)**	**Unit cell (Å)**	**Surface area ($m^2\ g^{-1}$)**
13X	1.0	2.8	4.0	n/a
NaY	0.8	5.5	24.6	700
NaY80	0.6	80	24.2	780

Sources: 13X (Sigma-Aldrich), NaY (Wako Chemicals) and NaY80 (Zeolyst International).

The aluminum content of the zeolites evaluated in this study seems to control the adsorption process, as evidenced by the increase in adsorption capacity with increasing molar Si/Al ratio. Similar trends were observed in sorption studies of sodium dodecylbenzene sulfonate with zeolites of varying Si contents [33]. No adsorption of the alkylbenzene sulfonate surfactant occurred on low siliceous NaY zeolites, while sorption was enhanced on the high siliceous NaY zeolites. Hydrophobic interactions appear to play an important role on the adsorption of PFOS since the most hydrophobic zeolite, NaY80, showed the highest adsorption capacity. It is well established that the silica content is the main parameter determining the hydrophobic properties of zeolites. High-silica zeolites such as NaY80 have hydrophobic surfaces properties, while low-silica zeolites such as NaY and 13X preferentially adsorb polar molecules. Although PFOS is a strong acid (estimated pKa -3.27) [34] which is completely dissociated under environmental conditions, the compound has a dual hydrophobic-hydrophilic nature.

The entrance of micropores of the zeolites 13X and NaY is 1.0 and 0.8 nm in diameter, respectively, while the zeolite NaY80 is 0.6 nm in diameter. The smaller pore aperture of the zeolite showing the best sorptive capacity, NaY80, suggests that the poor sorptive capacity of the two other zeolites was not due to molecular exclusion.

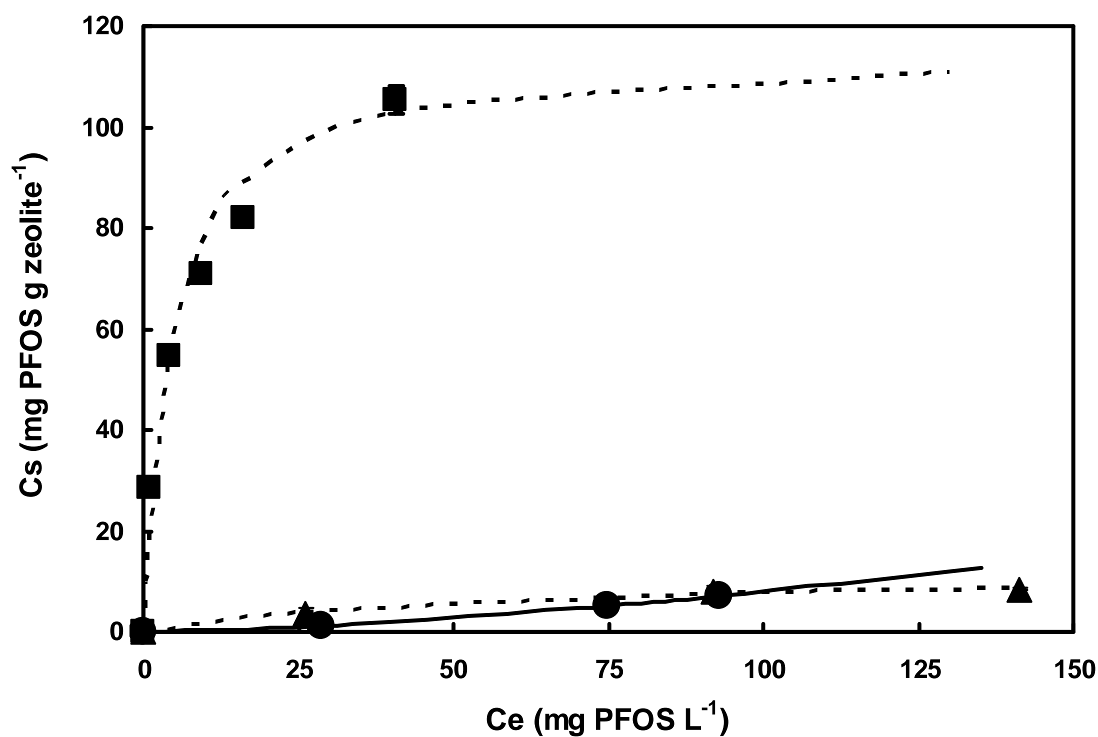


Figure 7.4 Adsorption isotherms of PFOS onto various zeolite types: NaY80 (Si/Al 80) (■); NaY (Si/Al 5.5) (●), and 13X (Si/Al 2.8). Experimental data fit to Langmuir model (dashed line); experimental data fit to Freundlich model (solid line). Error bars (shown if larger that the symbols) represent standard deviations of duplicate assays.

7.4.4 Adsorption of PFOS onto wastewater treatment sludge

Biosorption has been proposed as an alternative to replace current removal processes of hazardous contaminants from wastewaters [35]. The potential of wastewater treatment sludge as an adsorbent has received attention due to its ability to sorb hydrophobic organic compounds and low-cost as compared to conventional adsorbents. PFOS and related perfluoroalkyl surfactants have been detected in sewage sludge from several wastewater treatment plants at ng g^{-1} concentrations, suggesting partitioning of these compounds from the aqueous phase to the biosolids during the treatment process [13, 14, 36-39].

Figure 7.5 shows the isotherms for the adsorption of PFOS by two sludge samples obtained from different wastewater treatment plants, i*.e.,* anaerobic granular sludge and anaerobically digested sewage sludge (ADS). The isotherm for return aerobic activated sludge (RAS) is not shown since PFOS adsorption was negligible even at high aqueous equilibrium concentrations (up to 90 mg L^{-1}).

The results obtained for ADS show that adsorption appears to reach saturation as the equilibrium concentration of PFOS in solution approaches 14 mg L^{-1}, suggesting monolayer coverage of active sites. In contrast, the anaerobic granular sludge data fit a linear Freundlich isotherm (n = 1.083) spanning over the whole range of equilibrium concentrations evaluated (up to 80.9 mg PFOS L^{-1}) (Table 7.2). Kennedy *et al.* [40] studied the sorption of chlorophenols by anaerobic granular sludge and observed linear isotherms for the majority of chlorophenols. This linear adsorption behavior was

attributed to the highly porous structure of granular anaerobic sludge which would allow penetration of the adsorbate to sorption sites in the inner surface.

The K_d values determined in this study for ADS ranged from 77 to 277 L kg^{-1} dry-sludge, and for anaerobic granular sludge from 143 to 209 L kg^{-1} dry-sludge. A K_d of 120 L kg^{-1} was previously reported for the adsorption of PFOS onto municipal sewage treatment sludge [41]. However, the K_d values determined for the adsorption of PFOS onto biosolids are several orders of magnitude lower compared to those of bioaccumulative organic pollutants such as polychlorinated biphenyls and organochlorine pesticides [42], indicating that the perfluoroalkyl surfactant has a lower tendency to partition onto the sludge.

The results obtained clearly demonstrate that the type of the sludge strongly influences the degree of PFOS sorption and suggest that characteristics other than organic matter content must contribute to controlling sorption of PFOS onto wastewater treatment sludge. Indeed, given the dual oleophobic–hydrophobic nature of PFOS, it is unlikely that the adsorption mechanism obeys simple hydrophobic partitioning paradigms. Higgins and coworkers confirmed the importance of hydrophobic interactions on the adsorption of PFOS by sediment, but these authors also demonstrated that electrostatic interactions play a role in the adsorption of anionic PFC surfactants [30, 43].

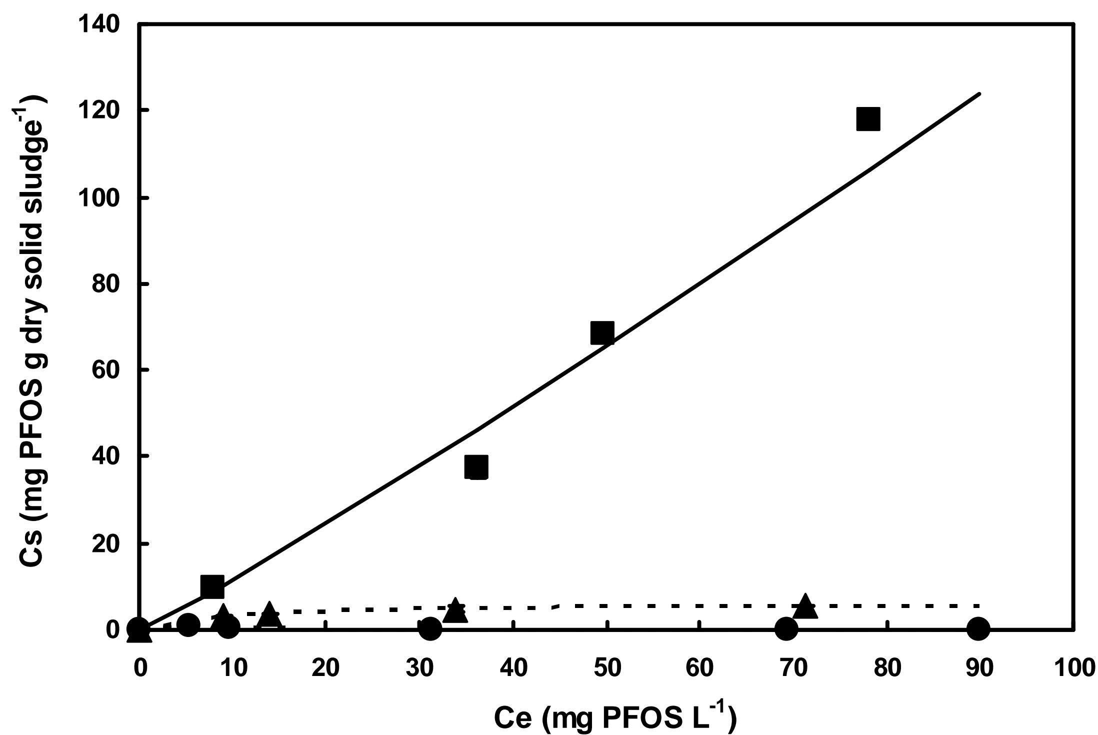


Figure 7.5 Adsorption isotherms of PFOS onto different wastewater treatment sludges: anaerobic granular sludge (■), anaerobic digested sludge or ADS (▲), aerobic activated sludge or RAS (●). Experimental data fit to Langmuir model (dashed line); experimental data fit to Freundlich model (solid line). Error bars (shown if larger that the symbols) represent standard deviations of duplicate assays.

7.5 Conclusions

The adsorption of PFOS, PFOA and PFBS from aqueous solutions onto granular activated carbon was demonstrated in this study. The substitution of the sulfonic group by a carboxylic group as well as a decreasing fluorocarbon chain contributed to a weaker adsorption of the PFCs by activated carbon, as evidenced by the stronger adsorption of PFOS compared to PFOA and PFBS. Activated carbon provided a high adsorptive capacity for PFC surfactants when these compounds were present at low mg L^{-1} concentrations; however, the adsorptive capacity was only moderate at higher PFC concentrations. Hydrophobic zeolites with a high silica content (e.g. zeolite NaY80, Si/Al = 80) were also found to adsorb PFOS, while low silica zeolites (Si/Al < 5.5) showed very poor sorption capacity. Furthermore, biosorption of PFOS by various wastewater treatment sludges was demonstrated. Comparison of the sorption capacity of GAC with that of various zeolites and wastewater treatment sludges demonstrated that the affinity of the various sorbents for PFOS decreased as follows: GAC > hydrophobic zeolite > anaerobic granular sludge > activated sludge. Taken together these results indicate that GAC sorption is a promising treatment for the removal of anionic perfluoroalkyl surfactants from dilute aqueous streams. These findings also suggest that some removal of PFOS by biosorption should be expected during biological wastewater treatment, and that extent of partitioning will depend on the characteristics of the sludge.

7.6 Acknowledgments

This study was conducted with financial support from the Semiconductor Research Corporation/Sematech Engineering Research Center for Environmentally Benign Semiconductor Manufacturing.

7.7 References

1. Kissa, E., *Fluorinated surfactants and repellants*. Marcel Dekker, Inc.: New York, 2001.
2. Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J., Perfluoroalkyl acids: A review of monitoring and toxicological findings. *Toxicol. Sci.* **2007,** *99*, (2), 366-394.
3. Ellis, D. A.; Mabury, S. A.; Martin, J. W.; Muir, D. C. G., Thermolysis of fluoropolymers as a potential source of halogenated organic acids in the environment. *Nature* **2001,** *412*, (6844), 321-324.
4. Ellis, D. A.; Martin, J. W.; De Silva, A. O.; Mabury, S. A.; Hurley, M. D.; Andersen, M. P. S.; Wallington, T. J., Degradation of fluorotelomer alcohols: A likely atmospheric source of perfluorinated carboxylic acids. *Environ. Sci. Technol.* **2004,** *38*, (12), 3316-3321.

5. Dinglasan, M. J. A.; Ye, Y.; Edwards, E. A.; Mabury, S. A., Fluorotelomer alcohol biodegradation yields poly- and perfluorinated acids. *Environ. Sci. Technol.* **2004,** *38*, (10), 2857-2864.

6. Dimitrov, S.; Kamenska, V.; Walker, J. D.; Windle, W.; Purdy, R.; Lewis, M.; Mekenyan, O., Predicting the biodegradation products of perfluorinated chemicals using CATABOL. *Sar and Qsar in Environmental Research* **2004,** *15*, (1), 69-82.

7. Wang, N.; Szostek, B.; Buck, R. C.; Folsom, P. W.; Sulecki, L. M.; Capka, V.; Berti, W. R.; Gannon, J. T., Fluorotelomer alcohol biodegradation - Direct evidence that perfluorinated carbon chains breakdown. *Environ. Sci. Technol.* **2005,** *39*, (19), 7516-7528.

8. Houde, M.; Martin, J. W.; Letcher, R. J.; Solomon, K. R.; Muir, D. C. G., Biological monitoring of polyfluoroalkyl substances: A review. *Environ. Sci. Technol.* **2006,** *40*, (11), 3463-3473.

9. 3M, Technical data bulletin. Environmental health, safety and regulatory (ehsr) profile of perfluorobutane sulfonate (PFBS). *3M, St Paul, MN.* **2002**.

10. Martin, J. W.; Mabury, S. A.; Solomon, K. R.; Muir, D. C. G., Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (Oncorhynchus mykiss). *Environ. Toxicol. Chem.* **2003,** *22*, (1), 196-204.

11. Skutlarek, D.; Exner, M.; Farber, H., Perfluorinated surfactants in surface and drinking water. *Environ. Sci. Pollut. Res.* **2006,** *13*, (5), 299-307.

12. Alzaga, R.; Bayona, J. M., Determination of perfluorocarboxylic acids in aqueous matrices by ion-pair solid-phase microextraction-in-port derivatization-gas

chromatography-negative ion chemical ionization mass spectrometry. *J. Chromatogr. A* **2004,** *1042*, (1-2), 155-162.

13. Higgins, C. P.; Field, J. A.; Criddle, C. S.; Luthy, R. G., Quantitative determination of perfluorochemicals in sediments and domestic sludge. *Environ. Sci. Technol.* **2005,** *39*, (11), 3946-3956.

14. Sinclair, E.; Kannan, K., Mass loading and fate of perfluoroalkyl surfactants in wastewater treatment plants. *Environ. Sci. Technol.* **2006,** *40*, (5), 1408-1414.

15. Moody, C. A.; Hebert, G. N.; Strauss, S. H.; Field, J. A., Occurrence and persistence of perfluorooctanesulfonate and other perfluorinated surfactants in groundwater at a fire-training area at Wurtsmith Air Force Base, Michigan, USA. *J. Environ. Monit.* **2003,** *5*, (2), 341-345.

16. Anonymous, Ground-water sampling for PFOA near the Washington Co. landfill. *Minnesota Pollution Control Agency, Minnesota Department of Health. St. Paul, MN* **2004**.

17. Tang, C. Y. Y.; Fu, Q. S.; Robertson, A. P.; Criddle, C. S.; Leckie, J. O., Use of reverse osmosis membranes to remove perfluorooctane sulfonate (PFOS) from semiconductor wastewater. *Environ. Sci. Technol.* **2006,** *40*, (23), 7343-7349.

18. Anonymous, Environmental Protection Agency tentatively OKs PFOS for semiconductor industry. *Solid State Technology* **2002,** *45*, (32).

19. NICNAS, Potassium perfluorobutane sulfonate. Existing chemical hazard assessment report. *Department of Health and Ageing, National Industrial*

Chemicals Notification and Assessment Scheme (NICNAS), Australian Government, Sidney, Australia **2005**.

20. Carter, K. E.; Farrell, J., Oxidative destruction of perfluorooctane sulfonate using boron-doped diamond film electrodes. *Environ. Sci. Technol.* **2008,** *42*, (16), 6111-6115.

21. Moriwaki, H.; Takagi, Y.; Tanaka, M.; Tsuruho, K.; Okitsu, K.; Maeda, Y., Sonochemical decomposition of perfluorooctane sulfonate and perfluorooctanoic acid. *Environ. Sci. Technol.* **2005,** *39*, (9), 3388-3392.

22. Hori, H.; Nagaoka, Y.; Yamamoto, A.; Sano, T.; Yamashita, N.; Taniyasu, S.; Kutsuna, S.; Osaka, I.; Arakawa, R., Efficient decomposition of environmentally persistent perfluorooctanesulfonate and related fluorochemicals using zerovalent iron in subcritical water. *Environ. Sci. Technol.* **2006,** *40*, (3), 1049-1054.

23. Ihara, Y., Adsorption of Anionic Surfactants and Related-Compounds from Aqueous-Solution onto Activated Carbon and Synthetic Adsorbent. *J. Appl. Polym. Sci.* **1992,** *44*, (10), 1837-1840.

24. Zor, S., Investigation of the adsorption of anionic surfactants at different pH values by means of active carbon and the kinetics of adsorption. *J. Serb. Chem. Soc.* **2004,** *69*, (1), 25-32.

25. Gauden, P. A.; Szmechtig-Gauden, E.; Rychlicki, G.; Duber, S.; Garbacz, J. K.; Buczkowski, R., Changes of the porous structure of activated carbons applied in a filter bed pilot operation. *J. Colloid Interface Sci.* **2006,** *295*, (2), 327-347.

26. Huling, S. G.; Jones, P. K.; Ela, W. P.; Arnold, R. G., Fenton-driven chemical regeneration of MTBE-spent GAC. *Water Res.* **2005,** *39*, (10), 2145-2153.

27. Dobbs, R. A.; Cohen, J. M. *Carbon adsorption isotherm of toxic organics*; EPA-600/8-80-023; EPA-600/8-80-023, U.S. Environmental Protection Agency: 1980.

28. Haghseresht, F.; Nouri, S.; Finnerty, J. J.; Lu, G. Q., Effects of surface chemistry on aromatic compound adsorption from dilute aqueous solutions by activated carbon. *Journal of Physical Chemistry B* **2002,** *106*, (42), 10935-10943.

29. Dabrowski, A.; Podkoscielny, P.; Hubicki, Z.; Barczak, M., Adsorption of phenolic compounds by activated carbon - a critical review. *Chemosphere* **2005,** *58*, (8), 1049-1070.

30. Higgins, C. P.; Luthy, R. G., Sorption of perfluorinated surfactants on sediments. *Environ. Sci. Technol.* **2006,** *40*, (23), 7251-7256.

31. Bailey, S. E.; Olin, T. J.; Bricka, R. M.; Adrian, D. D., A review of potentially low-cost sorbents for heavy metals. *Water Res.* **1999,** *33*, (11), 2469-2479.

32. Tao, Y. S.; Kanoh, H.; Abrams, L.; Kaneko, K., Mesopore-modified zeolites: Preparation, characterization, and applications. *Chem. Rev.* **2006,** *106*, (3), 896-910.

33. Kawai, T.; Tsutsumi, K., Adsorption characteristics of surfactants and phenol on modified zeolites from their aqueous-solutions. *Colloid and Polymer Science* **1995,** *273*, (8), 787-792.

34. Brooke, D.; Footitt, A.; Nwaogu, T. A., Environmental risk evaluation report: Perfluorooctanesulphonate (PFOS). *Environment Agency, Chemicals Assessment Section, Wallingford, UK* **2004**.

35. Aksu, Z., Application of biosorption for the removal of organic pollutants: A review. *Process Biochem.* **2005,** *40*, (3-4), 997-1026.

36. Boulanger, B.; Vargo, J. D.; Schnoor, J. L.; Hornbuckle, K. C., Evaluation of perfluorooctane surfactants in a wastewater treatment system and in a commercial surface protection product. *Environ. Sci. Technol.* **2005,** *39*, (15), 5524-5530.

37. Nakata, H.; Kannan, K.; Nasu, T.; Cho, H. S.; Sinclair, E.; Takemura, A., Perfluorinated contaminants in sediments and aquatic organisms collected from shallow water and tidal flat areas of the Ariake Sea, Japan: Environmental fate of perfluorooctane sulfonate in aquatic ecosystems. *Environ. Sci. Technol.* **2006,** *40*, (16), 4916-4921.

38. Schultz, M. M.; Barofsky, D. F.; Field, J. A., Quantitative determination of fluorinated alkyl substances by large-volume-injection liquid chromatography tandem mass spectrometry - Characterization of municipal wastewaters. *Environ. Sci. Technol.* **2006,** *40*, (1), 289-295.

39. Loganathan, B. G.; Sajwan, K. S.; Sinclair, E.; Kumar, K. S.; Kannan, K., Perfluoroalkyl sulfonates and perfluorocarboxylates in two wastewater treatment facilities in Kentucky and Georgia. *Water Res.* **2007,** *41*, (20), 4611-4620.

40. Kennedy, K. J.; Lu, J. H.; Mohn, W. W., Biosorption of Chlorophenols to Anaerobic Granular Sludge. *Water Res.* **1992,** *26*, (8), 1085-1092.

41. Ellefson, M., Soil adsorption/desorption study of potassium perlfuorooctane sulfonate (PFOS). *EPA Docket AR226-1030a030; 3M Company: Maplewood, MN, USA*. **2001**.

42. Katsoyiannis, A.; Samara, C., Persistent organic pollutants (POPs) in the conventional activated sludge treatment process: fate and mass balance. *Environ. Res.* **2005,** *97*, 245-257.

43. Higgins, C. P.; Luthy, R. G., Modeling sorption of anionic surfactants onto sediment materials: An a priori approach for perfluoroalkyl surfactants and linear alkylbenzene sulfonates (vol 110, pg 14054, 2006). *Environ. Sci. Technol.* **2007,** *41*, (17), 6316-6316.

CHAPTER 8

Toxicity of Fluoride to Microorganisms in Biological Wastewater Treatment Systems

8.1 Abstract

Fluoride is a common contaminant in a variety of industrial wastewaters. Available information on the potential toxicity of fluoride to microorganisms implicated on biological wastewater treatment is very limited. The objective of this study was to evaluate the inhibitory effect of fluoride towards the main microbial populations responsible for the removal of organic constituents and nutrients in wastewater treatment processes. The results of short-term batch bioassays indicated that the toxicity of fluoride varied widely depending on the microbial population. Anaerobic microorganisms involved in various metabolic steps in anaerobic digestion processes were found to be very sensitive to the presence of fluoride. The concentrations of fluoride causing 50% metabolic inhibition (IC_{50}) of propionate- and butyrate-degrading microorganisms as well mesophilic and thermophilic acetate-utilizing methanogens ranged from 18 to 43 mg L^{-1}. Fluoride was also inhibitory to microbial nitrification, albeit at relatively high levels (IC_{50}

= 149 mg L^{-1}). Nitrifying bacteria appeared to adapt rapidly to fluoride, and a near complete recovery of their metabolic activity was observed after only 4 d of exposure to high fluoride levels (up to 500 mg L^{-1}). All other microbial populations evaluated in this study, *i.e.*, glucose fermenters, aerobic glucose-degrading heterotrophs denitrifying bacteria, and H_2-utilizing methanogens tolerated fluoride at very high concentrations (> 500 mg L^{-1}). Fluoride exerted moderate to low inhibition towards the aquatic biomonitoring species, water fleas (*Ceriodaphnia dubia*) and fathead minnows (*Pimephales promelas*), as indicated by 50% lethal concentrations (96 h-LC_{50}) of 85 and 200 mg L^{-1}, respectively.

Key words: fluoride, microbial toxicity, glucose, nitrification, denitrification, methanogenesis, fermentation and biomonitoring.

This chapter is in preparation for submission as a paper entitled *"Toxicity of Fluoride to Microorganisms in Biological Wastewater Treatment Systems"*

8.2 Introduction

Fluoride is a widespread environmental contaminant and is estimated to be the 13th most abundant element on the earth crust [1]. The public health benefits and risks of fluoride from drinking water and other sources has been a source of controversy in recent years. Although, it is beneficial to human health at low concentrations (0.7 to 1.2 mg L^{-1}) by affording protection against dental caries, at concentrations exceeding the federal drinking water standard (4 mg L^{-1}), fluoride has been reported to cause skeletal and dental fluorosis [2]. Fluoride is ubiquitous in the environment as it is a component of most types of soils. Concentrations of inorganic fluoride in unpolluted surface water generally range from 0.01 to 0.30 mg L^{-1}, but considerably higher concentrations may be found in regions impacted by geothermal or volcanic activity [3].

Human activities effluents can also contribute to increase the concentration of fluoride in aquatic environments. Fluoride is often present in a variety of untreated industrial effluents, including those from chemical plants manufacturing organofluorine compounds, aluminum smelters, phosphate-fertilizers, semiconductor manufacturing, glass and brick-making industries and coal power plants [3-7]. The concentrations of fluoride in untreated industrial wastewaters vary widely, and concentrations as high as 500 to 2000 mg L^{-1} have been reported in effluents from semiconductor industry operations in Taiwan [8, 9]. In contrast, fluoride levels in municipal wastewaters receiving fluoride-bearing industrial effluents are generally low because industrial effluents will be diluted by wastewaters from residential and industrial sources. In

addition, significant removal of fluoride can occur by precipitation with calcium (II), which is a common wastewater contaminant. Nonetheless, although calcium forms insoluble salts with fluoride (CaF_2, K_{sp}= 3.9 10^{-11}), the precipitation of calcium fluoride only reduces fluoride to concentrations of approximately 20 mg L^{-1} [10].

In spite of the significance of fluoride as a wastewater contaminant, published data on the inhibitory effect of fluoride to microbial populations present in wastewater treatment systems are very limited. Nitrification is the only process that has been studied in some detail [11-13]. In contrast with the limited understanding of the potential inhibitory effects of fluoride on wastewater treatment microorganisms, the impact of fluoride on oral bacteria of interest to dentistry are well documented by a vast body of literature [14, 15]. Oral bacteria are inhibited by fluoride at concentrations in the range of 10 to 1,600 mg L^{-1}. Inhibition of soil microorganisms by inorganic fluoride, resulting in increased accumulation of soil organic matter, has also been reported in several studies [16-18]. In one study microbial biomass and enzymatic activity in soil were decreased substantially at water-extractable fluoride concentrations exceeding 20 μg F^- g^{-1} soil [17]. These findings suggest that, if present at sufficient concentration, fluoride might have a negative impact on biological wastewater treatment systems and higher aquatic organisms.

The objective of this study is to evaluate the inhibitory effect of inorganic fluoride towards the main microbial populations involved in organic matter and nitrogen nutrient removal in wastewater treatment plants. The acute toxic impact of fluoride against two

organisms commonly used in effluent biomonitoring, *i.e.,* fathead minnows (*Pimephales promelas*) and water fleas (*Ceriodaphnia dubia*), was also evaluated in this study.

8.3 Materials and Methods

8.3.1 Chemicals

Potassium nitrate (> 99.0% purity) and sodium acetate (99.0%) were obtained from Spectrum Chemicals and Laboratory Products (Gardena, CA, USA). Sodium fluoride (99.0%), sodium nitrite (> 99.5%), acetic acid (≥99.7%), propionic acid (≥99.5%) and butyric acid (≥ 99.0%) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Ammonium bicarbonate was obtained from MP Biomedicals (Solon, OH, USA). Phenol (99.0% ACS grade) was obtained from EMD chemicals (Gibbstown, NJ). D-glucose anhydrous was purchased from Mallinckrodt Chemical (Paris, KY). N_2/CO_2 and H_2/CO_2 gases (80/20, v/v) were delivered from US Air (Phoenix, AZ, USA). Methane (> 99.99%) was obtained from Scotty Specialty Gases (San Bernardino, CA, USA). All chemicals were used as received.

8.3.2 Sludge sources

Three different mesophilic inocula were evaluated in this study, including two types of methanogenic granular sludge (Eerbeek and Aviko sludge) and anaerobically digested sewage sludge (Ina Road sludge). Eerbeek sludge was obtained from an industrial

anaerobic sludge blanket (UASB) reactor treating recycle paper effluent (Industriewater, Eerbeek, The Netherlands), and the Aviko sludge from a UASB reactor treating potato processing wastewater (Aviko, Steenderen, The Netherlands). Both inocula were washed and sieved to remove fine particles and they were stored under nitrogen gas at 4ºC. The content of volatile suspended solids (VSS) in the Eerbeek and Aviko sludges was 12.9% and 11.5%, respectively. The Ina Road sludge (1.5% VSS) was obtained from an anaerobic sewage sludge digester at the Ina Road Municipal Wastewater Treatment plant, Tucson, Arizona. A thermophilic inoculum (Hyperion sludge, 1.55% VSS) was also utilized in the methanogenic studies, which was obtained from an anaerobic digestor treating sewage sludge at the Hyperion municipal wastewater treatment plant, Los Angeles, CA.

The inoculum utilized in the nitrification assays, Randolph Park sludge, was obtained from the nitrification stage of a full-scale sewage treatment facility (Randolph Park Wastewater Reclamation Facility, Tucson, Arizona). The VSS content in the sludge was 4.8%. Glucose-degrading enrichment cultures were obtained under anaerobic and aerobic conditions using Aviko sludge and Randolph Park sludge, respectively. The enrichment cultures were developed by transferring the culture to fresh glucose containing basal medium at a rate of 5% (v/v) when the glucose was consumed. The aerobic sewage sludge, Randolph Park sludge II (0.13% VSS), was obtained from the aeration tank of the Randolph Park Wastewater Reclamation Facility, in Tucson, Arizona. All sludge samples were stored under nitrogen gas in a refrigerator at 4°C.

8.3.3 Culture media

The composition of the basal mineral medium supplied in the nitrification bioassays (BM-1) was (in mg L^{-1}): $NaH_2PO_4 \bullet H_2O$ (1,200); Na_2HPO_4 (715), and 1 mL L^{-1} of trace element solution. The basal mineral medium employed in the denitrification bioassays (BM-2) contained (in mg L^{-1}): K_2HPO_4 (250); $(NH_4)HCO_3$ (417); $NaHCO_3$ (1,500); yeast extract (10) and trace element solution (1 mL L^{-1}). The basal mineral medium utilized in both the aerobic heterotrophic toxicity and fermentation bioassays (BM-3) contained (in mg L^{-1}): NH_4Cl (280); $NaHCO_3$ (5,000); K_2HPO_4 (250); KH_2PO_4 (2,050); $CaSO_4 \bullet 2\ H_2O$ (10), $MgSO_4 \bullet 7\ H_2O$ (100), yeast extract (50), and 1 mL L^{-1} of trace element solution. The basal mineral medium used in all other anaerobic bioassays (BM-4) contained (in mg L^{-1}): K_2HPO_4 (37); $CaCl_2 \bullet 2\ H_2O$ (10); $MgSO_4 \bullet 7\ H_2O$ (10); $MgCl_2 \bullet 6\ H_2O$ (78); NH_4Cl (669); $NaHCO_3$ (3,003); yeast extract (20), and 1 mL L^{-1} of trace element solution. The trace element solution contained (in mg L^{-1}): H_3BO_3 (50), $FeCl_2 \bullet 4\ H_2O$ (2,000), $ZnCl_2$ (50), $MnCl_2 \bullet 4H_2O$ (50), $(NH_4)_6Mo_7O_{24} \bullet 4H_2O$ (50), $AlCl_3 \bullet 6\ H_2O$ (90), $CoCl_2 \bullet 6\ H_2O$ (2000), $NiCl_2 \bullet 6\ H_2O$ (50), $CuCl_2 \bullet 2\ H_2O$ (30), $NaSeO_3 \bullet 5\ H_2O$ (100), EDTA (1,000), resazurin (200) and 36% HCl (1 mL L^{-1}). All media were adjusted to pH 7.2 with HCl or NaOH, as required.

8.3.4 Microbial toxicity assays

All microbial toxicity assays (with the exception of the Microtox® assay) were incubated in a climate-controlled chamber at 30±2˚C in an orbital shaker (110 r.p.m.). In all bioassays the desired amount of F^- was added to flasks using neutralized concentrated stock solutions of sodium fluoride. The maximum specific glucose consumption (mg glucose-degraded g^{-1} VSS d^{-1}), nitrifying (mg NH_4^+-consumed g^{-1} VSS d^{-1}), denitrifying (mg NO_3^- g^{-1} VSS d^{-1}) and methanogenic (mg CH_4-COD g^{-1} VSS d^{-1}) activities were calculated from the slope of the glucose consumption, ammonium concentration, nitrate concentration and cumulative methane production; respectively, and biomass concentration versus time (d), as the mean value of triplicate or duplicate assays. The initial concentrations of F^- causing 20, 50 and 80% reduction in activity compared to an uninhibited control were referred to as IC_{20}, IC_{50} and IC_{80}, respectively.

<u>*Nitrification toxicity assays*</u>

Maximum specific nitrifying activities measurements were performed in serum flasks (165 mL) supplied with 50 mL of culture medium. Basal medium BM-1, ammonium (61 mg L^{-1}, added as $(NH_4)HCO_3$,) and 0.5 g VSS L^{-1} of Randolph Park sludge were added to each vial followed by addition of F^-. Substrate controls were based on assays where no toxicant was added. The headspace content was replenished daily with air to prevent

oxygen limitation. Liquid samples were taken periodically and analyzed for ammonia. Nitrite and nitrate concentrations were also determined in selected samples.

Denitrification toxicity assays

Maximum specific denitrifying activities measurements were carried out in duplicate serum flasks (165 mL) supplied with 100 mL of culture medium. Basal medium BM-2 (95 mL), nitrate (1,010 mg L^{-1} as KNO_3), electron accepting substrate (450 mg L^{-1} acetate as sodium acetate) and microbial inoculum (1.0 g VSS L^{-1} of Eerbeek sludge) were added to each of the vials. Following addition of fluoride, flasks were flushed with N_2:CO_2 gas (80:20, v/v), sealed with butyl rubber stoppers and aluminum crimp seals, prior to incubation. Duplicate substrate controls were based on assays where no toxicant was added. Liquid samples were taken periodically to determine nitrate utilization.

Toxicity to aerobic heterotrophs

Microbial inhibition of fluoride towards aerobic heterotrophs was determined in shaken batch bioassays provided with glucose as the substrate. Tests were carried out in triplicate in Erlenmeyer flasks (125 mL) supplied with 50 mL of culture medium. The inoculum (5% v/v of glucose-degrading enrichment culture obtained from Randolph Park aerobic

sludge) was transferred to flasks containing basal medium BM-3 and glucose (1 g L^{-1}) followed by addition of F^-. Controls were based on assays where no toxicant was added. All flasks were loosely capped with a cotton prop to allow air transport. Liquid samples were withdrawn from each flask at different time intervals and then centrifuged.

Inhibition of glucose fermentation

Tests were carried out in triplicate in serum flasks (165 mL) supplied with 50 mL of culture medium. All flasks were sealed with butyl rubber stoppers and aluminum crimp seals, and then the headspace was flushed with N_2:CO_2 gas (80:20, v/v) to exclude oxygen from the assay. Controls were based on assays where no toxicant was added. Two sets of shaken batch toxicity bioassays were conducted in order to evaluate the impact of fluoride on the growth (test A) and the metabolic activity (test B) of glucose fermentors, respectively. In test A, 5% v/v of the glucose-degrading enrichment culture was transferred to flasks containing basal medium BM-3 spiked with glucose (1 g L^{-1}). Flasks were incubated overnight to ensure that the sludge was adapted to the medium. On the following day, the medium was re-spiked with glucose (2 g L^{-1}) using a concentrated stock solution followed by addition of F^-. Liquid samples were obtained periodically to monitor the glucose consumption. In test B, 1.5 g VSS L^{-1} of Aviko granular sludge was transferred to vials containing basal medium BM-3 spiked with glucose (3 g L^{-1}). Following addition of the toxicant, the headspace was flushed with N_2:CO_2 gas (80:20,

v/v) and flasks were incubated overnight. Glucose readings were taken on the following day.

Methanogenic toxicity assays

Maximum specific hydrogenotrophic and acetoclastic methanogenic activities measurements were conducted at 30±2 ºC (mesophilic assays), and 55±2ºC (thermophilic assays). Eerbeek sludge or Ina Road anaerobically-digested sludge (mesophilic inocula) or Hyperion thermophilic sludge were added to serum flasks (165 mL) containing 25 mL of basal medium BM-4 to reach a final concentration of 1.5 g VSS L^{-1}. All flasks were sealed with butyl rubber stoppers and aluminum crimp seals, and their headspace was flushed with N_2:CO_2 gas (80:20, v/v). The culture medium in acetoclastic assays was amended with acetate at a final concentration of 1,876 mg L^{-1} (as sodium acetate) In assays utilizing hydrogen, H_2/CO_2 was supplied by pressurizing bottles with 152 kPa of H_2/CO_2 (80:20, v/v). The flasks were pre-incubated overnight to ensure that the sludge was adapted to the medium conditions. On the following day, the headspace of acetoclastic assays was again flushed with N_2/CO_2. For the assays utilizing hydrogen, H_2/CO_2 was also added again. Then, F^- was added as NaF. Triplicate substrate controls were based on assays where no toxicant was added.

The methane content in the headspace of each flask was determined periodically until 80% or more of the substrate in the controls was depleted. Subsequently, a second

feeding of the substrate (acetate or H_2/CO_2) was supplied in some assays to test the possible impact of extended exposure to fluoride on the metabolic activity of the methanogens. The methane content in the headspace of each flask was again determined periodically during the several days. The duration of the first feeding in the acetoclastic and hydrogenotrophic mesophilic methanogenic assays inoculated with Ina Road sludge was 15.3 d. In assays inoculated with Eerbeek granular sludge and fed H_2 and acetate, respectively, the first feeding extended for 2.5 and 4.6 d. In the case of the thermophilic assays, the duration of the first feeding in the acetoclastic and hydrogenotrophic methanogenic assays was 9.4 and 2.5 d, respectively.

Toxicity to anaerobic propionate and butyrate-degrading bacteria.

The inhibitory effect of fluoride to anaerobic propionate- and butyrate-degrading bacteria was assayed following the methodology described above for the methanogenic assays, with some modifications. Aviko anaerobic sludge (1.5 g VSS L^{-1}) was added to a serum flask (165 mL) containing 50 mL of BM-4. Propionate and butyrate were spiked from concentrated stocks (pH 7.2) to attain a final concentration of 1.4 and 1.1 g L^{-1}, respectively, which is equivalent to 2.1 and 2.0 g chemical oxygen demand (COD) L^{-1}. The methane production and the volatile fatty acids (VFA, *i.e.,* acetate, propionate and butyrate) consumption were monitored periodically.

8.3.5 Aquatic toxicity

Microtox® assay

The assays were conducted using the Microtox® Toxicity Analyzer model 500 and lyophilized cultures of the luminescent, marine bacterium *Vibrio fischeri* NRRL-B-11177 (AZUR Environmental, Carlsbad, California, USA). The test quantifies the inhibition of luminescence caused by different concentrations of the suspected toxicant. Bacterial bioluminescence is related directly to cell respiration. Microbial inhibition results in a decreased rate of respiration and a corresponding decrease in the rate of luminescence. The inhibition of bacterial light emission was measured at 25ºC in triplicate experiments as recommended elsewhere [19]. Briefly, 2.5 mL of sample was diluted with 250 µL of a Microtox® reagent, then approximately 1 ml of test sample was added to 100 µl of the reconstituted bacteria. Luminescence readings were taken prior to adding the aqueous samples and then at 5, 15 and 30 min of exposure. The Microtox® data acquisition software MTX7 was used to calculate the values causing 20, 50, and 80% reduction in bioluminescence.

Toxicity to biomonitoring species

The acute toxicity of F^- towards the water flea, *Ceriodaphnia dubia,* and the fish species, *Pimephales promenas* (common name "fathead minnows"), was determined according to the protocol described in EPA-821-R-02-012 [20]. Briefly, the test involved exposure of the organisms to the toxicant in a well- defined medium for 96 h at a temperature of 25ºC. The medium was renewed after 48 h of exposure. Survival, temperature, dissolved oxygen, conductivity, and pH were measured daily during each trial. All end-points were expressed as lethal concentrations (LC) and they were determined using the Probit method.

8.3.6 Analytical methods

The concentration of acetate, propionate, and butyrate in liquid samples as well as the methane content in the headspace of the serum flasks was determined by gas chromatography using an HP5290 Series II system (Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector (GC-FID). The GC was fitted with a DB-FFAP column (J&W Scientific, Palo Alto, CA) capillary column. The temperature of the column, the injector port and the detector was 140, 180 and 275°C, respectively. The carrier gas was helium at a flow rate of 9.3 mL min^{-1} and a split flow of 32.4 mL min^{-1}. Samples for measuring methane content (100 µL) in the headspace were collected using a

pressure-lock gas syringe. Sugars were determined colorimetrically [21]. Briefly, 0.5 mL of centrifuged sample was transferred into a test tube and then 0.5 mL of 5% (v/v) phenol and 2.5 mL of concentrated sulfuric acid were added. The sample was vortexed and allowed to rest for 7 min. Subsequently, the tube was heated at 45ºC for 20 min. The solution was allowed to cool down and then the concentration of glucose in the bioassays was determined by measuring the color intensity of the sample at 490 nm. Calibration curves containing glucose concentrations ranging from 2.9 to 14.3 mg L^{-1} were previously constructed. Blank samples were analyzed to correct for background noise.

Nitrate, nitrite and fluoride were analyzed by suppressed conductivity ion chromatography using a Dionex 3000 system (Sunnyvale, CA, USA) fitted with a Dionex IonPac AS18 analytical column (4 x 250 mm) and an AG18 guard column (4 x 40 mm). The column was maintained at 35ºC. The eluent used was 10 mM KOH at a flow rate of 1.0 mL min^{-1}. The injection volume was 25 µL. Before measurement, all samples were either, centrifuged (10,000 rpm) for 10 min or passed through a membrane filter (0.45 µm). Routine analyses of F^- were conducted using a VWR SympHony fluoride-selective combination electrode. Ammonium was determined using an Orion Thermo combination ion-selective electrode. The pH was determined immediately after sampling with an Orion model 310 PerpHecT pH-meter with a PerpHecT ROSS glass combination electrode. Volatile suspended solids and other analytical parameters were determined according to *Standard Methods for Examination of Water and Wastewater* [22].

8.4 Results and Discussion

Microbial toxicity assays

8.4.1 Nitrification and denitrification toxicity assays

An illustrative example of the time course of ammonium consumption for the nitrifying activity assay amended with 0, 50, 90, 130, 180, 230 and 300 mg F^{-} L^{-1} is shown in Figure 8.1. The maximum specific nitrifying activities in treatments containing fluoride were normalized based on the activity of the control treatment lacking fluoride. The normalized activity of nitrifying microorganism as a function of the initial fluoride concentration is plotted in Figure 8.2A. The same procedure was utilized to calculate the microbial activities of the different microorganisms evaluated in this study. The IC_{20}, IC_{50} and IC_{80}, values determined are summarized in Table 8.1.

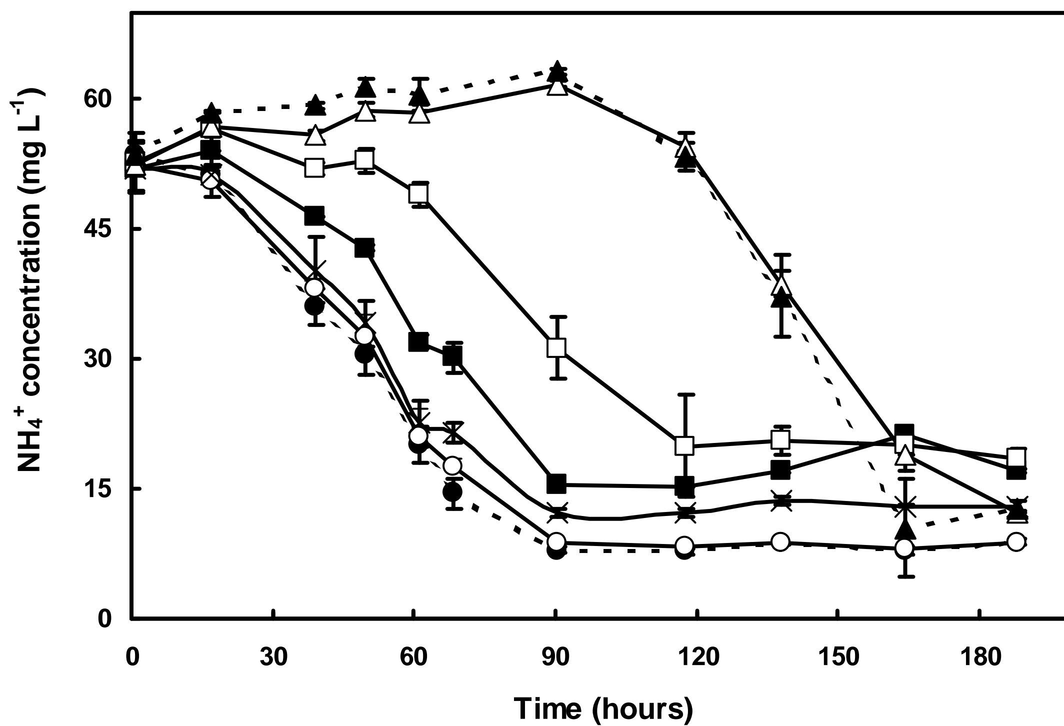


Figure 8.1 Time course of ammonium consumption by a mixed microbial culture obtained form the nitrification stage of a full-scale municipal wastewater treatment plant (0.5 g VSS L^{-1}) in the presence of increasing F^- concentrations (in mg L^{-1}): (●) 0, (○) 50, (ж) 90, (■) 130, (□) 180, (Δ) 230, and (▲) 300.

Table 8.1 Inhibitory effect of sodium fluoride on the key microbial populations in biological wastewater treatment systems. IC_{20}, IC_{50} and IC_{80} are the concentrations of fluoride causing 20, 50 and 80% decrease in the activity of the target microorganisms, respectively.

Substrate	Redox Conditions	Toxicity	Inoculum*	1st feeding IC_{20}	1st feeding IC_{50}	1st feeding IC_{80}	2nd feeding IC_{20}	2nd feeding IC_{50}	2nd feeding IC_{80}
				mg L^{-1}					
Mesophilic methanogens									
Acetate	Anaerobic	Activity (methang.)	Eerbeek sludge	40.0	160.0	500.0	15.0	30.3	60
Acetate	Anaerobic	Activity (methang.)	Ina Road sludge	17.5	34.5	93.0	12.4	25.8	35.7
H_2	Anaerobic	Activity (methang.)	Eerbeek sludge	815	> 815	> 815	520.0	645.0	795.0
H_2	Anaerobic	Activity (methang.)	Ina Road sludge	30.0	82.0	390.0	805.0	1005.0	1125.0
Thermophilic methanogens									
Acetate	Anaerobic	Activity (methang.)	Hyperion sludge	7.2	18.1	39.2	29.5	42.6	62.9
H_2	Anaerobic	Activity (methang.)	Hyperion sludge	218.6	432.6	> 600	> 600	> 600	> 600
Anaerobic propionate-utilizing microorganisms									
Propionate	Anaerobic	Activity (propionate removal)	Eerbeek sluge	10.5	36.5	62.0	------	------	------
Anaerobic butyrate-utilizing microorganisms									
Butyrate	Anaerobic	Activity (butyrate removal)	Eerbeek sludge	17.5	25.5	34.8	------	------	------

Table 8.1 *(continued)*. Inhibitory effect of sodium fluoride on the key microbial populations in biological wastewater treatment systems. IC_{20}, IC_{50} and IC_{80} are the concentrations of fluoride causing 20, 50 and 80% decrease in the activity of the target microorganisms, respectively.

Substrate	Redox Conditions	Toxicity	Inoculum*	1st feeding			2nd feeding		
				IC_{20}	IC_{50}	IC_{80}	IC_{20}	IC_{50}	IC_{80}
				mg L^{-1}					
Denitrification									
Nitrate	Anoxic	Activity (nitrate reduction)	Eerbeek sludge	> 800.0[a]	> 800.0[a]	> 800.0[a]			
Nitrification									
Ammonium	Aerobic	Activity (NH_4^+ consumption)	Nitrifying sludge	104.3	148.8	179.8			
Heterotrophic aerobes									
Glucose	Aerobic	Growth	Randolph Park	> 539.0[b]	> 539.0[b]	> 539.0[b]			
Glucose fermenters									
Glucose	Anaerobic	Growth	Aviko sludge	150.5	> 540.0	> 540.0			
Glucose	Anaerobic	Activity	Enrich. culture	87.0	325.0	539.0			
Microtox									
Complex medium	Aerobic	Activity (luminiscence)	*Vibrio fischeri*	> 136.0[c]	> 136.0[c]	> 136.0[c]			

[a,b,c] Not toxic at the highest concentration tested, *i.e.,* 800, 539 and 136 mg L^{-1}, respectively.

Fluoride was found to inhibit nitrifying microorganisms in aerobic sewage sludge. Nonetheless, nitrifying bacteria appeared to become acclimated rapidly to this contaminant. The metabolic activity determined in assays that were initially inhibited by fluoride increased substantially with exposure time. Assays with fluoride concentrations of 230 mg L^{-1} and higher were completely inhibited during the initial 100 h of exposure, but their activity increased sharply thereafter reaching levels close to those observed in the fluoride-free controls. The observed recovery may be due to physiological acclimatization to fluoride or to a shift in the microbial population to another nitrifying strain which is less sensitive to the contaminant.

Literature data indicates that fluoride exerts relatively low inhibition towards nitrifying bacteria in activated sludge systems. Complete oxidation of 400 mg NH_4^+ L^{-1} in a synthetic coal gasification wastewater was observed in batch assays amended with 300 mg F^- L^{-1} [13]. CSTR experiments inoculated with a nitrifying culture enriched from municipal sewage sludge showed that fluoride concentrations of up to 2,430 mg NH_4^+ L^{-1} did only exert moderate inhibition (39%) of nitrification activity [12]. Carrera *et al.* [11] reported that elevated fluoride levels (630 mg L^{-1}) were required to cause 50% inhibition of the nitrifying activity of biomass in a lab-scale activated sludge reactor treating a high-strength ammonium wastewater (546 mg NH_4^+ L^{-1}). The wide variation observed among the various studies in the toxicity response of nitrification to fluoride may be due to differences in the experimental conditions, including ammonia concentration and other medium components, as well as variations in the microbial community structure of the inoculum, among others.

Regarding the toxic action of fluoride on denitrifying microorganisms, the contaminant did not exert any significant inhibitory effect on the activity of the mixed culture at concentrations as high as 800 mg L^{-1} (Figure 8.2B). We are not aware of any previous study from the literature reporting on the inhibitory effect of fluoride on denitrifying bacteria during biological wastewater treatment. However, fluoride in soils (up to 3,700 mg F^{-} kg^{-1}) has been reported to affect nitrate reduction, but the inhibitory effect varied considerably depending on the soil characteristics [18, 23].

These results suggest that biological processes for the removal of nitrogen from wastewater are not expected to be affected by fluoride unless the concentrations are exceedingly high.

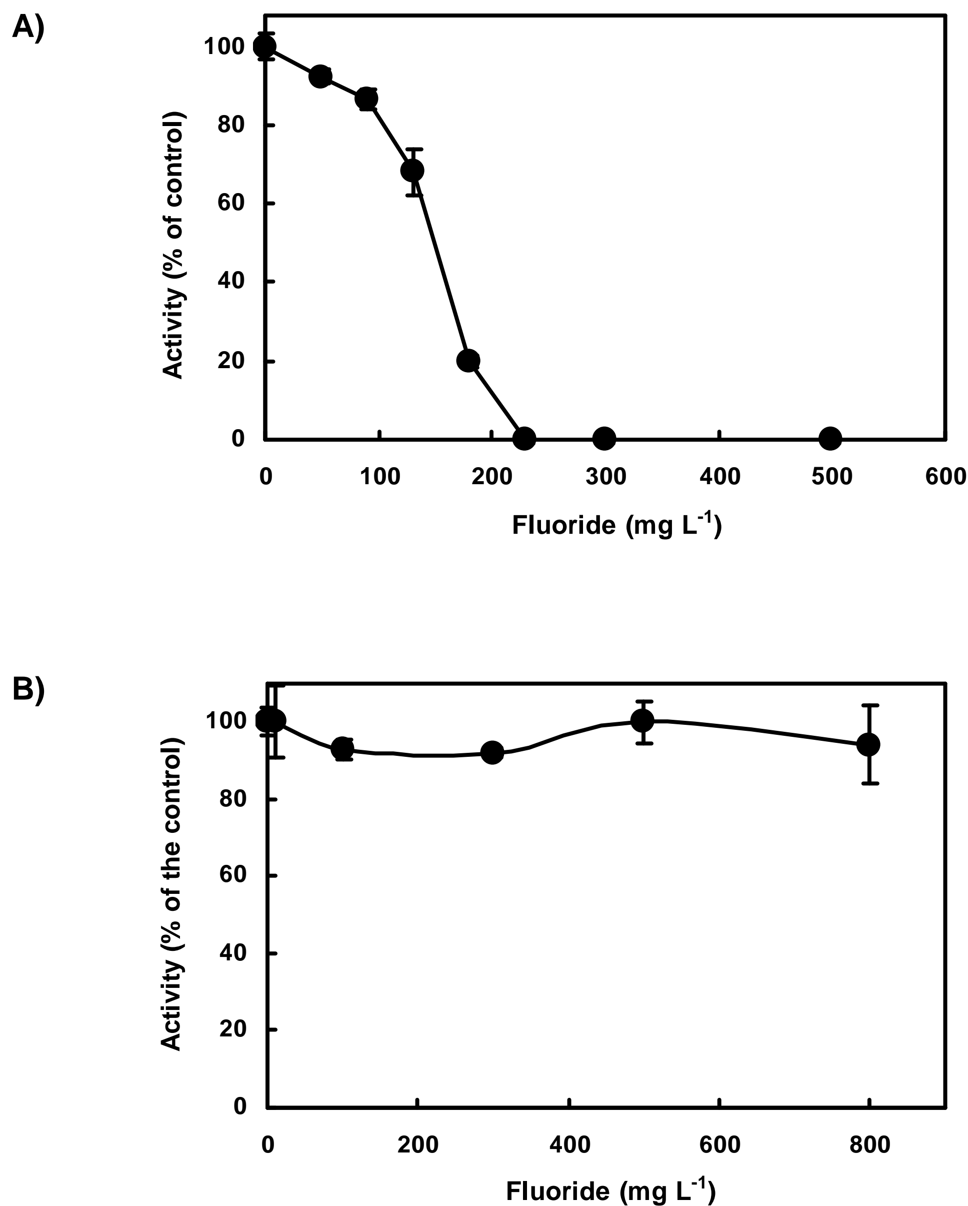


Figure 8.2 (A) Inhibitory effect of F^- on the nitrifying specific activity of a mixed microbial culture obtained from the nitrification stage of a full-scale municipal wastewater treatment plant (0.5 g VSS L^{-1}) as measured by ammonia depletion. (B) Inhibitory effect of F^- on the denitrifying specific activity of an industrial anaerobic granular sludge (1.0 g VSS L^{-1}) as determined by nitrate depletion. The nitrifying and denitrifying specific activities were normalized with respect to the control. Error bars (shown if larger than the symbols) represent standard deviations of duplicate assays.

8.4.2 Inhibition of aerobic heterotrophs and glucose fermenters by fluoride

Sodium fluoride did not display any inhibitory effect towards heterotrophic bacteria in aerobic sewage sludge (Figure 8.3A). Results from batch bioassays inoculated with a glucose-degrading enrichment culture showed no decrease in the glucose degradation rates at fluoride concentrations of up to 540 mg L^{-1} (Table 8.1). In agreement with our results, the only other study assessing the impact of fluoride (50-100 mg L^{-1}) on the removal of organic matter (ethanol and acetate) in the activated sludge process reported no significant effect on cell growth and chemical oxygen demand (COD) removal efficiency [24]. However, the presence of fluoride resulted in deterioration of the sludge settling properties as indicated by a 100 to 200% increase of the sludge volume index. The mechanisms contributing to the deterioration of the sludge settling ability in the presence of fluoride are unknown. Several studies from the dental literature have reported that fluoride can inhibit the formation of biolfims and other cell aggregates [14, 25, 26].

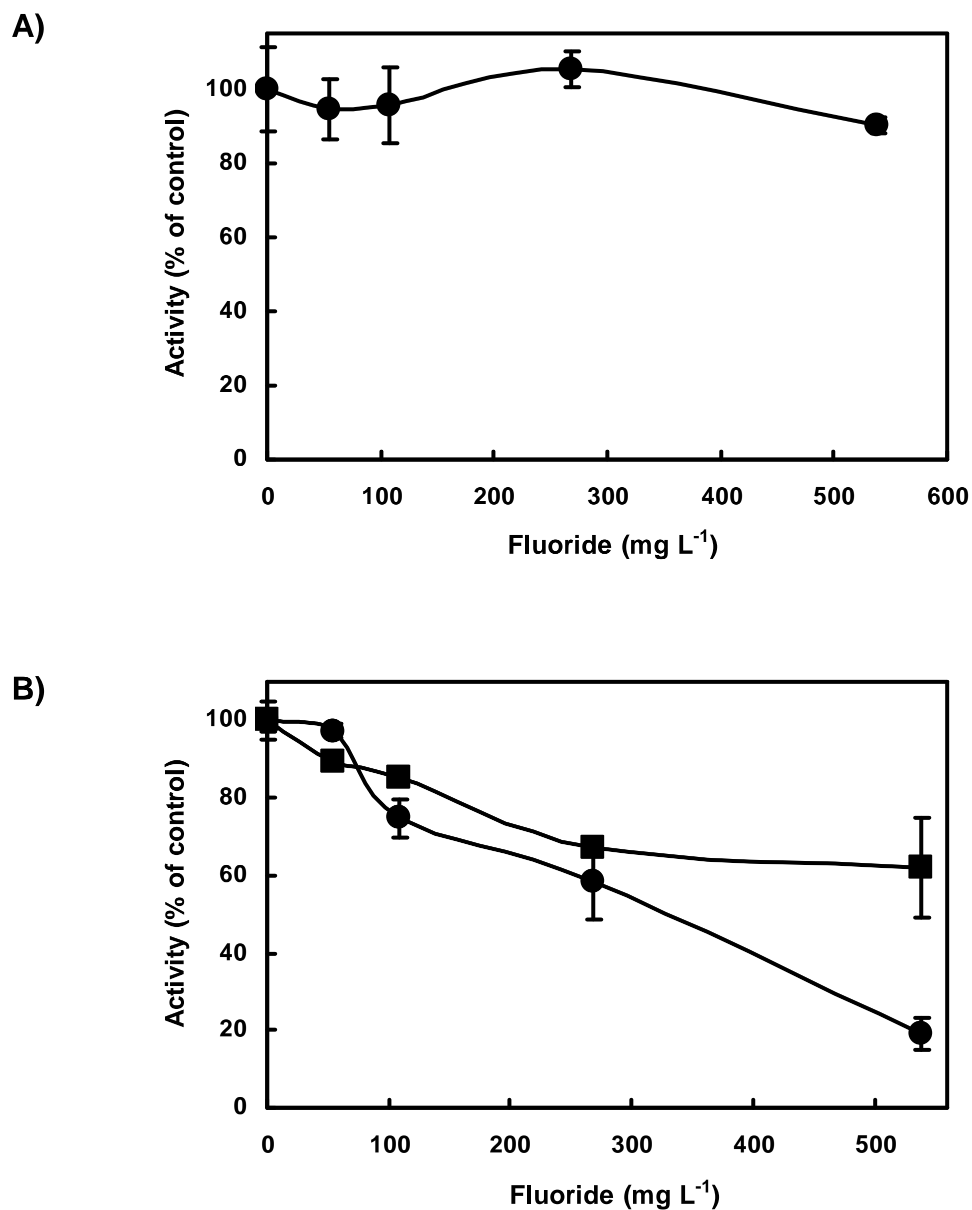


Figure 8.3. (A) Inhibitory effect of F^- on the glucose-degrading specific activity of an enrichment culture obtained from aerobic wastewater treatment sludge. (B) Inhibitory effect of F^-on the glucose fermentation by a mixed anaerobic culture inoculated with 1.5 g VSS L^{-1} anaerobic granular sludge (■), and an anaerobic glucose-degrading enrichment culture (●). The glucose-degrading specific activities were normalized with respect to the control in which no contaminant was added. Error bars (shown if larger than the symbols) represent standard deviations of triplicate assays.

Fluoride was observed to cause inhibition of glucose-fermenting microorganisms (Figure 8.3B). The higher inhibition observed in the assay with the enrichment culture suggests a greater impact of fluoride on the growth as compared to the metabolic activity of glucose fermenters. Inhibition of fermentation in anaerobic wastewater treatment sludge has not been reported in the literature. Nonetheless, there are numerous studies that confirm the toxic action of fluoride on glucose incorporation and carbohydrate metabolism by oral bacteria [14, 15]. The minimum inhibitory concentrations reported to impair carbohydrate degradation by *Streptococcus* species and other oral bacteria vary widely from 5 to 1,600 mg L^{-1} [27-31].

8.4.3 Toxicity to anaerobic microorganisms: methanogens and microorganisms involved in propionate- and butyrate degradation

Acetoclastic methanogens were particularly susceptible to the inhibitory effect of fluoride. Figures 8.4A and 8.4B revealed a sharp decrease in the specific methanogenic activity of mesophilic and thermophilic acetoclastic microorganisms with increasing fluoride concentrations. The IC_{50} values determined in the assays with mesophilic and thermophilic anaerobically-digested sludges were 34.5 and 7.2 mg L^{-1}, respectively (Table 8.1). Acetoclastic methanogens present in the mesophilic granular sludge appeared to be more tolerant to fluoride as demonstrated by the IC_{50} value of 160 mg L^{-1}. In the case of H_2-utilizing methanogens, fluoride caused a decrease in the rate of methane

production by granular and thermophilic sludges but only when present at very high concentrations (Figure 8.4D). On the other hand, hydrogenotrophic methanogens in mesophilic anaerobically-digested sludge were more sensitive to the presence of fluoride (Figure 8.4C).

The impact of extending the time of exposure on the methanogenic inhibition of fluoride was investigated by supplying a second feeding of substrate to the bioassay once that the initially supplied substrate had been depleted. In the case of acetoclastic methanogens, the inhibitory effect of fluoride increased with increasing time of exposure, particularly in the granular sludge as demonstrated by a 5.3-fold increase in the IC_{50} value of the second feeding (Table 8.1). In contrast, H_2-utilizing methanogens were found to be more tolerant to fluoride at relatively high concentrations after prolonged exposure times. The mechanisms responsible for the substantial decrease in the inhibitory effects of fluoride towards hydrogenotrophic methanogens with incubation time are unclear. A possible explanation is that fluoride exposure may have caused a shift in microbial population, resulting in the dominance of these microorganisms, which were less sensitive to fluoride.

To the best of our knowledge, there are no previous literature reports concerned with the inhibitory effects of fluoride towards methanogenic microorganisms. The relatively low fluoride inhibiting values observed in this study are of particular concern in view of the slow growth kinetics of acetate-utilizing methanogens (doubling times range from 1 to 7 d [32]). An active acetoclastic methanogenic population is essential for the degradation of acetate which, in turn, is required to attain adequate removal of

biodegradable organic matter during wastewater treatment. In practice, this means that fairly long time periods might be required for the recovery from an incidental toxicity exposure. For instance, Zayed & Winter [33] reported that recovery of methanogenesis following exposure to 10-20 mg L^{-1} of copper was attained after 35 or 47 d after the toxic shock.

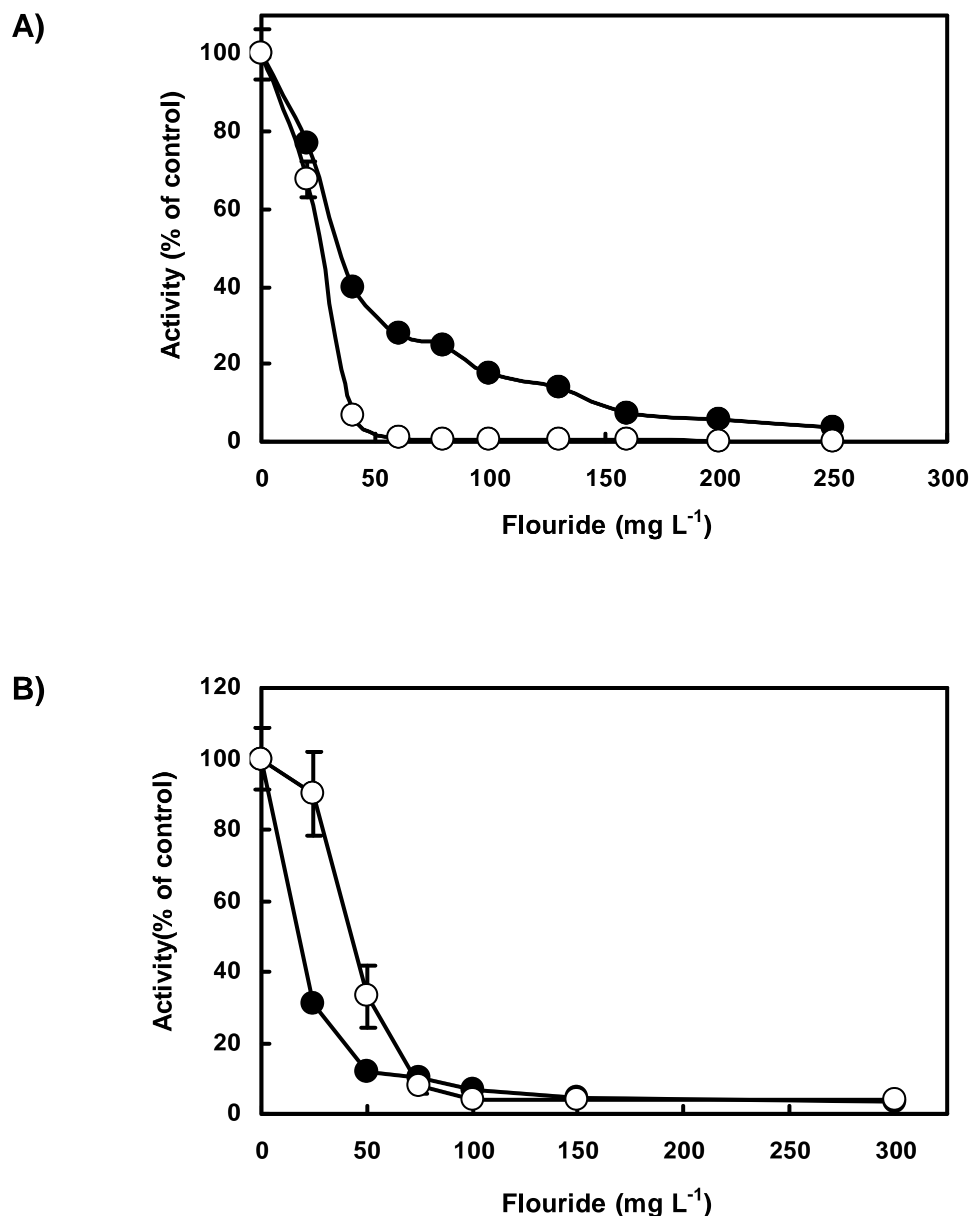


Figure 8.4. Inhibitory effect of F^- on the activity of mesophilic and thermophilic acetoclastic (A and B) and hydrogenotrophic (C and D) methanogens, respectively. Bioassays were conducted with Ina Road anaerobically digested sludge (A and C) and Hyperion anaerobically-digested thermophilic sludge (B and D), respectively. Legend: (●) First substrate feeding and (○) second substrate feeding. The methanogenic activities were normalized with respect to the control. Error bars (shown if larger than the symbols) represent standard deviations of triplicate assays.

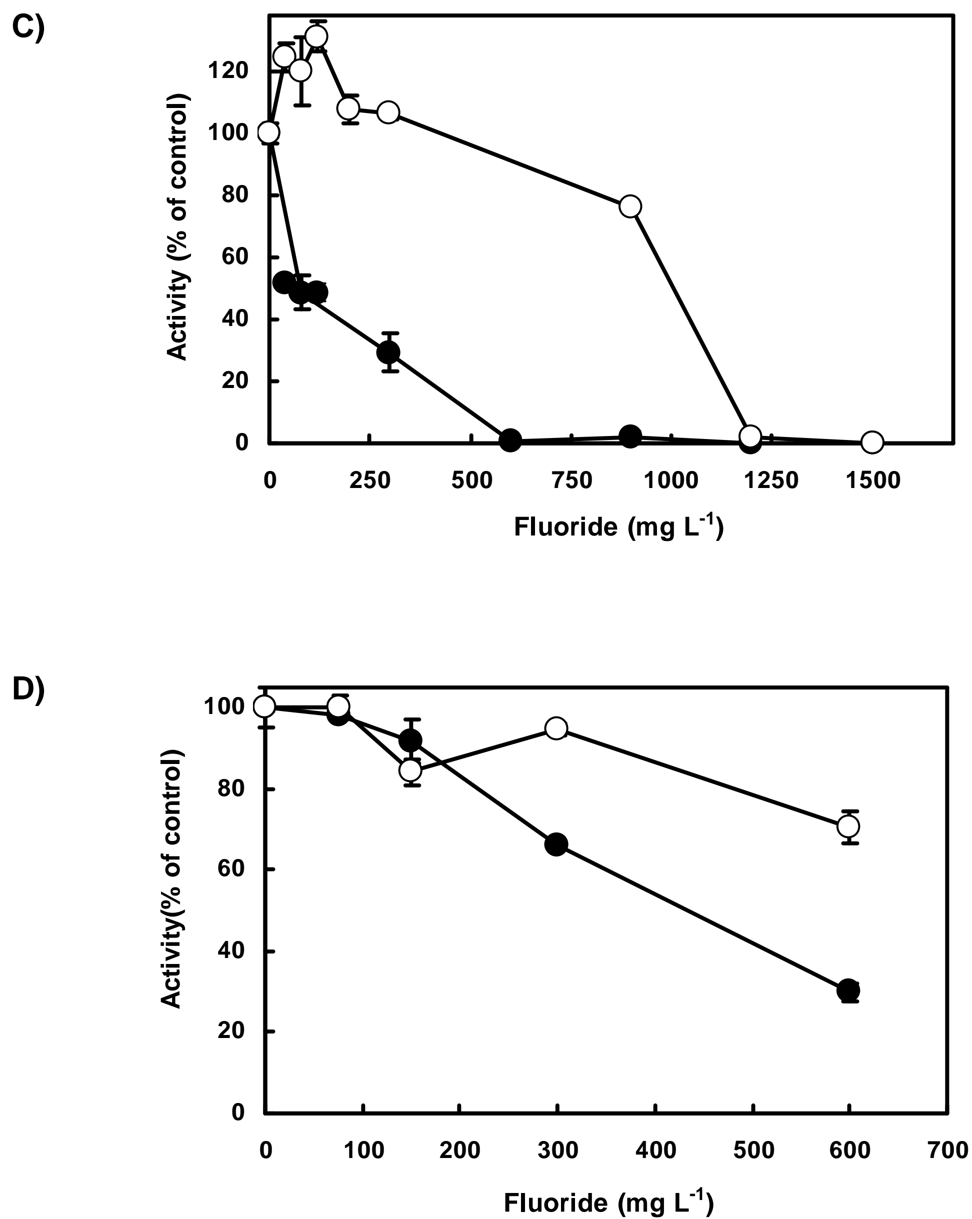


Figure 8.4. *(continued)* Inhibitory effect of F^- on the activity of mesophilic and thermophilic acetoclastic (A and B) and hydrogenotrophic (C and D) methanogens, respectively. Bioassays were conducted with Ina Road anaerobically digested sludge (A and C) and Hyperion anaerobically-digested thermophilic sludge (B and D), respectively. Legend: (●) First substrate feeding and (○) second substrate feeding. The methanogenic activities were normalized with respect to the control. Error bars (shown if larger than the symbols) represent standard deviations of triplicate assays.

Similarly to acetoclastic methanogens, anaerobic microorganisms that utilize propionate and butyrate were very sensitive to fluoride as evidenced by the relatively low IC_{50} values determined for these microbial populations, 36.5 and 25.5 mg L^{-1}, respectively (Table 8.1). As shown in Figures 8.5A and 8.5B, the utilization rate of VFA steeply decreased with increasing fluoride concentrations. This decrease was accompanied by a reduction in the rate of methanogenesis.

The high toxicity of fluoride towards anaerobic microbial populations involved in VFA degradation is also a concern because anaerobic digestion is commonly applied for the management of excess sludge in municipal wastewater treatment systems [34]. The inhibition of acetogenic microorganisms would prevent the effective degradation of VFA (*e.g.* propionate, butyrate, etc.) into acetate and, therefore, compromise the anaerobic digestion process. The apparent tolerance of H_2-utilizing methanogens to fluoride does not preclude inhibition of anaerobic sludge digestion by this ion, since only about one-third of the electron equivalents in complex organic matter are channeled through H_2 gas.

The mechanisms responsible for the microbial toxicity of fluoride towards acetate, propionate and butyrate-utilizing microorganisms in anaerobic reactors are not well understood. Nonetheless, fluoride is known to inhibit the action of various microbial enzymes including the proton-extruding ATPase, the glycolytic enzyme enolase, and urease [14, 35]. Furthermore, F^-/HF can bind to many enzymes, including heme-containing enzymes, copper-based oxidases and other metalloenzymes, affecting metabolism. Fluoride can form complexes with metals such as aluminum or beryllium,

leading to compounds that can mimic phosphate (*e.g.* AlF_4^- and BeF_3^- H_2O) and interfere with a variety of enzymes, *e.g.,* phosphatases and pyrophosphatases [14].

Pyrophosphatase (PPase) plays an important role in the energy metabolism of methanogens [36, 37]. Fluoride is a potent inhibitor of PPase in various microorganisms [38, 39]. Variation in the susceptibility to fluoride of pyrophosphatase (PPase) enzymes from acetoclastic and hydrogenotrophic methanogens might have contributed to the considerable differences observed in the response of these methanogens to fluoride. A pyrophosphatase has been isolated from *Methanothrix soehngenii*, a common acetoclastic methanogen in anaerobic reactors, that is not inhibited by fluoride [40]. The composition of the medium could also affect inhibition by fluoride. Studies with the methanogen, *Methanococcus jannaschii***,** have shown that prior exposure to specific metallic ions (*e.g*., Mn^{2+} or Co^{2+}) increased the tolerance against inhibition by sodium fluoride, to which the enzyme was otherwise very sensitive [41].

The inhibitory effect of organic compounds containing fluoride such as methyl fluoride (CH_3F) is well documented. CH_3F is known to be a specific inhibitor of acetoclastic methanogens and anaerobic mixed cultures that produce acetate as an intermediate, but this organofluorine compound is not or only mildly inhibitory to hydrogenotrophic methanogens [42-44]. The different sensitivity of acetate- and H_2-utilizing methanogens towards CH_3F is in agreement with the toxic response observed in this study for these two methanogenic trophic groups to fluoride, suggesting the possibility that microbial methylation of fluoride might have occurred leading to the formation of toxic CH_3F. This biotransformation has not been reported earlier [45, 46].

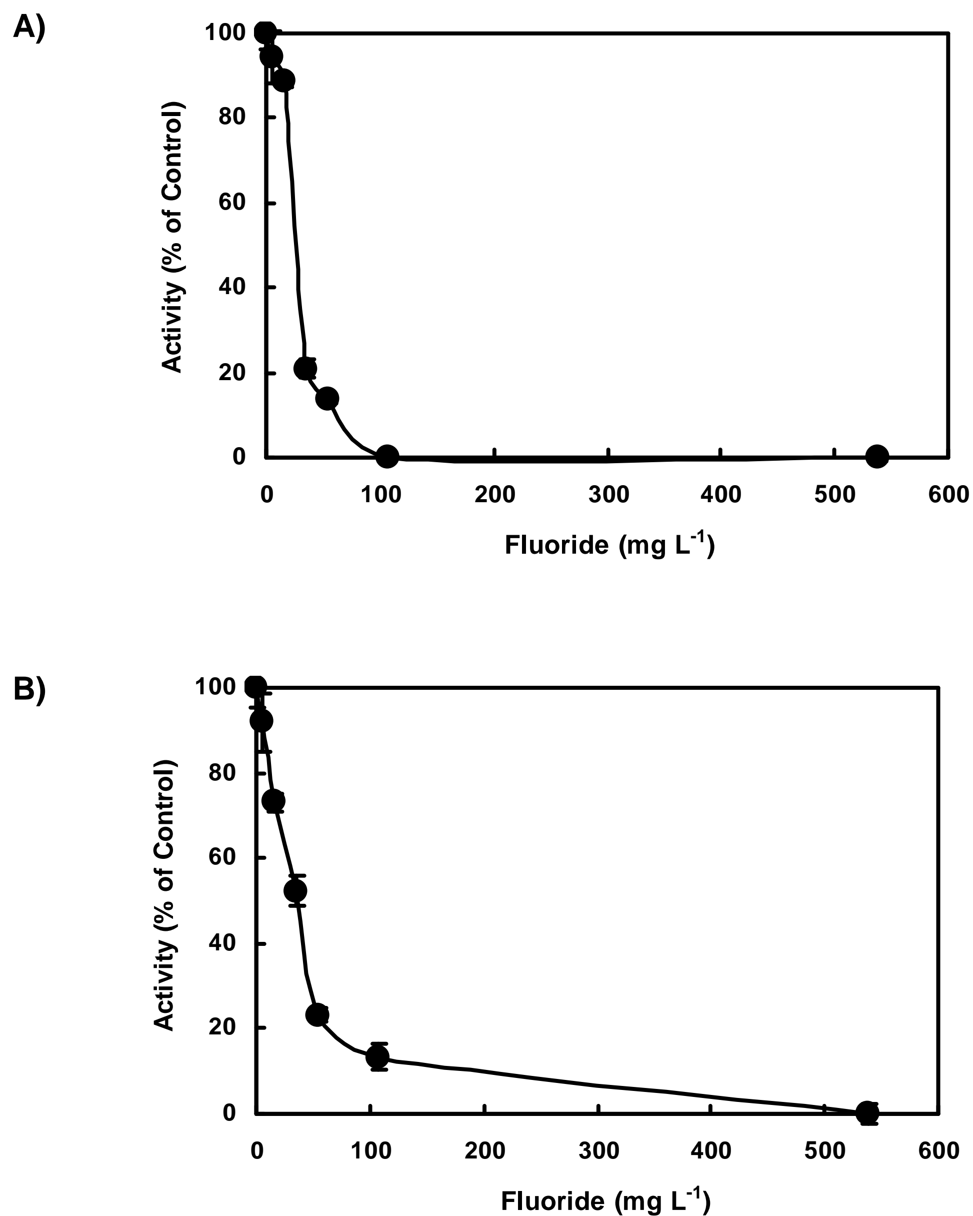


Figure 8.5. Inhibitory effect of F^- on the maximum specific activity of propionate (A) and butyrate (B) utilization by mesophilic anaerobic consortium (Aviko sludge). The activities were normalized with respect to the control. Error bars (shown if larger than the symbols) represent standard deviations of triplicate assays.

8.4.4 Inhibition of aquatic organisms by fluoride

Fluoride did not exert any inhibitory effect in the Microtox® assay at the highest concentration tested (136 mg L^{-1}) at exposure times ranging from 5 to 30 min (results not shown). Microtox® is a highly sensitive assay which is widely applied to monitor the toxicity of effluents and evaluate the toxic effects of chemical compounds. The results of the test have been shown to correlate well with toxicity values for fish, crustaceans and algae for a wide range of organic and inorganic chemicals [47, 48].

Standardized acute inhibition bioassays with water fleas (*Ceriodaphnia dubia*) and fathead minnows (*Pimephales promelas*) confirmed that fluoride is a weak to moderate inhibitor of these aquatic organisms (Table 8.2). For instance, the 96 h-LC_{50} values determined for *C. dubia* and *P. promelas* were 85 and 200 mg F^{-} L^{-}, respectively. These findings are agreement with several literature studies that have reported LC_{50} values for fluoride against various water flea species (*C. dubia, C. pulchella,* and *Daphnia magna*) ranging from 83 to 680 mg L^{-1} [3, 49, 50]. The LC_{50} value determined for fluoride in our bioassays with *P. promelas* are also in the range of those reported by Smith and coworkers [51] for this fish species, which ranged from 180 to 315 mg L^{-1}. LC_{50} values determined for other fish species, including brown trout (*Salmo trutta fario*), rainbow trout *(Oncorhynchus mykiss*; previously known as *Salmo gairdneri*), European carp (*Cyprinus carpio*), and sheepshead minnow (*Cyprinodon variegatus*), are in the same range varying from 51 to 830 mg F^{-} L^{-1}, depending on the species and duration of exposure [3, 52-54].

In addition to lethal effects, fluoride can cause other adverse effects on fish, including delays in the hatching of fertilized eggs and behavioral alterations delaying migration with fish loss, at relatively low concentrations. Delays in egg hatching varying from 1 h up to 10 d have been observed at fluoride concentrations ranging from 1.5 to 16.7 mg L^{-1}, depending on the fish species [55, 56]. Furthermore, a field study conducted in the Columbia river concluded that low concentrations of fluoride (0.5 mg L^{-1}) could adversely affect the upstream migration of Chinook and Coho salmon (*Oncorhynchus tshawytscha* and *O. kisutch*) [57]. In contrast with this finding, healthy populations of rainbow trout are known to exist in natural habitats (Firehole river at Yellowstone Park, and Walter and Pyramid lakes in Nevada) containing concentrations of fluoride as high as 13 to 14 mg L^{-1} [54].

Table 8.2 96-h lethal concentrations of fluoride towards two organisms commonly used in effluent biomonitoring, water fleas (*Ceriodaphnia dubia*) and fathead minnow (*Pimephales promelas*). LC_{20}, LC_{50}, LC_{80} are the concentrations of the toxic lethal to 20, 50 and 80% of the test organisms, respectively.

Organism	**LC_{20}**	**LC_{50} mg L^{-1}**	**LC_{80}**
Ceriodaphnia dubia	76	85	96
Pimephales promelas	172	200	231

8.5 Conclusions

Fluoride is inhibitory towards key microbial populations in anaerobic sludge digestion, *i.e.,* mesophilic and thermophilic acetoclastic methanogens, as well as propionate- and butyrate degraders, at concentrations lower than those found in some fluoride-containing industrial effluents. In contrast, very high concentrations of soluble fluoride (> 500 mg L^{-1}) can be tolerated by microbial communities involved in the aerobic activated sludge and in denitrification processes without significant inhibitory impact. Nitrification processes are somewhat more sensitive but they appear to acclimate rapidly to fluoride. Finally, fluoride was only found to be inhibitory to aquatic species when present at relatively high levels.

8.6 Acknowledgements

This study was conducted with financial support from the Semiconductor Research Corporation/Sematech Engineering Research Center for Environmentally Benign Semiconductor Manufacturing.

8.7 References

1. Manson, B.; Moore, C. B. In *Principles of geochemistry*, 1982; New York, Wiley: 1982; p 350.
2. *Fluoride in drinking water: a scientific review of EPA's standards*. The National Academies Press: Washington, DC, 2006; p 1 - 467.
3. Camargo, J. A., Fluoride toxicity to aquatic organisms: a review. *Chemosphere* **2003,** *50*, (3), 251-264.
4. Fuge, R.; Andrews, M. J., Fluorine in the Uk Environment. *Environmental Geochemistry and Health* **1988,** *10*, (3-4), 96-104.
5. Shen, F.; Chen, X. M.; Gao, P.; Chen, G. H., Electrochemical removal of fluoride ions from industrial wastewater. *Chemical Engineering Science* **2003,** *58*, (3-6), 987-993.
6. Skjelkvale, B. L., Water Chemistry in Areas with High Deposition of Fluoride. *Science of the Total Environment* **1994,** *152*, (2), 105-112.
7. Sujana, M. G.; Thakur, R. S.; Rao, S. B., Removal of fluoride from aqueous solution by using alum sludge. *Journal of Colloid and Interface Science* **1998,** *206*, (1), 94-101.
8. Hu, C. Y.; Lo, S. L.; Kuan, W. H.; Lee, Y. D., Removal of fluoride from semiconductor wastewater by electrocoagulation-flotation. *Water Research* **2005,** *39*, (5), 895-901.

9. Huang, C. J.; Liu, J. C., Precipitate flotation of fluoride-containing wastewater from a semiconductor manufacturer. *Water Research* **1999,** *33*, (16), 3403-3412.

10. Toyoda, A.; Taira, T., A new method for treating fluorine wastewater to reduce sludge and running costs. *Ieee Transactions on Semiconductor Manufacturing* **2000,** *13*, (3), 305-309.

11. Carrera, J.; Torrijos, M.; Baeza, J. A.; Lafuente, J.; Vicent, T., Inhibition of nitrification by fluoride in high-strength ammonium wastewater in activated sludge. *Process Biochemistry* **2003,** *39*, (1), 73-79.

12. Clarkson, W. W.; Collins, A. G.; Sheehan, P. L., Effect of Fluoride on Nitrification of a Concentrated Industrial-Waste. *Applied and Environmental Microbiology* **1989,** *55*, (1), 240-245.

13. Whang, G. D., Effects of fluoride, boron, arsenic and thiocyanate on ammonia and thyocyanate oxidation in extended aeration sludge process. *PhD dissertation, Texas University, Austin, TX* **1985**, 167.

14. Marquis, R. E.; Clock, S. A.; Mota-Meira, M., Fluoride and organic weak acids as modulators of microbial physiology. *Fems Microbiology Reviews* **2003,** *26*, (5), 493-510.

15. Wiegand, A.; Buchalla, W.; Attin, T., Review on fluoride-releasing restorative materials - Fluoride release and uptake characteristics, antibacterial activity and influence on caries formation. *Dental Materials* **2007,** *23*, (3), 343-362.

16. Rao, D. N.; Pal, D., Effect of Fluoride Pollution on Organic-Matter Content of Soil. *Plant and Soil* **1978,** *49*, (3), 653-656.

17. Tscherko, D.; Kandeler, E., Ecotoxicological effects of fluorine deposits on microbial biomass and enzyme activity in grassland. *European Journal of Soil Science* **1997,** *48*, (2), 329-335.

18. Wilke, B. M., Fluoride-Induced Changes in Chemical-Properties and Microbial Activity of Mull, Moder and Mor Soils. *Biology and Fertility of Soils* **1987,** *5*, (1), 49-55.

19. Bulich, A. A.; Isenberg, D. L., Use of the Luminescent Bacterial System for the Rapid Assessment of Aquatic Toxicity. *Isa Transactions* **1981,** *20*, (1), 29-33.

20. EPA, Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. In 5th ed.; Washington, DC, 2002.

21. Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F., Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **1956,** *28*, (3), 350 - 356.

22. AHPA, *Standard methods for the examination of water and wastewater*. 20 th ed.; American Public Health Association (AHPA): Washington D C, 1998.

23. Ottow, J.; Kottas, P., Einflup anthropogener Fluorkontamination auf den Bodenmetabolismus. *Daten Dokumente Unweltschultz* **1984,** *36*, 145 - 154.

24. Singh, M.; Kar, D., Effect of Fluoride on the Settleability of Biomass from an Activated-Sludge Plant Treating Petrochemical Effluent. *Journal of Fermentation and Bioengineering* **1989,** *67*, (5), 366-368.

25. Cao, J. K.; Doyle, R. J., Fluoride modifies adhesion of Streptococcus pyogenes. *Fems Immunology and Medical Microbiology* **2002,** *32*, (2), 175-177.

26. Embleton, J. V.; Newman, H. N.; Wilson, M., Amine and tin fluoride inhibition of Streptococcus sanguis adhesion under continuous flow. *Oral Microbiology and Immunology* **2001,** *16*, (3), 182-184.

27. Bradshawa, D. J.; Marsha, P. D.; Hodgson, R. J.; Visser, J. M., Effects of glucose and fluoride on competition and metabolism within in vitro dental bacterial communities and biofilms. *Caries Research* **2002,** *36*, (2), 81-86.

28. Eisenberg, A. D.; Oldershaw, M. D.; Curzon, M. E. J.; Handelman, S. L., Effects of Fluoride, Lithium, and Strontium on Growth and Acid Production of Mutans Streptococci and Actinomyces-Viscosus. *Caries Research* **1991,** *25*, (3), 179-184.

29. Ekenback, S. B.; Linder, L. E.; Sund, M. L.; Lonnies, H., Effect of fluoride on glucose incorporation and metabolism in biofilm cells of Streptococcus mutans. *European Journal of Oral Sciences* **2001,** *109*, (3), 182-186.

30. LenanderLumikari, M.; Loimaranta, V.; Hannuksela, S.; Tenovuo, J.; Ekstrand, J., Combined inhibitory effect of fluoride and hypothiocyanite on the viability and glucose metabolism of Streptococcus mutans, serotype c. *Oral Microbiology and Immunology* **1997,** *12*, (4), 231-235.

31. Maltz, M.; Emilson, C. G., Susceptibility of Oral Bacteria to Various Fluoride Salts. *Journal of Dental Research* **1982,** *61*, (6), 786-790.

32. Mara, D.; Horan, H. J., *Handbook of Water and Wastewater Microbiology*. 10 th ed.; Academic Press: 2003.

33. Zayed, G.; Winter, J., Inhibition of methane production from whey by heavy metals - protective effect of sulfide. *Applied Microbiology and Biotechnology* **2000,** *53*, (6), 726-731.

34. Chen, Y.; Cheng, J. J.; Creamer, K. S., Inhibition of anaerobic digestion process: A review. *Bioresource Technology* **2008,** *99*, (10), 4044-4064.

35. Hamilton, I. R.; Ellwood, D. C., Effects of Fluoride on Carbohydrate-Metabolism by Washed Cells of Streptococcus-Mutans Grown at Various Ph Values in a Chemostat. *Infection and Immunity* **1978,** *19*, (2), 434-442.

36. Roth, R.; Bachofen, R., Characterization and Partial-Purification of an Atpase and Inorganic Pyrophosphatase of the Archaebacterium Methanobacterium-Thermoautotrophicum. *Biochimica Et Biophysica Acta-General Subjects* **1994,** *1201*, (2), 271-283.

37. Smith, K. S.; Ingram-Smith, C., Methanosaeta, the forgotten methanogen? *Trends in Microbiology* **2007,** *15*, (4), 150-155.

38. Smirnova, I. N.; Baikov, A. A., 2-Step Mechanism of Inhibition of Inorganic Pyrophosphatase by Fluoride. *Biochemistry-Moscow* **1983,** *48*, (10), 1414-1423.

39. Tominaga, N.; Mori, T., Purification and Characterization of Inorganic Pyrophosphatase from Thiobacillus-Thiooxidans. *Journal of Biochemistry* **1977,** *81*, (2), 477-483.

40. Jetten, M. S. M.; Fluit, T. J.; Stams, A. J. M.; Zehnder, A. J. B., A Fluoride-Insensitive Inorganic Pyrophosphatase Isolated from Methanothrix-Soehngenii. *Archives of Microbiology* **1992,** *157*, (3), 284-289.

41. Kuhn, N. J.; Wadeson, A.; Ward, S.; Young, T. W., Methanococcus jannaschii ORF mj0608 codes for a class C inorganic pyrophosphatase protected by Co2+ or Mn2+ ions against fluoride inhibition. *Archives of Biochemistry and Biophysics* **2000,** *379*, (2), 292-298.

42. Conrad, R.; Klose, M., How specific is the inhibition by methyl fluoride of acetoclastic methanogenesis in anoxic rice field soil? *Fems Microbiology Ecology* **1999,** *30*, (1), 47-56.

43. Frenzel, P.; Bosse, U., Methyl fluoride, an inhibitor of methane oxidation and methane production. *Fems Microbiology Ecology* **1996,** *21*, (1), 25-36.

44. Janssen, P. H.; Frenzel, P., Inhibition of methanogenesis by methyl fluoride: Studies of pure and defined mixed cultures of anaerobic bacteria and archaea. *Applied and Environmental Microbiology* **1997,** *63*, (11), 4552-4557.

45. Murphy, C. D.; Schaffrath, C.; O'Hagan, D., Fluorinated natural products: the biosynthesis of fluoroacetate and 4-fluorothreonine in Streptomyces cattleya. *Chemosphere* **2003,** *52*, (2), 455-461.

46. O'Hagan, D.; Schaffrath, C.; Cobb, S. L.; Hamilton, J. T. G.; Murphy, C. D., Biosynthesis of an organofluorine molecule - A fluorinase enzyme has been discovered that catalyses carbon-fluorine bond formation. *Nature* **2002,** *416*, (6878), 279-279.

47. Blum, D. J. W.; Speece, R. E., A Database of Chemical Toxicity to Environmental Bacteria and Its Use in Interspecies Comparisons and Correlations.

Research Journal of the Water Pollution Control Federation **1991,** *63*, (3), 198-207.

48. Kaiser, K. L. E.; Esterby, S. R., Regression and Cluster-Analysis of the Acute Toxicity of 267 Chemicals to 6 Species of Biota and the Octanol Water Partition-Coefficient. *Science of the Total Environment* **1991,** *109*, 499-514.

49. Hickey, C. W., Sensitivity of 4 New-Zealand Cladoceran Species and Daphnia-Magna to Aquatic Toxicants. *New Zealand Journal of Marine and Freshwater Research* **1989,** *23*, (1), 131-137.

50. Leblanc, G. A., Acute Toxicity of Priority Pollutants to Water Flea (Daphnia-Magna). *Bulletin of Environmental Contamination and Toxicology* **1980,** *24*, (5), 684-691.

51. Smith, L. R.; Holsen, T. M.; Ibay, N. C.; Block, R. M.; Deleon, A. B., Studies on the Acute Toxicity of Fluoride-Ion to Stickleback, Fathead Minnow, and Rainbow-Trout. *Chemosphere* **1985,** *14*, (9), 1383-1389.

52. Camargo, J. A.; Tarazona, J. V., Short-Term Toxicity of Fluoride-Ion (F-) in Soft-Water to Rainbow-Trout (Salmo-Gairdneri) and Brown Trout (Salmo-Trutta-Fario). *Fluoride* **1991,** *24*, (2), 76-83.

53. Heitmuller, P. T.; Hollister, T. A.; Parrish, P. R., Acute Toxicity of 54 Industrial-Chemicals to Sheepshead Minnows (Cyprinodon-Variegatus). *Bulletin of Environmental Contamination and Toxicology* **1981,** *27*, (5), 596-604.

54. Sigler, W. F.; Neuhold, J. M., Fluoride intoxication in fish: a review. *Journal of Wildlife Diseases* **1972,** *8*, (3), 252 - 254.

55. Ellis, M.; Westfall, B. A.; Ellis, M. D. *Determination of water quality*; Fish and Wildlife Services, Department of Interior, USA: 1948.

56. Pillai, K. S.; Mane, U. H., The Effect of Fluoride on Fertilized-Eggs of a Fresh-Water Fish, Catla-Catla (Hamilton). *Toxicology Letters* **1984,** *22*, (2), 139-144.

57. Damkaer, D. M.; Dey, D. B., Evidence for fluoride effects on salmon passage at John Day Dam, Columbia River, 1982-1986. *North American Journal of Fisheries Management* **1989,** *9*, 154 - 162.

CONCLUSIONS AND FUTURE DIRECTIONS

A review of the literature indicated that perfluorooctane sulfonate (PFOS) and derivatives are emerging contaminants widely used in industrial process due to their excellent surface-active properties and chemical and thermal stability. PFOS has been shown to be environmentally persistent, toxic and a possible carcinogen. In this dissertation new techniques were explored for their potential use in the environmental monitoring of PFOS and related compounds. ^{19}F NMR spectroscopy and HPLC-suppressed conductivity detection were identified as promising analytical methods that could be used on a routine basis. Structural information of perfluorinated compounds in environmental samples could be obtained without matrix interferences by ^{19}F NMR spectroscopy, while quantification can be conducted in an efficient and cost-effective manner with HPLC-suppressed conductivity detection.

The presence of PFOS in sewage sludge from municipal wastewater treatment plants in Arizona was evaluated. Sludge samples were extracted and the extract was concentrated by means of solid phase extraction for analysis in LC-MS/MS that confirmed the presence of PFOS in municipal sludge, supporting the growing concensus that perfluorinated compounds are widespread in the environment. Additional research is recommended to evaluate the presence of PFOS and related pollutants in highly industrialized areas of the state and if needed establish regulatory actions.

The dissertation evaluated biological, chemical and physico-chemical methods to treat PFOS. PFOS was shown to be chemically reductively defluorinated by a vitamin B_{12}

catalyst in the presence of Ti(III) as a reducing agent. This constituted the first time that the reductive dehalogenation was reported at near ambient conditions. Branched isomers were more susceptible to reductive dehalogenation compared to linear isomers. These research results provide unique insights into developing new strategies to design more biodegradable perfluorinated compounds based on branched isomers. Since the vitamin B_{12} catalyst mimics enzymatic reductive dehalogenation, the results suggest that microbial reductive dehalogenation of PFOS might be possible. However, the challenge is to find sediments containing PFOS-degrading microbes. Since, PFOS and derivatives displayed resistance to microbial degradation even by microorganisms present in sludge from semiconductor wastewaters. Further biodegradation studies should be oriented to sludge/sediments from highly contaminated sites to increase the likelihood of finding these PFOS-degrading organisms.

Sorptive techniques of removing PFOS were studied with granular activated carbon, zeolites and wastewater sludge. Of the sorbents tested only activated carbon showed an excellent potential for the removal of perfluorinated surfactants from industrial effluents. Continuous column experiments are recommended to evaluate the feasibility of utilizing this sorbent to remove PFOS before disposal of industrial effluent into municipal wastewaters.

Since fluoride was the main transformation product from the reductive dehalogenation of PFOS, the microbial toxicity of fluoride towards microorganisms of biological wastewater treatment was evaluated. The most sensitive organisms were those involved in metabolizing volatile fatty acids under anaerobic conditions. Nonetheless, the

inhibiting concentrations were higher than fluoride concentrations expected in the wastewater. Fluoride-bearing industrial effluents will be diluted by wastewaters from residential and industrial sources. In addition, significant removal of this contaminant can occur by precipitation with calcium (II), which is a common wastewater contaminant. Thus inhibition of anaerobic microorganism involved in the digestion process is not a concern in biological treatment of semiconductor wastewaters.