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GORDONIA SP. NB4-1Y: MICROBIAL DEGRADATION OF 6:2 FLUOROTELOMER SULFONAMIDE ALKYL BETAINE (FTAB) AND 6:2 FLUOROTELOMER SULFONATE (FTSA)

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Gordonia sp. NB4-1Y: Microbial Degradation of 6:2 Fluorotelomer Sulfonamide Alkyl Betaine (FTAB) and 6:2 Fluorotelomer Sulfonate (FTSA)

by

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ABSTRACT

The purpose of this study was to identify and quantify breakdown products of the environmental contaminants 6:2 fluorotelomer sulfonamide alkyl betaine (FTAB) and 6:2 fluorotelomer sulfonate (FTSA), when added as the sole-sulfur source to cultures of the soil bacterium, *Gordonia* sp. NB4-1Y. FTAB is used in aqueous film-forming firefighting foams and it is known that FTSA will appear, upon the initial degradation of FTAB, in soil, aquatic ecosystems, and ground water with suspected adverse health effects in animals and humans. The majority of existing data on FTAB biodegradation is from analysis of contaminated soil and water samples, and metabolism by mixed microbial communities in sewage treatment plants, rather than from studies involving pure cultures. In an effort to understand the fundamental biochemical processes driving bacterial FTAB and FTSA metabolism, sulfur-limited pure cultures of NB4-1Y were analyzed for both volatile and water-soluble breakdown products using a Dionex Ultimate 3000 UHPLC chain coupled to a Thermo Q-Exactive Orbitrap mass spectrometer over 7 days. Over 168 h, 99.97 % of 60-µM FTSA was degraded into eleven major breakdown products, with a mol % recovery of 88.22, while 70.42 % of 60-µM FTAB was degraded into ten major breakdown products, with a mol % recovery of 84.65. The products detected indicate that NB4-1Y may be using two different approaches for FTSA desulfonation, with approximately 55 mol % of breakdown products being assigned to a major pathway that begins with the conversion of FTSA to 5:2-FT-ketone, with 5:2-FT-ketone being the major product at 43.62 mol %. The minor pathway, hypothesized to begin with conversion of FTSA to 5:3-FTCA, accounted for less than 1.0 mol % of products after 168-hrs of incubation. FTSA is a proposed intermediate of FTAB breakdown by NB4-1Y, but was not detected, possibly due to rapid utilization under sulfur-limiting conditions. As with FTSA breakdown, 20.07 % of FTAB products were assigned to the

major pathway, while less than 1.0 mol % were assigned to the minor pathway. Future research will involve isolating enzymes involved in FTAB and FTSA metabolism, verifying their function *in vitro*, and designing molecular tools for monitoring their metabolism in the environment by diverse microbial communities.

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1 INTRODUCTION

1.1 *Gordonia* spp.

The genus of microorganisms known as *Gordonia* of the family Gordoniaceae, are mycolic acid-containing, Gram-positive, soil bacteria of the order Actinomycetales (Drzyzga 2012). As of 2012, the *Gordonia* genus comprised of 32 species isolated from a wide-range of environmental habitats, much like their mycolic acid-containing relatives *Mycobacteria* and *Rhodococcus* (Drzyzga 2012). Strains have been isolated from contaminated soil and wastewater, estuary sand, mangrove rhizosphere, oil-producing wells, biofilters, activated sludge, and clinical samples (Drzyzga 2012). The specific strain of *Gordonia* discussed in this thesis, NB4-1Y, was isolated from enriched vermicompost by Van Hamme et al. (2013) using *bis*-(3-pentafluorophenylpropyl)-sulfide (PFPS) as a novel sulfur source. Unusual compounds, such as PFPS or other non-naturally occurring compounds, have been used previously to isolate unique bacterial strains (Arenskotter et al. 2004), an example being *Rhodococcus* sp. JVH1, also isolated for its C—S bond cleaving ability using PFPS (Van Hamme et al. 2004).

Gordonia have received much interest from researchers in the environmental, industrial, biotechnological, and medical fields (Drzyzga 2012). *Gordonia* possess unique abilities to degrade xenobiotics, toxic environmental pollutants, hydrocarbons, and natural compounds that are not readily biodegradable (Arenskotter et al. 2004). The plethora of chemical compounds that the *Gordonia* genus is capable of transforming, degrading, and synthesizing make them a strong candidate for a variety of environmental and industrial applications (Drzyzga 2012). In fact, as of October 2011, over 65 patents have been registered in relation to the *Gordonia* species (Drzyzga 2012).

This study focusses on the preliminary steps involved in developing potential bioremediation technologies for anthropogenic fluorinated chemicals. Here, the bacterial strain NB4-1Y of the *Gordonia* genus is examined for its capability to metabolize the heavily fluorinated environmental contaminants, 6:2 fluorotelomer sulfonamide alkyl betaine (FTAB) and 6:2 fluorotelomer sulfonate (FTSA).

1.2 Per- and Poly-fluorinated Contaminants

Incorporating fluorine into organic compounds can increase stability and alter chemical properties such as lipophilicity and acidity (Murphy 2010). Specific to this thesis, per- and poly-fluoroalkyl substances (PFASs) are a group of aliphatic fluorinated chemicals in which all (per-) or nearly all (poly-) carbons are fluorinated, exclusive of the terminal functional group (Boiteux et al. 2017) (Table 7-2). Consequently, PFASs exhibit unique characteristics that have been imparted into various products to increase qualities like oil and water repellency, and reduction in surface tension such as in fire-fighting foams (Houtz et al. 2016).

The manufacturing of PFAS-based surfactants and polymers has been occurring at large scale since the 1950's (Butt et al. 2014; Houtz et al. 2016), although environmental concerns have emerged only due to their global detection in the environment, biota, and humans (Boiteux et al. 2017). Concerns about PFASs in the environment are related to:

1. The strength of the carbon-fluorine bond and associated resistance to hydrolysis, photolysis, and biodegradation resulting in their persistence and bioaccumulation in water, soil, and the environment (Boiteux et al. 2017);

2. Suspected adverse health effects (Krafft and Riess 2015) and toxicity (Phillips et al. 2007; Newsted et al. 2008);
3. Their incomplete removal during sewage treatment (Higgins et al. 2005; Loganathan et al. 2007);
4. Their widespread use in surfactants, grease and water repellents, lubricants, and of particular interest to this study, aqueous film-forming foams (AFFFs) (Clarke and Smith 2011).

AFFF products containing fluorinated surfactants are used in industrial and military firefighting to extinguish hydrocarbon-fuel fires (Zhang et al. 2016). These products typically contain a per- or poly-fluoroalkylthiol carbon chain appended with an alkylamidosulfonate or alkylbetaine terminal group (e.g. FTAB found in Table 7-2) (Zhang et al. 2016). Upon the release of AFFF products such as Forafac® 1157, which contains FTAB (Moe et al. 2012), biodegradation studies have demonstrated that FTAB is degraded into 6:2 fluorotelomer sulfonamide (6:2-FTSAOA) in blue mussels (Moe et al. 2012) and FTSA in fire-fighting areas and laboratory microcosms (Schultz et al. 2004). FTSA is listed under the Unregulated Contaminant Monitoring Regulation (UCMR 3; US EPA enforces health standards for contaminants in drinking water) (Park et al. 2016) and has been observed in concentrations as high as 14,600-µg/L at military sites (Schultz et al. 2004). Degradation of FTSA begins with desulfonation to form 6:2 fluorotelomer aldehyde (FTAL) and the release of HSO_3^-, bypassing the formation of 6:2-FTOH in both activated sludge (Wang et al. 2011; Liu and Avendaño 2013) and in pure culture (Van Hamme et al. 2013). In activated sludge, the continued degradation of 6:2-FTAL continues along the well characterized pathways observed in 6:2-FTOH degradation (Wang et al. 2011).

1.3 6:2-FTOH Degradation Pathway in Diverse Microbial Communities

The aerobic biodegradation of fluorotelomer alcohols (FTOHs) such as 8:2-FTOH and 6:2-FTOH have been well studied (Dinglasan et al. 2004; Liu et al. 2010). In a review by Liu and Avendaño (2013), the aerobic biodegradation pathways of 6:2-FTOH demonstrated in both activated sludge and aerobic soils (Figure 1-1) are summarized. Analysis of 6:2-FTOH aerobic degradation using diverse microbial communities revealed that 6:2-FTOH was degraded into five terminal metabolites: PFBA, PFPeA, PFHxA, 5:3-FTCA, and 4:3-FTCA (Liu et al. 2010).

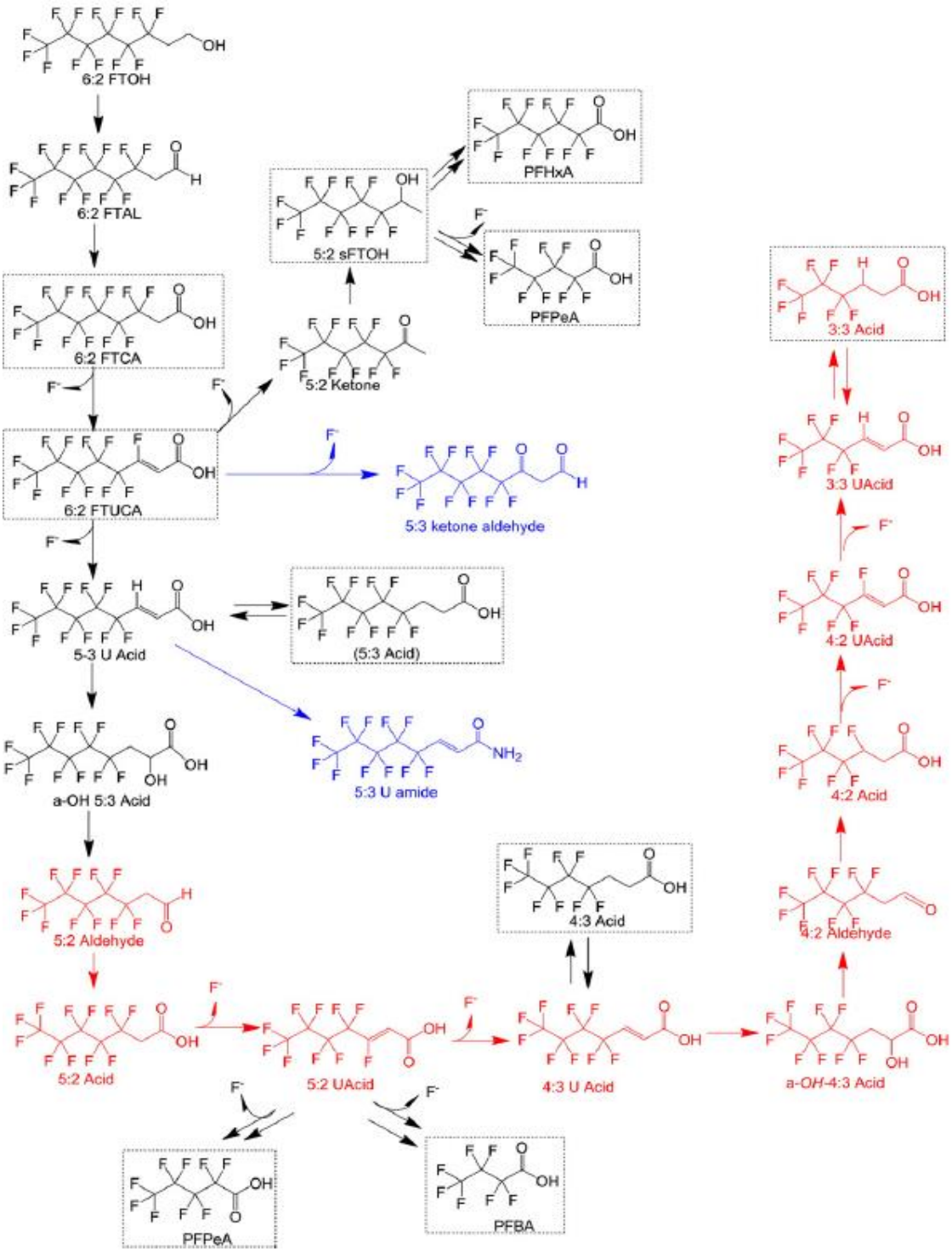


Figure 1-1: Aerobic biodegradation pathways of 6:2-FTOH in activated sludge and aerobic soil using diverse microbial cultures.
Double arrows indicate multiple transformation steps. Blue pathways have only been seen in soil (Liu et al. 2010), while red pathways have only been observed in sludge (Wang et al. 2012). Stable and semi-stable compounds are in dashed boxes. Figure from Liu and Avendaño (2013).

Van Hamme et al. (2013) indicate that as of April 2013, the majority of research has focused on the microbial degradation of PFASs utilizing mass loads in sewage treatment plants (Sinclair and Kannan 2006; Loganathan et al. 2007), *in vitro* using mixed sewage inocula (Dinglasan et al. 2004; Rhoads et al. 2008), or in soil microcosms (Russell et al. 2008). Further studies employing pure cultures are necessary in beginning to understand the biochemistry and molecular biology utilized by bacteria in PFAS degradation (Van Hamme et al. 2013).

As previously noted, FTSA degradation has been described to bypass 6:2-FTOH production and convert straight into 6:2-FTAL (Wang et al. 2011; Van Hamme et al. 2013). The metabolites described in Figure 1-1 from 6:2-FTAL degradation, provide a reference point for analysis of FTSA degradation using pure cultures of *Gordonia* sp. NB4-1Y.

1.4 FTSA Degradation Pathway in *Gordonia* sp. NB4-1Y Pure Cultures

Desulfonation is the initial step required for FTSA to be biologically and chemically transformed into downstream products (Zhang et al. 2016). Desulfonation of FTSA by microbes was determined to be the rate-limiting step for further biodegradation (Wang et al. 2011). Van Hamme et al. (2013) describe a potential biotransformation pathway for FTSA using pure cultures of *Gordonia* sp. NB4-1Y (Figure 1-2). Wang et al. (2011) propose a similar pathway using mixed bacterial cultures in closed bottles of activated sludge from waste water treatment plants (Figure 1-3). However, Wang et al. (2011) propose that 5:3-acid is a minor product of FTSA degradation,

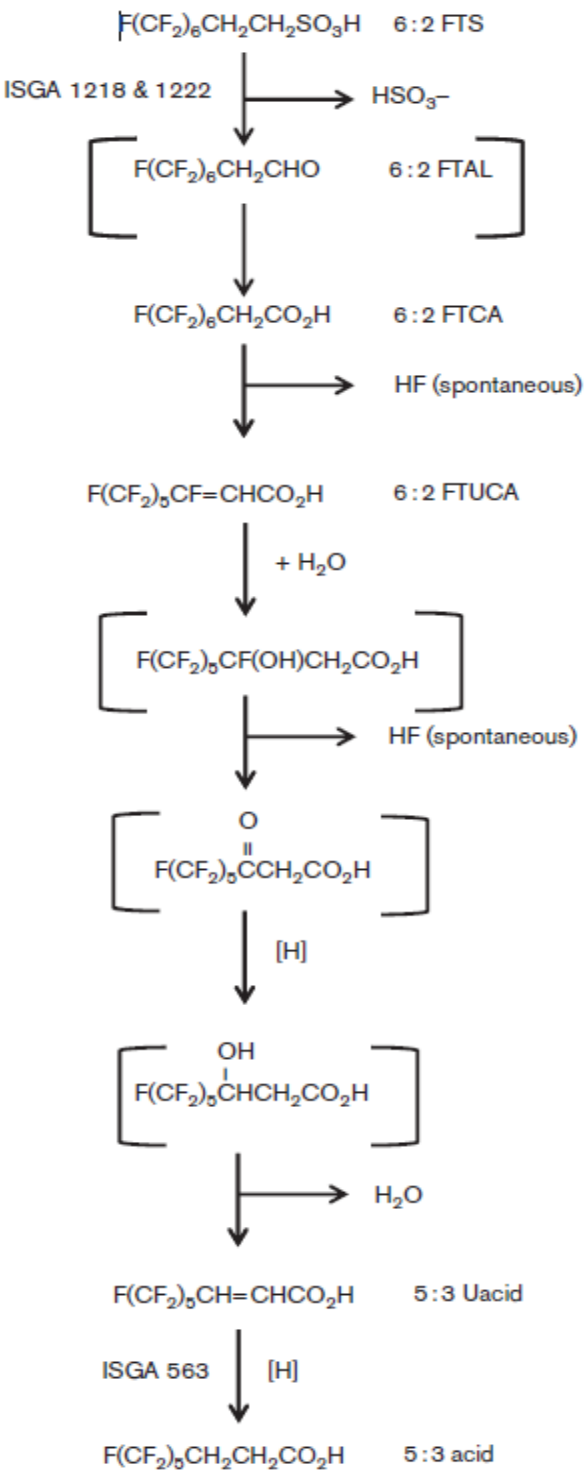


Figure 1-2: Proposed metabolic pathway for 6:2-FTSA in NB4-1Y pure cultures. Figure from Van Hamme et al. (2013).

and the volatile compounds 5:2-ketone and 5:2-sFTOH comprise the majority of transformation products. For the present study, culture vessels were designed to ensure volatile metabolites would be captured if NB4-1Y could produce them.

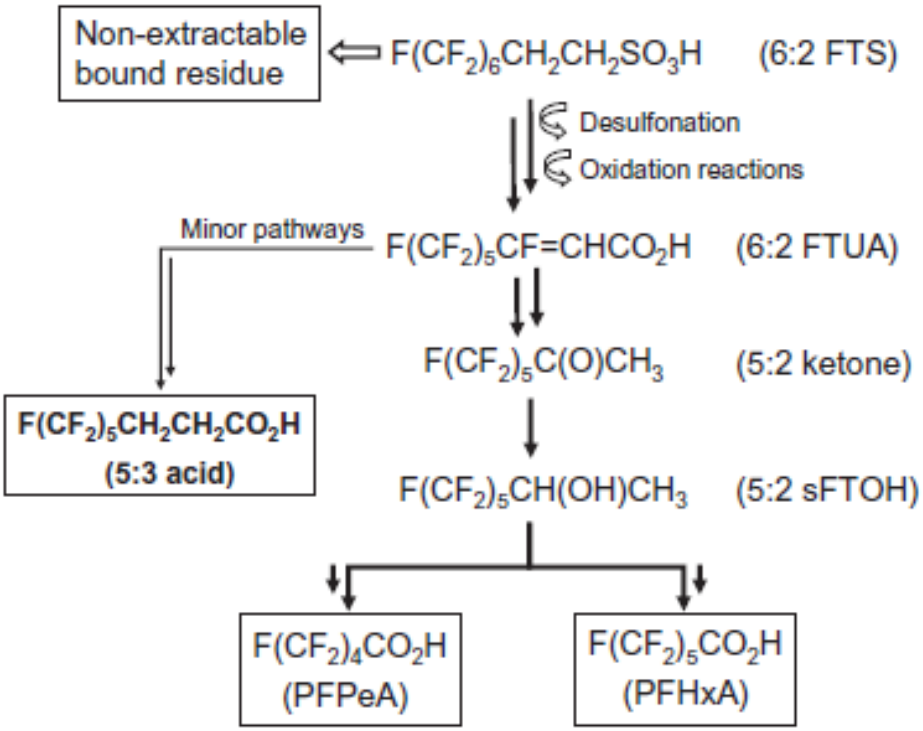


Figure 1-3: Proposed aerobic biotransformation pathway for 6:2-FTSA in activated sludge. Double arrows indicate multiple transofrmation steps. The analyte 5:3-acid (5:3-FTCA) is proposed to be part of a 'minor' pathway, with 5:2-ketone (5:2-FT-ketone) being inferred as the 'major' pathway. Figure from Wang et al. (2011).

1.5 Goals of this Study

This study set out with three goals in mind. First, we wanted to identify the majority of the metabolites produced by *Gordonia* sp. NB4-1Y when 6:2 fluorotelomer sulfonate or 6:2 fluorotelomer sulfonamide alkyl betaine are provided as the sole-sulfur source. Second, we wanted to quantify all metabolites produced during this degradation in order to generate a mass balance. The last goal of this study was to understand the degradation pathways *Gordonia* sp. NB4-1Y utilizes to metabolize FTSA and FTAB.

2 MATERIALS AND METHODS

2.1 Chemicals, Media, and Stock Solution Preparation

2.1.1 Chemicals

The following compounds were purchased from Fischer Chemical: KH_2PO_4 (Crystalline/Certified ACS, ≥ 99 % pure; 0.003 % sulfate max), K_2HPO_4 (Crystalline powder/Certified ACS, ≥ 98.0 % pure; 0.005 % sulfate max), NH_4Cl (Crystalline/Certified ACS, ≥ 99.5 % pure; 0.002 % sulfate max), $NaCH_3COO^-$ (Fused crystals/Certified ACS, ≥ 99 % pure; 0.003 % sulfate max), and acetonitrile (HPLC grade, submicron filtered, ≥ 99.9 % pure). $MgSO_4$ from J.T. Baker (anhydrous, powder; ≥ 99.0 % pure) was used in this experiment. De-ionized water (18-MΩ) for media and stock solutions was generated with a Barnstead Nanopure Diamond system. Anhydrous ethyl alcohol from Commercial Alcohols (100 % v/v) was used.

The ammonium salt of 6:2-FTSA was obtained from Synquest Laboratories (certified 98.0 % pure by titration with NaOH). A 0.27-g mL^{-1} solution of FTAB was provided by McGill University. Calibration standards for PFBA, PFPeA, PFHxA, PFHPa, 3:3-FTCA. 4:3-FTCA, 5:3-FTCA, 6:2-FTCA, 6:2-FTOH. 5:2-FT-ketone, 5:2-sFTOH, 6:2-FTSA, and 6:2-FTAB and labelled internal standards MPFBA, MPFPeA, MPFHxA, MPFOA, M62-FTCA, M62-FTS, and M62-FTUA came from Wellington Laboratories (Guelph ON (CAN)). Maxi-Clean 600-mg SPE C18 cartridges were purchased from GRACE (Deerfield IL (USA)).

2.1.2 Media and Stock Solution Preparation

Sulfur-free acetate (SFA) media was prepared according to Van Hamme et al. (2004) with the following composition per liter: 0.40-g potassium dibasic phosphate (KH_2PO_4), 1.60-g

potassium monobasic phosphate (K_2HPO_4), 1.55-g ammonium chloride (NH_4Cl), 5.00-g sodium acetate ($NaCH_3COO$), 0.165-g magnesium chloride ($MgCl_2$), 0.090-g calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), 5.00-mL Wolfe's minerals, and 1.00-mL Pfenning's vitamin solution. The appropriate masses for KH_2PO_4, K_2HPO_4, NH_4Cl, and $NaCH_3COO$ were added to a specific volume before being autoclaved at 121°C for 20 minutes. The solution was allowed to cool to room temperature before $MgCl_2$, $CaCl_2 \cdot H_2O$, Wolfe's minerals, and Pfenning's vitamin solution were added aseptically in a flowhood. Wolfe's minerals and Pfenning's vitamins solution were added as a 0.22-µM pore sized filter sterilized solution to cooled medium after autoclaving. Carbon-free media was prepared the same as sulfur-free media except for the addition of acetate. Stock solutions of $MgSO_4$, FTSA, and FTAB were prepared at 4-mM in a 50:50 mixture of anhydrous ethanol and 18-MΩ water. The FTSA solution was heated to 55°C and stirred overnight to fully dissolve the solution.

2.2 *Gordonia* sp. NB4-1Y Growth Optimization

2.2.1 Ethanol Toxicity Test

Gordonia sp. NB4-1Y cultures were grown using various concentrations of ethanol to determine an appropriate volume of ethanol to solubilize FTSA and FTAB in and henceforth, optimize growth conditions. A liquid inoculum culture containing 100-µL of anhydrous ethanol (1.0 % v/v), 25-µL of $MgSO_4$ stock, 375-µL of sterilized 18 MΩ water, and 9.50-mL of carbon-free media was inoculated from fresh plate culture and incubated for 92-hrs at 30°C with constant mixing. The range 0.5-5.0 % v/v of ethanol in a total culture volume of 15-mL was tested. Two replicates of each concentration contained 150-µL of liquid inoculum culture, 25-µL of $MgSO_4$

stock, 75-750 µL of anhydrous ethanol, 0-675 µL of sterilized 18 MΩ water, and 14.1-mL of carbon-free media. Optical density was measured at 600 nm every 24-hrs for 168-hrs.

2.2.2 Optimal FTSA & FTAB Concentration for Growth

Gordonia sp. NB4-1Y cultures were grown using various concentrations of FTSA or FTAB as the sole-sulfur source, to determine an optimal concentration for growth. A liquid inoculum culture containing 125-µL of FTSA or FTAB stock (50-µM of FTSA/FTAB), 125-µL of 50 % ethanol solution, and 9.75-mL of SFA medium was inoculated from plate culture and incubated for 92-hrs at 30^{o}C in a 50-mL rolling tube. Over a concentration range of 4 to 100-µM, two replicates for each specified concentration of FTSA or FTAB was tested over a 168-hr period. Rolling tube cultures contained 150-µL of inoculum, 15.0 to 375-µL of FTSA or FTAB stock, 0 to 360-µL of 50 % ethanol solution, and 14.475-mL of SFA media. Each culture had a starting ethanol concentration of 1.25 % v/v. Optical density readings were measured every 24-hrs of growth using a wavelength of 600 nm.

2.3 Time-Course Experiment

2.3.1 Inoculum Cultures

Inoculum cultures were prepared aseptically in sterilized 100-mL serum bottles with the following composition: 150-µL of their respective stock solution and 9850-µL of SFA. Two replicates were made for each condition: FTSA, FTAB, and $MgSO_4$. Two replicates for sterile FTSA, FTAB, $MgSO_4$ cultures were also made. Two replicates of a negative control were also made and contained 150-µL of 50 % ethanol solution and 9850-µL of SFA. Cultures were sealed air-tight with a butyl rubber septum and aluminum crimp cap. Cultures were grown for 72-hrs. at 30^{o}C and 150 rotations per minute.

2.3.2 Experimental Cultures

Experimental cultures were prepared in sterilized 100-mL serum bottles aseptically in a flowhood with the following composition: 200-µL of their respective inoculum culture, 300-µL of their respective chemical stock, and 19.50-mL of SFA media. Each culture contained 60-µM of target sulfur compound. Prior to inoculation, experimental cultures were sealed using a foam plug and incubated for 48-hrs. at 150 rotations per minute. After inoculation, bottles were sealed air-tight with a butyl rubber septum and aluminum crimp cap. Butyl rubber septa were sterilized using isopropanol prior to inserting two 20 G needles (Becton Dickinson and Company, Franklin NJ (USA)). A solid-phase C18 cartridge (GRACE 600-mg) was inserted into one needle while the other needle was inserted temporarily for purging cultures with sterilized air to replenish oxygen levels and capture volatile metabolites. A total of 25 replicates were produced for each condition, yielding 175 cultures total.

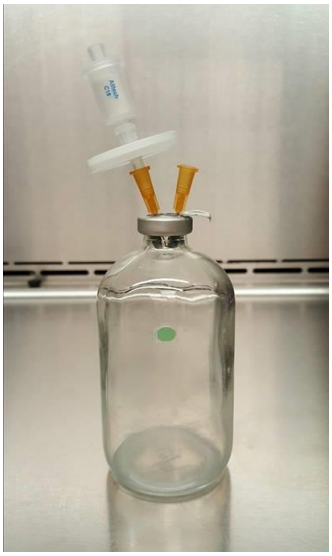


Figure 2-1: *Experimental culture setup.*

2.3.3 Purging Schedule

Oxygen levels for each culture condition were monitored using a Firesting O_2 Fibre-optic Oxygen Meter and Pyro Oxygen Logger software (Ohio Lumex Company Inc., Solon OH (USA)). One culture for each condition contained an oxygen sensor enabling the measurement of the depletion of oxygen levels. To ensure each culture was purged for the same length of time, the culture condition whose oxygen levels were depleted the greatest determined the length of time each culture was purged to fully replenish their oxygen levels.

Prior to purging, the surface of the butyl rubber septa were sterilized with isopropanol and a fresh 20G needle was inserted. An AquaClear 50 aquarium air pump (Rolf C. Hagen Corp., Mansfield MA (U.S.A)) customized with a HEPA-CAP^{TM} glass microfiber filter with polypropylene housing, two 0.30-µM membrane filters, and one 0.40-µM membrane filter was used to purge air into each culture. The pump had the following parameters: 120 Volts AC/60 Hz/3.5 watts, 2 x 2 500 cc/min, and a pounds per square inch (psi) of 2.5 lb/po^2. Purging time varied from 20 – 120 seconds and after purging, the purge needle was discarded.

2.3.4 Sample Extraction

After purging, four replicates from each condition (28 total) were taken for plating and extraction. Two replicates for each condition were plated aseptically on nutrient agar and incubated for 72-hrs at 30^{o}C in a INFORS HT Multitron Pro incubator. After plating, each replicate was treated with three volumes of acetonitrile (60-mL) and incubated for one hour at 30^{o}C, 150 rpm. A 10-mL aliquot of the supernatant was placed in a 50-mL polypropylene centrifuge tube and centrifuged in a Thermo Scientific ST 16R centrifuge at 4000 $\times g$ for ten minutes. Centrifuged samples were filtered using a syringe and 0.40-µM attachable membrane filter into a polypropylene sample vial. Aqueous samples were stored at -20^{o}C until analysis. Solid-phase C18 cartridges were removed after purging and eluted with 3-mL of acetonitrile into sample vials during the 1-hr incubation. C18 eluate samples were stored in polypropylene sample vials at -20^{o}C until analysis.

2.4 Analysis of Metabolites

2.4.1 Internal Standards and Instrumental Controls

Solutions spiked with 5-ppb of unlabeled perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), 3:3 fluorotelomer carboxylic acid (3:3-FTCA), 4:3 fluorotelomer carboxylic acid (4:3-FTCA), 5:3 fluorotelomer carboxylic acid (5:3-FTCA), 6:2 fluorotelomer carboxylic acid (6:2-FTCA), 6:2 fluorotelomer unsaturated acid (6:2-FTUA), FTSA, and FTAB, as well as 87.7-ppb of 5:2 secondary fluorotelomer alcohol (5:2-sFTOH), 5:2 fluorotelomer ketone (5:2-FT-ketone), and 6:2 fluorotelomer alcohol (6:2-FTOH) were used for quality control analysis. A linear range and instrument limit of detection (iLOD) for PFBA, PFPeA, PFHxA, PFHpA, 3:3-FTCA, 4:3-FTCA, 5:3-FTCA, 6:2-FTCA, 5:2-sFTOH, 5:2-FT-ketone, 6:2-FTOH, FTSA and FTAB was determined (Table 3-1).

Quality control samples were injected every 20 samples along the analytical sequence. Absolute area, the analyte to internal standard (IS) response ratio, retention time, and procedural blanks were monitored for each of the expected metabolites. Quality control is essential for ensuring the production of reliable data with a minimum amount of error, and to produce consistent, reliable data.

2.4.2 Analyte Identification and Quantification

Due to the lack of analytical standards for some of the investigated compounds, isotope-labelled ISs with similar chemical structure were used to quantify some compounds. Proposed by Backe et al. (2013), analytes can be classified into three categories based on the availability of

isotope-labelled ISs: quantitative (Qn) analytes, semi-quantitative (Sq), and qualitative (Ql). All of the analytes for this project were quantitative. Quantitative analytes are those of which the native analyte has a suitable corresponding IS available (Munoz et al. 2016). For example, MPFOA is used to quantify PFHpA as they are both similar in chemical structure (perfluoroalkyl carboxylates), a method optimized and validated by Munoz et al. (2016). All of the expected metabolites in this experiment are considered to be quantitative analytes, as ISs with similar molecular structure were available. An IS solution containing MPFBA, MPFPeA, MPFHxA, MPFOA, M62-FTS, M62-FTCA, and M62-FTUA was used to quantify the expected metabolites. Each component of the IS solution corresponds to one or several analytes. A summary of the quantitative analytes and their corresponding IS can be found in the Appendix: Table 7-2.

2.4.3 Sample Preparation

C18 eluate samples were diluted by a factor of 1.25 and contained 160-µL of eluate sample in ACN and 40-µL of an IS solution at 25-ng mL^{-1}. Aqueous samples were diluted one to two times. Dilution level 1 was a factor of 16 containing 50-µL of the extracted sample, 110-µL of ACN, and 40-µL of IS solution at 25-ng mL^{-1}. Dilution level 2 was a factor of 400 containing 40-µL of the diluted sample, 120-µL of ACN, and 40-µL of IS solution at 25-ng mL^{-1}.

2.4.4 Instrumental Analysis

Chromatographic operating conditions were adapted from a procedure developed by Munoz et al. (2016). The Dionex Ultimate 3000 LC chain was operated out of the University of Montreal. A Thermo Hypersil Gold aQ column (100 × 2.1 mm; dp = 1.9-µm) was used for analyte

separation and a Thermo Hypercarb column (20 x 2.1 mm; dp = 7 µM) was the delay column used. The aqueous mobile phase consisted of HPLC-water with 0.15 % acetic acid (v/v) and the organic phase consisted of acetonitrile with 0.15 % acetic acid (v/v). Flow rate was set at 0.55-mL min^{-1}, injection volume at 15-µL, and the column inlet temperature at 40^{o}C. Details on chromatographic gradient elution conditions are supplied in the Appendix: Table 7-2. The heated electrospray ionization source was used with the following conditions: sheath gas flow rate was 45 arbitrary units (a.u.), auxiliary gas flow rate was 10 a.u., sweep gas flow rate was 10 a.u., and capillary temperature was 320^{o}C. Spray voltage was either -3-kV or +3-kV operating in fast polarity-switching mode. Analyte detection was performed using a Q-Exactive Orbitrap mass spectrometer. Orbitrap parameters were set as follows: AGC was set at $5x10^{6}$, maximum injection time at 50-ms, and resolution at 70,000. The mass scan range was set at 150-700 *m/z* (full scan MS mode). A summary of the method parameters can be found in the Appendix: Table 7-2.

2.4.5 Quantitative Process

Analytes were quantified in the final diluted aqueous and C18 eluate samples using the analyte/IS ratios and worked back through dilution factors to determine the amounts in the original aqueous samples and C18 eluate. These ng mL^{-1} values were then converted to mol % to represent concentrations collected from the aqueous phase and C18 eluates.

3 RESULTS

3.1 *Gordonia* sp. NB4-1Y Growth Optimization

3.1.1 Ethanol Toxicity

Given that PFAS stock solutions were prepared in ethanol, the concentration of ethanol that *Gordonia* sp. NB4-1Y could tolerate was determined by exposing cultures to ethanol concentrations ranging from 0.5 to 2.5 % v/v and monitoring $OD_{600\ nm}$. At ethanol concentrations of 0.5 and 1.0 %, NB4-1Y grew to an $OD_{600\ nm}$ of 0.387 and 0.410 after 24-hrs, and 0.517 and 0.544 after 144-hrs, respectively. Cultures grown at an ethanol concentration of 2.5 % had an average $OD_{600\ nm}$ of 0.136 after 24-hrs and 0.620 after 144-hrs (Figure 3-1).

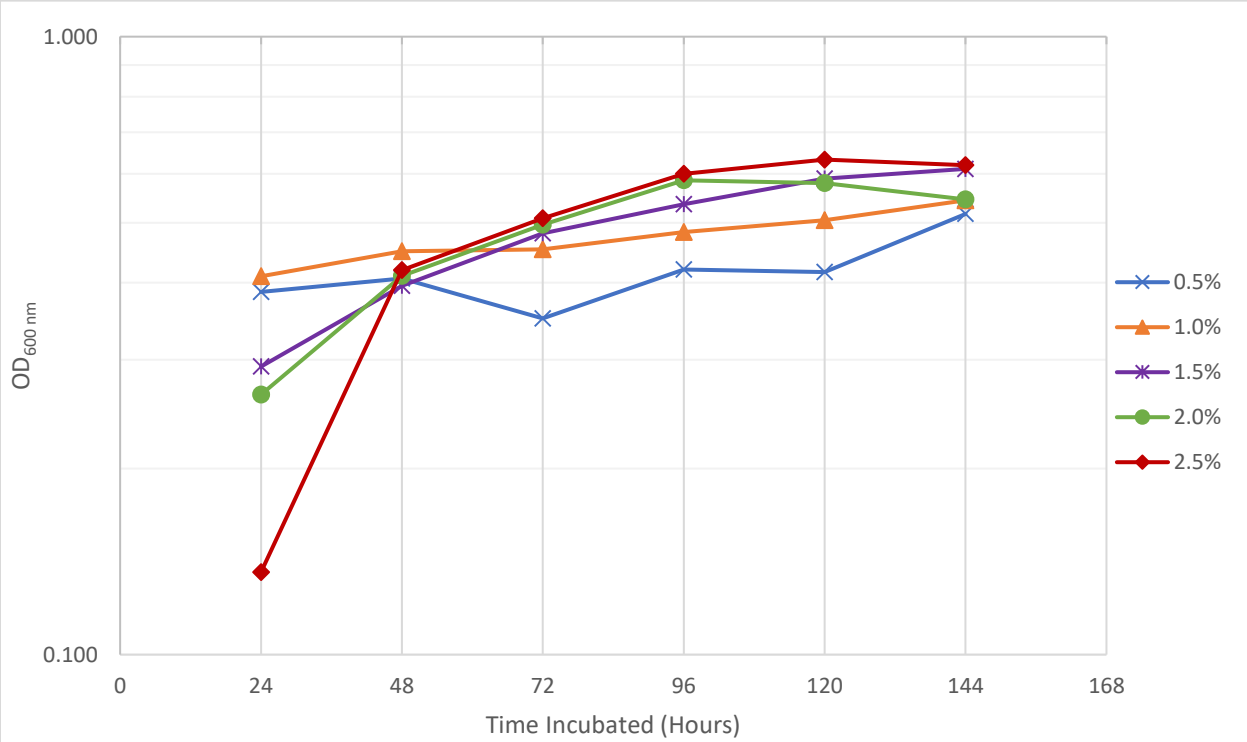


Figure 3-1: Determination of ethanol tolerance in *Gordonia* sp. NB4-1Y between 0.5-2.5 % v/v ethanol.
Exponential growth of NB4-1Y is delayed upon increasing ethanol concentration from 0.5-2.5 %.

Given that NB4-1Y appeared to tolerate 2.5 % v/v ethanol, the experiment was repeated with concentrations between 3.0 and 5.0 % v/v. At 3.0 % v/v ethanol, $OD_{600\ nm}$ values of 0.073 and 0.712 were observed after 24 and 144-hrs of incubation, respectively. Cultures grown at an ethanol concentration of 5.0 % v/v had an average absorbance of 0.008 and 0.079 after 24 and 144-hrs of incubation (Figure 3-2), an order of magnitude lower than at concentrations below 4.0 % v/v.

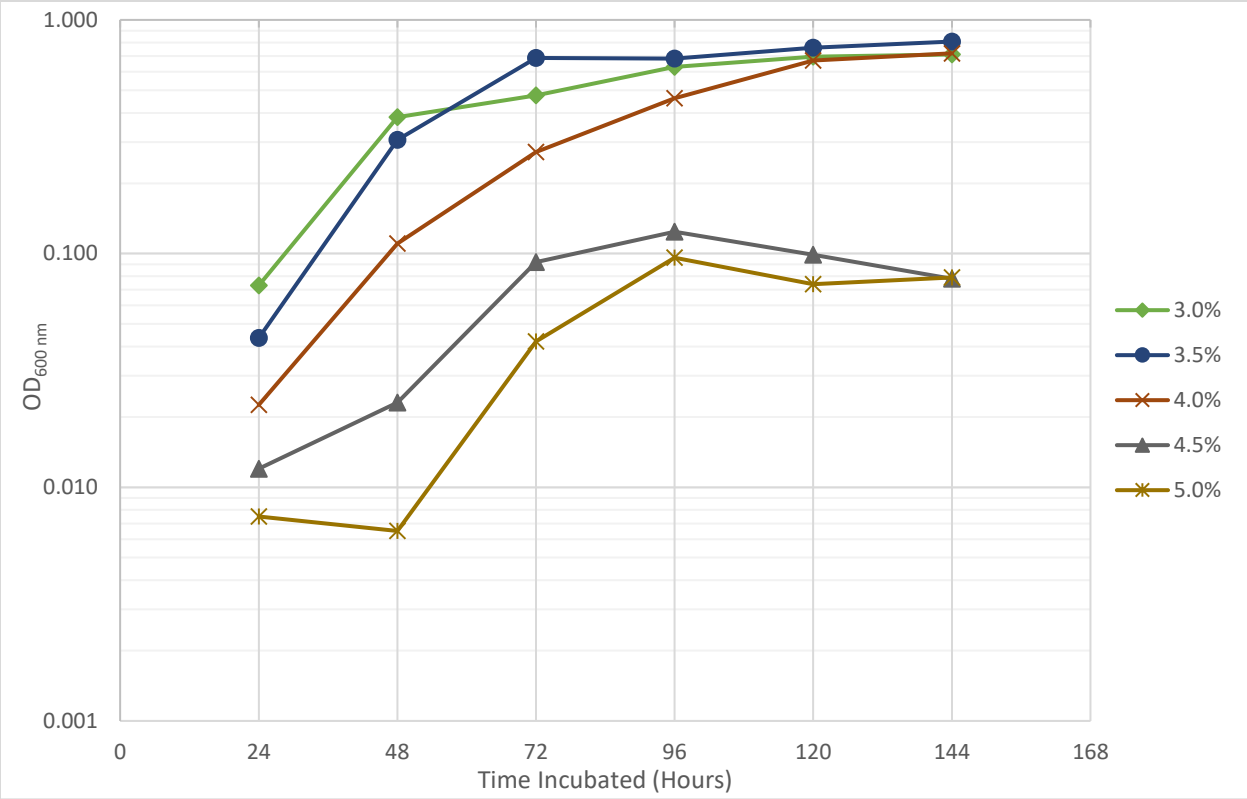


Figure 3-2: Determination of ethanol tolerance of *Gordonia* NB4-1Y between 3.0-5.0 % v/v ethanol.
Exponential growth is further delayed upon increasing ethanol concentration past 2.5 %. Toxicological effects are experience in ethanol concentrations greater than 4.0 %.

3.1.2 Optimum FTSA Concentration

In order to optimize conditions for detecting FTSA metabolites in NB4-1Y cultures, the organism was exposed to FTSA concentrations ranging from 4 to 100-µM. At 4-µM, NB4-1Y grew to an $OD_{600\ nm}$ of 0.039 after 24-hrs and 0.249 after 168-hrs of incubation. When supplied

with 100-µM FTSA, cultures grew to $OD_{600\ nm}$ 0.028 after 24-hrs and 0.565 after 168-hrs (Figure 3-3). There was a 52.8 % increase in overall growth by increasing the concentration of FTSA from 20-µM (0.207 after 168-hrs) to 40-µM (0.392 after 168-hrs) (Figure 3-3). A FTSA concentration of 60-µM was utilized in the growing of NB4-1Y cultures in this study.

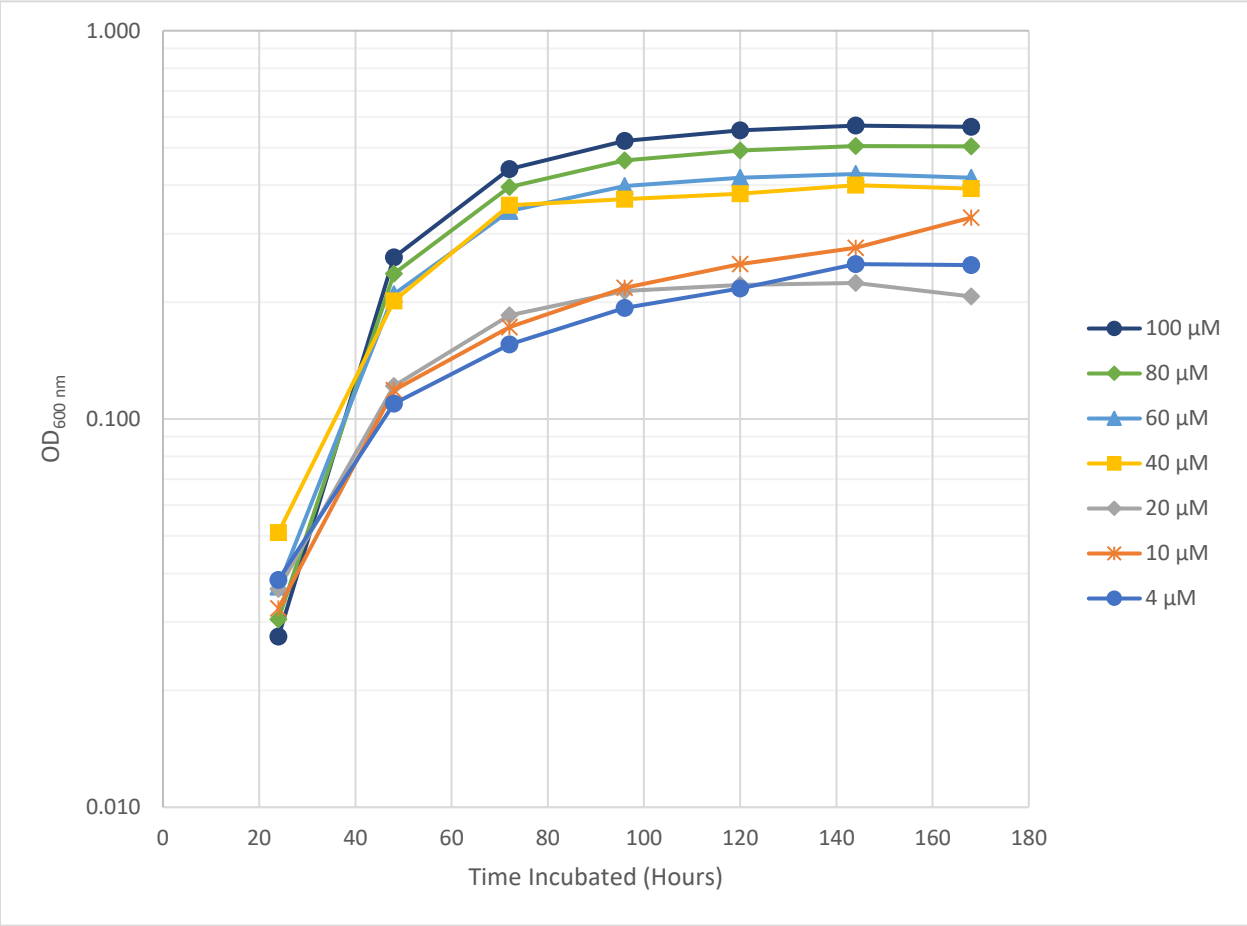


Figure 3-3: Determination of an optimal concentration of FTSA to be used as a sole-sulfur source for *Gordonia* sp. NB4-1Y.
No apparent toxicological effects are exhibited by NB4-1Y in the 4-100-µM range. An increase in overall growth is observed when increasing the concentration of FTSA from 20 to 40-µM.

3.1.3 Optimum FTAB Concentration

In order to optimize conditions for detecting FTAB metabolites in NB4-1Y cultures, the organism was exposed to FTAB concentrations ranging from 4 to 100-µM. At 4-µM, NB4-1Y grew to an $OD_{600\ nm}$ of 0.023 after 24-hrs and 0.095 after 168-hrs of incubation. When supplied

with 100-µM FTAB, cultures grew to an $OD_{600\ nm}$ of 0.035 after 24-hrs and 0.316 after 168-hrs (Figure 3-4). There was a 57.7 % increase in overall growth between increasing the concentration of FTAB from 20-µM (0.126 after 168-hrs) to 40-µM (0.218 after 168-hrs) (Figure 3-4). At a concentration of 80-µM, NB4-1Y exhibited increased growth (0.333), while at 100-µM NB4-1Y showed a slight decrease (0.316).

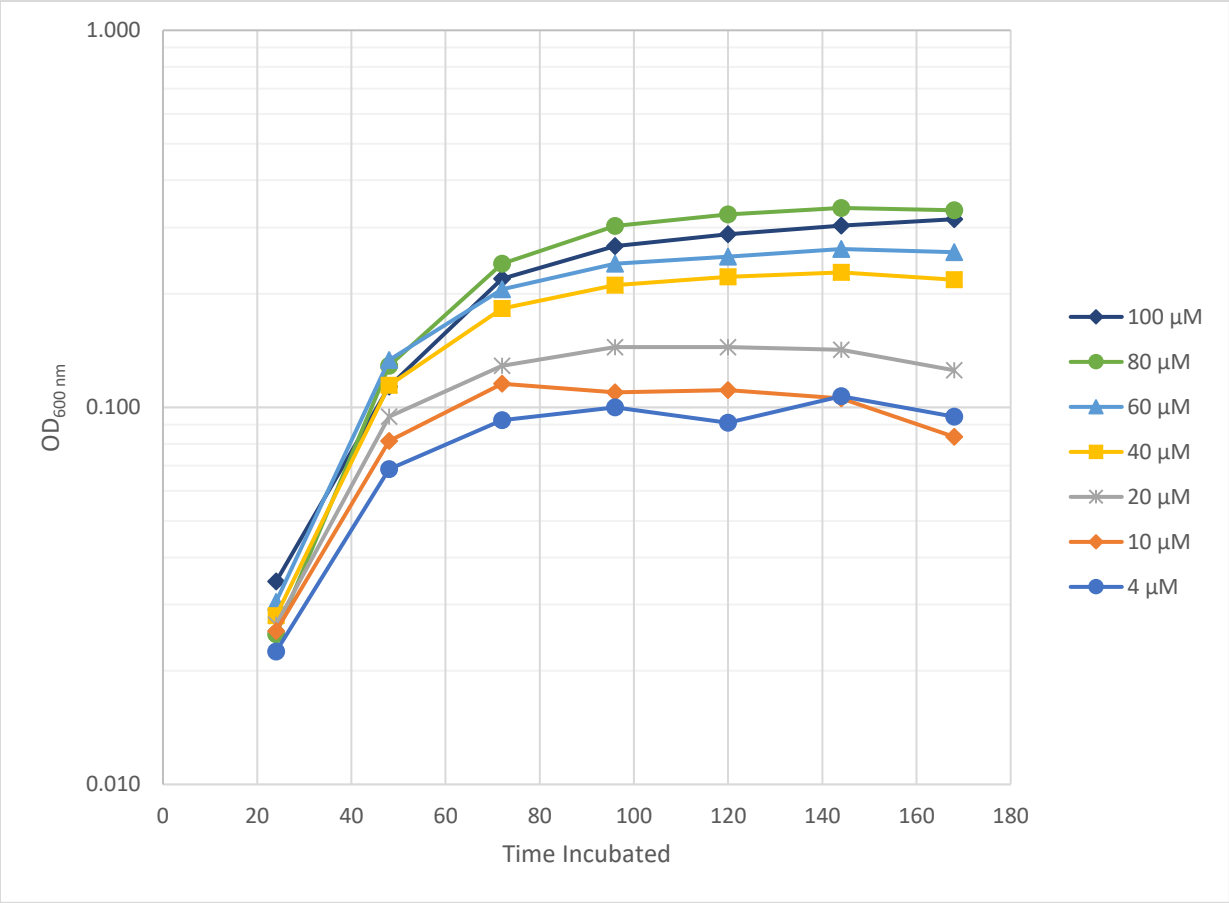


Figure 3-4: Determination of an optimal concentration of FTAB to be used as a sole-sulfur source for *Gordonia* sp. NB4-1Y.
No apparent toxicological effects are exhibited by NB4-1Y in the 4-100-µM range tested. A significant increase in overall growth is seen when increasing the concentration of FTAB from 20 to 40-µM.

3.2 Experimental Controls

3.2.1 Method Validation

3.2.1.1 Linearity and Instrumental Detection Limits

A linear range and instrumental limit of detection for FTSA, FTAB, and the twelve expected metabolites was determined prior to sample analysis. The coefficient of determination (R^2) for each analyte was >0.9920 and the instrumental limit of detection for the compounds ranged from 0.04 to 8-ng mL^{-1} (Table 3-1).

Table 3-1: Linearity and instrumental detection limits for the expected fluorinated metabolites, FTSA, and FTAB.

Compound	R^2	Linear Range (ng mL^{-1})	iLOD (ng mL^{-1})
PFBA	0.9962	0.29-34	0.09
PFPeA	0.9959	0.29-34	0.09
PFHxA	0.9970	0.06-34	0.04
PFHpA	0.9986	0.29-34	0.08
6:2-FTAB	0.9994	0.29-34	0.18
6:2-FTSA	0.9928	0.29-34	0.17
3:3-FTCA	0.9927	0.29-34	0.2
4:3-FTCA	0.9970	0.29-34	0.16
5:3-FTCA	0.9973	0.29-34	0.27
6:2-FTCA	0.9966	0.06-34	0.07
6:2-FTUA	0.9965	0.06-34	0.06
6:2-FTOH	0.9931	10-600	8
5:2-sFTOH	0.9938	1-600	0.9
5:2-FT-ketone	0.9956	1-600	1

3.2.2 Quality Control

3.2.2.1 Absolute Area

In this study, control charts for absolute area, analyte/IS response ratio, and retention time were utilized to evaluate the performance of the LC-MS/MS and ensure precise and accurate measurements of each analyte. Control charts are a visual representation of the confidence interval of some important parameter which represents the quality assurance or the integrity of the study. Confidence intervals are a range of values so defined that there is a specified probability that the value of a parameter will lie within it. For example, analysis of a solution containing 5-ng mL^{-1} of PFBA should yield a constant response every time a solution with 5-ng mL^{-1} of PFBA is analyzed.

PFBA, PFPeA, PFHxA, PFHxA, 3:3-FTCA, 4:3-FTCA, 5:3-FTCA, 6:2-FTUA, and 6:2-FTSA showed relatively constant values for absolute area throughout quality control while 6:2-FTCA, 5:2-FT-ketone, 5:2-sFTOH, 6:2-FTAB, and 6:2-FTOH fluctuated. The control chart for

PFBA, PFPeA, PFHxA, and PFHpA is presented here (Figure 3-5), and others can be found in the Appendix: Figure 7-1 to Figure 7-4.

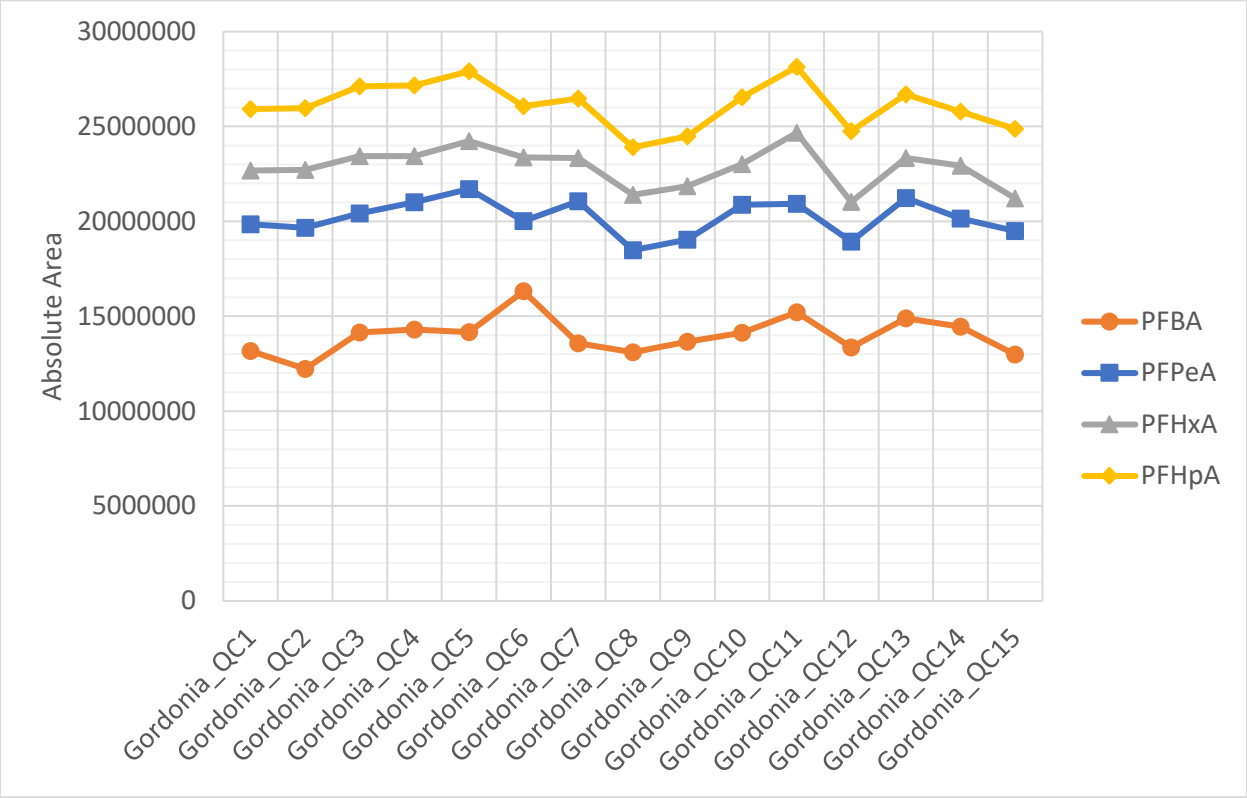


Figure 3-5: Illustration of quality control approach along the analytical sequence; control chart used to monitor the signal received upon the detection of 5-ng mL^{-1} PFBA, PFPeA, PFHxA, and PFHpA.

3.2.2.2 Analyte to Internal Standard Response Ratio

Control charts for the analyte/IS response ratio were monitored throughout the experiment to ensure accurate quantification of each analyte. PFBA, PFPeA, PFHxA, PFHpA, 3:3-FTCA, 4:3-FTCA, 5:3-FTCA, 6:2-FTUA, 5:2-FT-ketone, 6:2-FTSA, 5:2-sFTOH, 6:2-FTOH showed relatively constant analyte/IS ratios throughout quality control analysis, while 6:2-FTCA and 6:2-FTAB fluctuated. The control chart for PFBA, PFPeA, PFHxA, and PFHpA is presented here

(Figure 3-6) and the remaining control charts can be found in the Appendix: Figure 7-5 to Figure 7-9.

Figure 3-6: Illustration of quality control approach along the analytical sequence; control chart used to monitor the signal received upon the detection of 5-ng mL^{-1} PFBA, PFPeA, PFHxA, and PFHpA.

3.2.2.3 Retention Time

The retention time among the 14 analytes in the quality control samples varied very little (Figure 3-7).

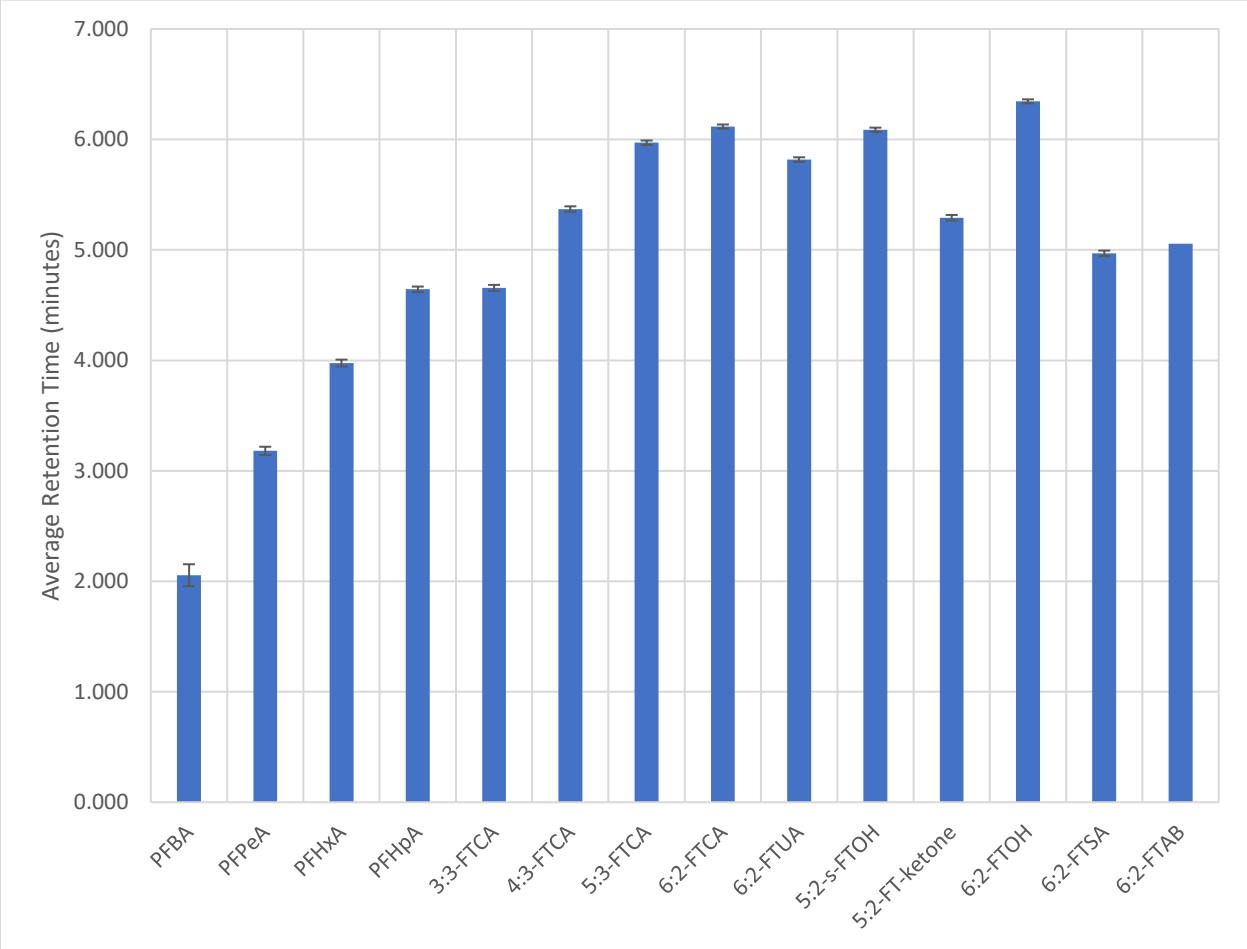
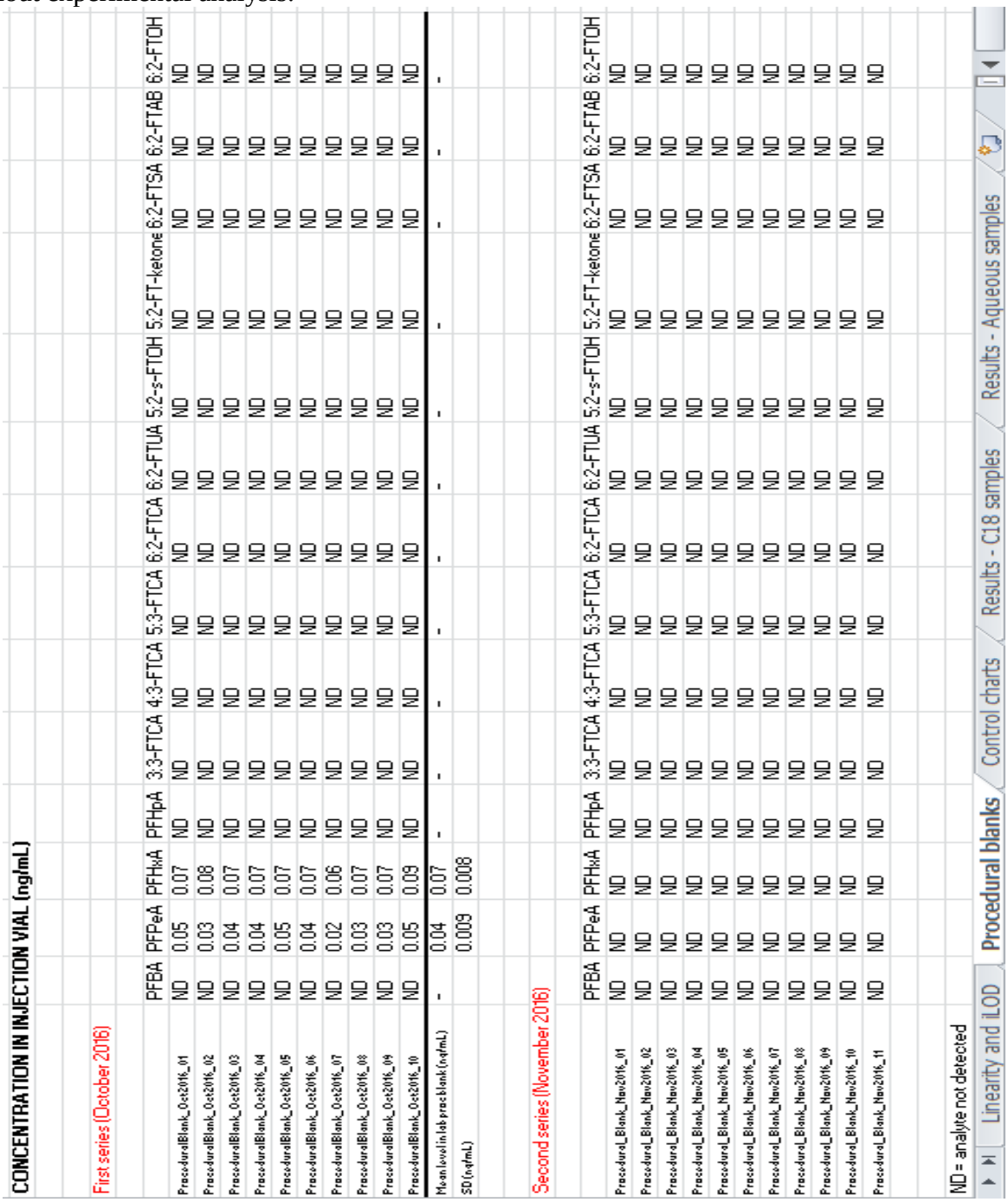


Figure 3-7: Illustration of quality control approach along the analytical sequence; average retention time and standard deviation (n = 15) with an average relative standard deviation (RSD) of 0.8 % of monitored PFAs throughout analytical analysis.

3.2.2.4 Procedural Blanks

Procedural blanks were conducted throughout experimental analysis to monitor for trace amounts of carryover and for method and procedure validation.

Table 3-2: Procedural blank analysis conducted during the months of October and November throughout experimental analysis.



CONCENTRATION IN INJECTION VIAL (ng/mL)

First series (October 2016)

	PFBA	PFPeA	PFHxA	PFHpA	3:3-FTCA	4:3-FTCA	5:3-FTCA	6:2-FTCA	6:2-FTUA	5:2-s-FTOH	5:2-FT-ketone	6:2-FTSA	6:2-FTAB	6:2-FTOH
ProceduralBlank_Oct2016_01	ND	0.05	0.07	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ProceduralBlank_Oct2016_02	ND	0.03	0.08	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ProceduralBlank_Oct2016_03	ND	0.04	0.07	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ProceduralBlank_Oct2016_04	ND	0.04	0.07	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ProceduralBlank_Oct2016_05	ND	0.05	0.07	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ProceduralBlank_Oct2016_06	ND	0.04	0.07	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ProceduralBlank_Oct2016_07	ND	0.02	0.06	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ProceduralBlank_Oct2016_08	ND	0.03	0.07	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ProceduralBlank_Oct2016_09	ND	0.03	0.07	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ProceduralBlank_Oct2016_10	ND	0.05	0.09	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mean level in lab prep blank (ng/mL)	-	0.04	0.07	-	-	-	-	-	-	-	-	-	-	-
SD (ng/mL)		0.009	0.008											

Second series (November 2016)

	PFBA	PFPeA	PFHxA	PFHpA	3:3-FTCA	4:3-FTCA	5:3-FTCA	6:2-FTCA	6:2-FTUA	5:2-s-FTOH	5:2-FT-ketone	6:2-FTSA	6:2-FTAB	6:2-FTOH
ProceduralBlank_Nov2016_01	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ProceduralBlank_Nov2016_02	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ProceduralBlank_Nov2016_03	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ProceduralBlank_Nov2016_04	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ProceduralBlank_Nov2016_05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ProceduralBlank_Nov2016_06	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ProceduralBlank_Nov2016_07	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ProceduralBlank_Nov2016_08	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ProceduralBlank_Nov2016_09	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ProceduralBlank_Nov2016_10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ProceduralBlank_Nov2016_11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND = analyte not detected

3.3 Experimental Results

3.3.1 FTSA Liquid Cultures and C18 Eluates

3.3.1.1 Initial Steps in FTSA Degradation

The initial breakdown products of FTSA (6:2-FTCA, 6:2-FTUA, 6:2-FTOH) were quantified over a 168-hr incubation period, during which time NB4-1Y degraded the FTSA from 100.55 to 0.03 mol % (Figure 3-8). The metabolites 6:2-FTCA and 6:2-FTUA were present at the beginning of incubation (0-hr) and their concentrations increased in the aqueous phase from 0.02 to 4.28 mol % and 0.45 to 13.68 mol %, respectively, over the 168-hr incubation period. 6:2-FTOH was found to increase in concentration in the eluate of the C18 cartridges from 0.17 to 4.14 mol % between 96 and 168-hrs of incubation (Figure 3-8). In sterile FTSA cultures, the concentration of FTSA ranged from 90.27 mol % at 0-hr to 129.75 mol % after 72-hrs, to 105.11 mol % after 168-hrs of incubation (Figure 3-8), averaging 103.53 mol %. Additional degradation products were

quantified and split into two groups, major and minor degradation pathways, as presented below.

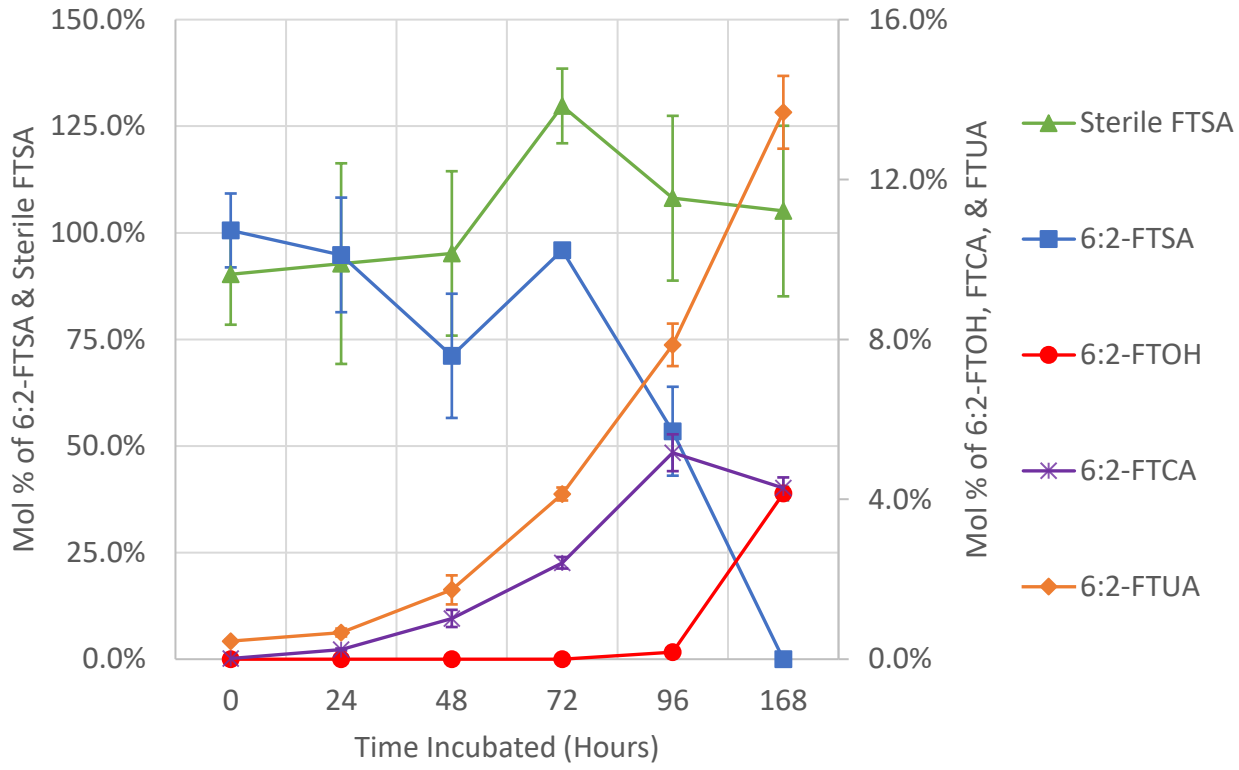


Figure 3-8: Time course of FTSA degradation in NB4-1Y cultures and production of breakdown products 6:2-FTOH, 6:2-FTCA, 6:2-FTUA in aqueous samples and their respective C18 eluates over a 168-hr time period.
FTSA, 6:2-FTCA, and 6:2-FTUA are present in the aqueous phase of inoculated FTSA cultures, while 6:2-FTOH (red) was identified in the C18 eluate of inoculated FTSA cultures. Sterile FTSA (green) represents the quantification of FTSA present in sterile FTSA cultures. Four replicates for each time point were analyzed (n=4).

3.3.1.2 Major Degradation Pathway Products

NB4-1Y converted 43.68 mol % of total FTSA into the major metabolite 5:2-FT-ketone, which was recovered from C18 cartridges (Figure 3-9). 5:2-FT-ketone first appeared after 24-hrs of incubation. Approximately 8.97 mol % of total FTSA was converted into 5:2-sFTOH after 168-hrs of incubation and first appeared after 96-hrs. PFHxA was present as 0.097 mol % beginning at 0-hr and increased to 0.534 mol % after 168-hrs. In the aqueous phase, PFPeA appeared after 72-

hrs yielding 0.002 mol % and increased to 0.075 mol % after 168-hrs (Figure 3-9).

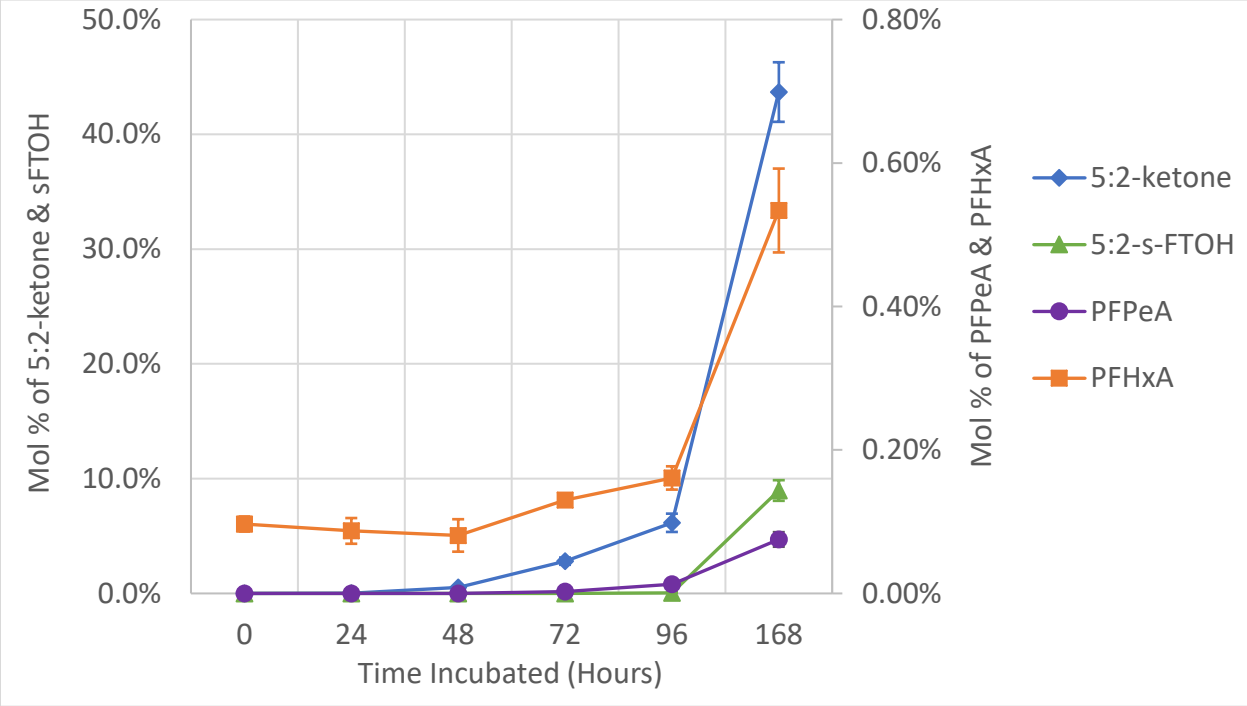


Figure 3-9: Major degradation pathway products of inoculated FTSA cultures and C18 eluate over a 168-hr time period.
5:2-FT-ketone and 5:2-sFTOH are present in C18 eluate while PFPeA and PFHxA are present in inoculated FTSA cultures. Four replicates for each time point were analyzed (n=4).

3.3.1.3 Minor Degradation Pathway Products

The metabolite 5:3-FTCA appeared after 48-hrs yielding 0.099 mol %, peaked at 0.726 mol % after 96-hrs, and finished at 0.351 mol % after 168-hrs of incubation. The metabolites PFBA and 4:3-FTCA were first identified after 168-hrs yielding 0.050 and 0.020 mol %, respectively

(Figure 3-10).

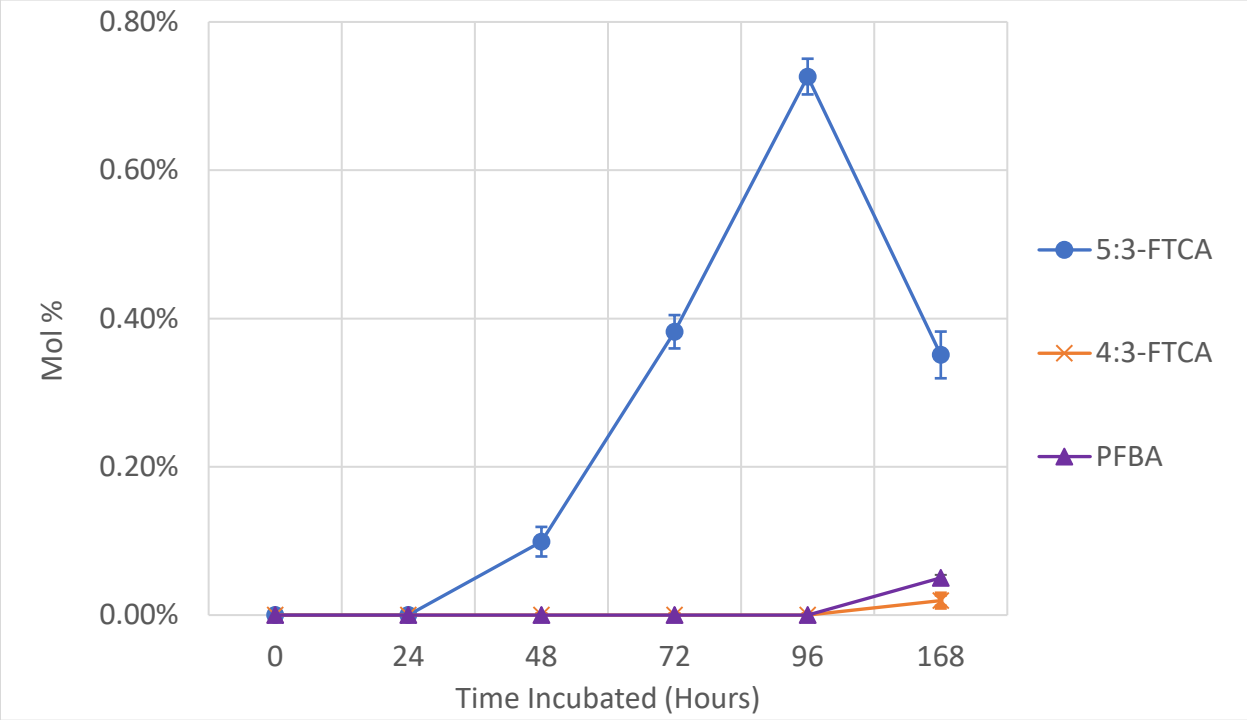


Figure 3-10: Minor degradation pathway products of inoculated FTSA cultures over a 168-hr time period.
5:3-FTCA, 4:3-FTCA, and PFBA were identified in the aqueous phase in extremely small quantities. Four replicates for each time point were analyzed (n=4).

3.3.1.4 Percent Recovery

After 168-hrs, more than 50 % of the metabolites produced from FTSA degradation were found in the volatile phase (eluted from C18 cartridges), while approximately 1/5 of the total FTSA was converted to water-soluble metabolites. The percent recovery for FTSA and the expected metabolites ranged from 74.09-105.92 % (Table 3-3), yielding an average recovery rate of 88.21 %.

Table 3-3: Percent recovery of fluorinated analytes and concentration of analytes identified in the aqueous and volatile phase in inoculated FTSA cultures at specific time points.

HOURS INCUBATED	AQUEOUS TOTAL (µm)	C18 ELUATE TOTAL (µm)	TOTAL (µM)	% RECOVERY
0	60.76	0.00	60.76	101.27
24	57.57	0.00	57.58	95.96
48	44.52	0.32	44.84	74.73
72	61.86	1.69	63.55	105.92
96	40.61	3.84	44.45	74.09
168	12.26	34.13	46.39	77.31

3.3.2 FTAB Liquid Cultures and C18 Eluates

3.3.2.1 Initial Steps in FTAB Degradation

Over the 168-hr incubation period, *Gordonia* sp. NB4-1Y converted more than 50 % of the FTAB present in the culture. Degradation of FTAB appeared to occur between 96 and 168-hrs of incubation (Figure 3-11). FTSA was present in the initial solution at a concentration of 0.93 mol % and was completely degraded after 72-hrs. The volatile metabolite 6:2-FTOH increased from 0.197 – 2.421 mol % from the time it appeared at 96-hrs to 168-hrs. 6:2-FTCA was initially present at 0.009 mol % and increased to 7.467 mol % after 168-hrs. 6:2-FTUA was initially present at 0.015 mol % and increased to yield 10.791 mol % after 168-hrs. In sterile FTAB cultures, the

concentration of FTAB ranged from 65.42 – 109.73 mol % (Figure 3-11), averaging 83.05 mol %.

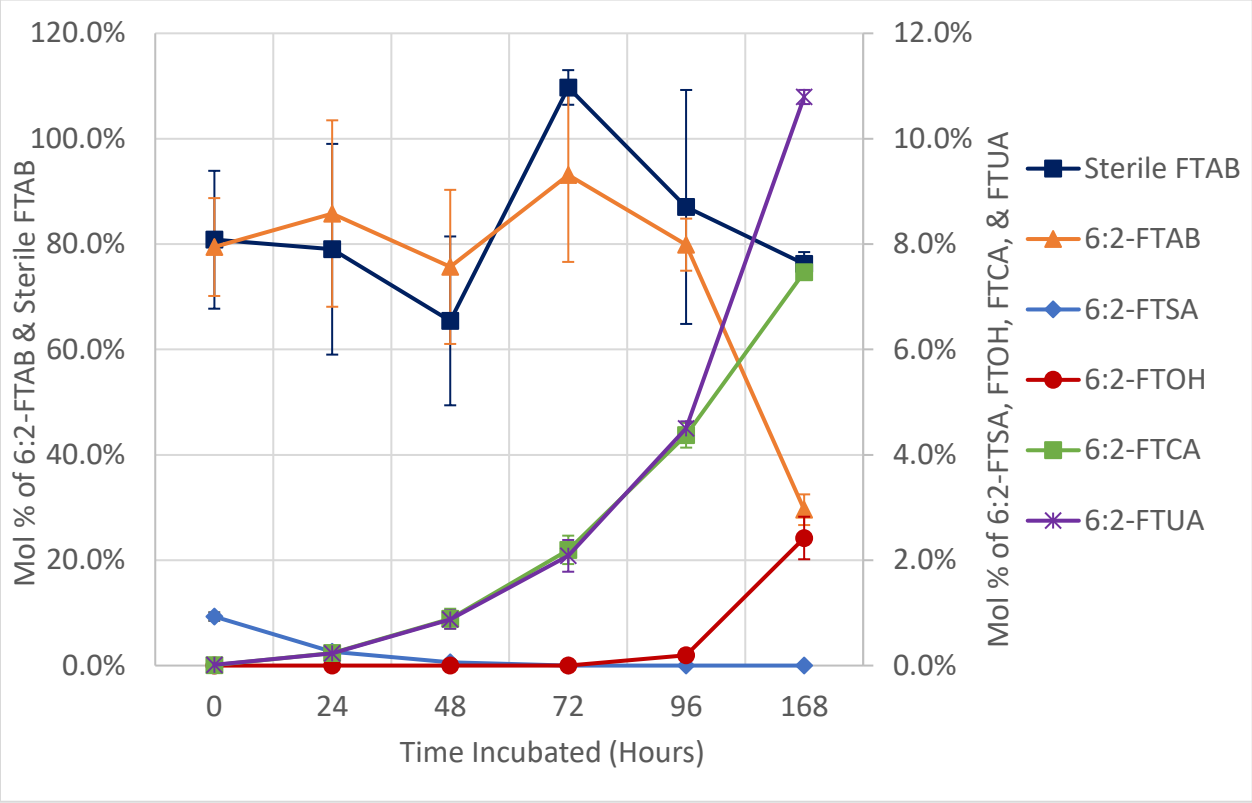


Figure 3-11: FTAB degradation in NB4-1Y cultures and production of 6:2-FTOH, 6:2-FTCA, 6:2-FTUA in aqueous samples and their respective C18 eluates over a 168-hr time period.
FTAB, FTSA, 6:2-FTCA, and 6:2-FTUA are present in the aqueous phase of inoculated FTAB cultures, while 6:2-FTOH (red) was identified in C18 eluate. Sterile FTAB (dark blue) represents the quantification of FTAB present in sterile FTAB cultures. Four replicates for each time point were analyzed (n=4).

3.3.2.2 Major Degradation Pathway Products

Gordonia sp. NB4-1Y converted 18.349 mol % of FTAB into volatile 5:2-ketone after 168-hrs of incubation. 5:2-ketone was first eluted from C18 cartridges after 24-hrs of incubation, yielding 0.008 mol %. The metabolite 5:2-sFTOH first appeared after 96-hrs and increased from 0.024 – 1.247 mol % after 168-hrs (Figure 3-12). PFPeA and PFHxA were detected at low

concentrations, yielding 0.001 and 0.026 mol %, respectively, after 168-hrs (Figure 3-12).

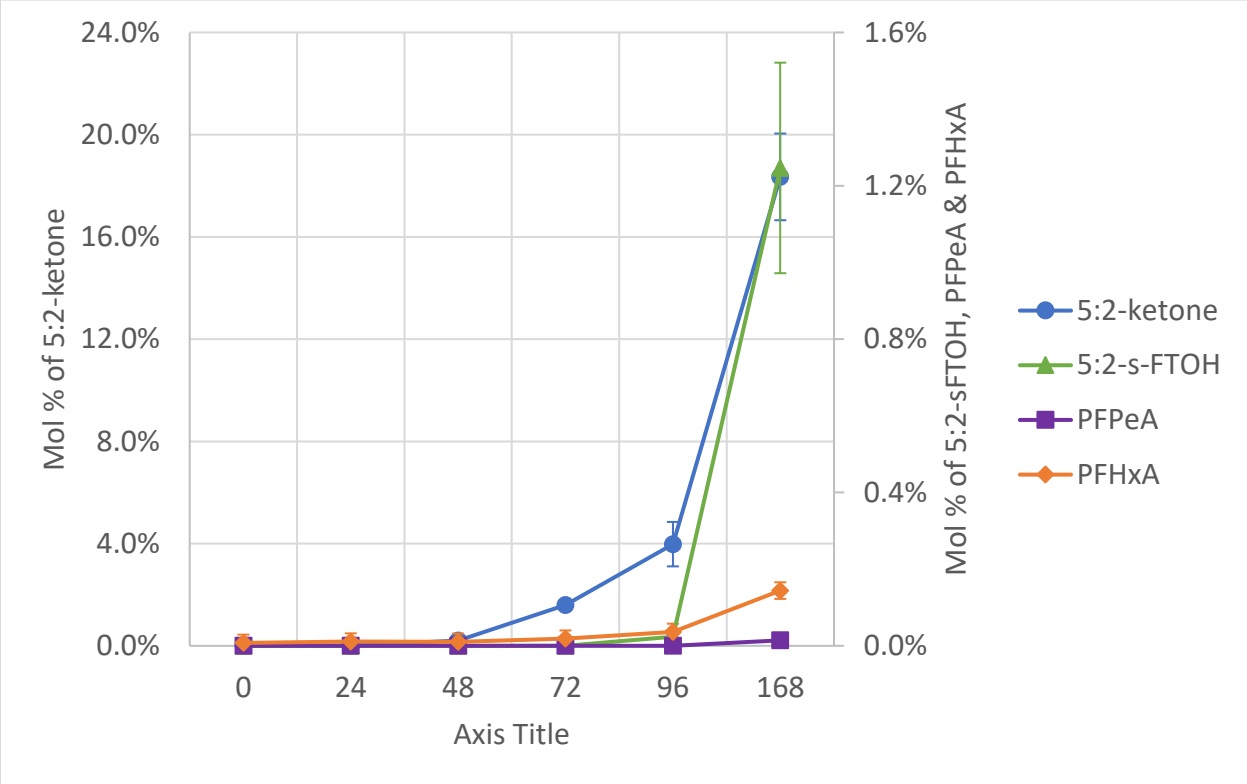


Figure 3-12: Major degradation pathway products of inoculated FTAB cultures and C18 eluate over a 168-hr time period.
PFPeA and PFHxA were identified in the aqueous phase, while 5:2-FT-ketone and 5:2-sFTOH were eluted from C18 cartridges. Four replicates for each time point were analyzed (n=4).

3.3.2.3 Minor Degradation Pathway

The metabolite 5:3-FTCA slowly increased 10-fold from 0.040 mol % at 48-hrs to 0.413 mol % at 168-hrs (Figure 3-13). The metabolites 4:3-FTCA and PFBA were both identified after

168-hrs of incubation and were quantified at 0.018 mol % of the total FTAB present in the cultures.

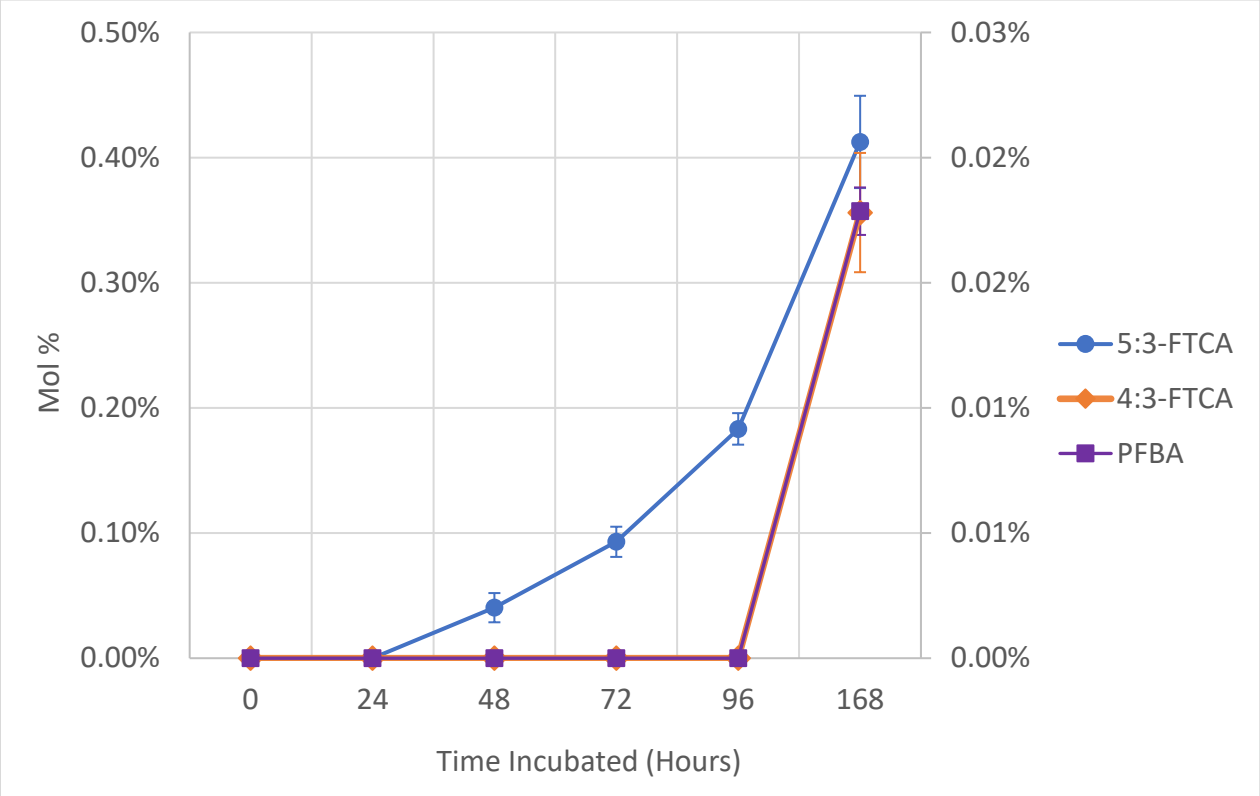


Figure 3-13: Minor degradation pathway products of inoculated FTAB cultures over a 168-hr time period.
5:3-FTCA, 4:3-FTCA, and PFBA were identified in the aqueous phase in extremely small quantities. Four replicates for each time point were analyzed (n=4).

3.3.2.4 Percent Recovery

After 168-hrs, 13.22 of the total 60-µM present was in the volatile phase and eluted from C18 cartridges, while 29.24-µM was identified in the aqueous phase (Table 3-4). The percent recovery of FTAB and the expected metabolites ranged from 70.77 – 99.09 % (Table 3-4), yielding an average recovery rate of 84.64 %.

Table 3-4: Percent recovery of fluorinated analytes and proportion of analytes identified in the aqueous and volatile phase in inoculated FTAB cultures at specific time points.

HOURS INCUBATED	AQUEOUS TOTAL (µm)	C18 TOTAL (µm)	TOTAL (µm)	% RECOVERY
0	48.24	0.00	48.24	80.39
24	51.91	0.01	51.92	86.53
48	46.53	0.12	46.65	77.75
72	58.50	0.96	59.46	99.09
96	53.47	2.52	55.99	93.32
168	29.24	13.23	42.47	70.78

4 DISCUSSION

4.1 *Gordonia* sp. NB4-1Y Growth Optimization

4.1.1 Ethanol Toxicity

Prior to investigating the degradation of specific fluorochemicals by NB4-1Y, the concentration of ethanol found to be toxic to the microorganisms needed to be determined, as both FTSA and FTAB were dissolved in a 50 % ethanol solution. Analysis indicated that no significant hindrance to growth occurred until 4. 5 % v/v ethanol. In fact, an ethanol concentration as high as 4.0 % increased the total amount of growth after 168-hrs ($OD_{600\ nm}$ of 0.720) (Figure 3-1). While it was safe to grow NB4-1Y in media containing up to 4.0 % v/v ethanol, a reduction in the initial growth rate was observed at higher concentrations. After 24-hrs, cultures grown in 0.5 % v/v ethanol exhibited an $OD_{600\ nm}$ of 0.387, while cultures grown at 4.0 % v/v ethanol had an $OD_{600\ nm}$ of 0.023 (Figure 3-1). The first sign of this decline can be seen in cultures grown with as little as 1.5 % v/v ethanol, and a decrease in initial growth rate is seen at 2.5 % v/v ethanol (Figure 3-1).

These results are indicative that *Gordonia* sp. NB4-1Y are capable of growing at ethanol concentrations of up to 4.0 % v/v, but as ethanol concentration increases, NB4-1Y may take longer to adjust to their environment before proliferation can occur. With these results, an ethanol concentration of 1.25 % v/v was utilized to ensure cultures experienced limited ethanol toxicity.

4.1.2 Optimum FTSA Concentration

Determining an optimum concentration of FTSA and FTAB was necessary to access the toxicity of the heavily fluorinated compounds towards NB4-1Y and to identify a suitable concentration for quantification of breakdown products. NB4-1Y did not appear to suffer any toxic effects when exposed to FTSA concentration as high as 100-µM, and total growth of the cultures seemed to profit from the increased presence of FTSA. Additionally, based on Figure 3-2, the

growth rate of NB4-1Y in the exponential phase (between 24-48-hrs) appeared to increase as the concentration of FTSA increased. It is also important to note the increase in total growth NB4-1Y exhibits upon increasing the concentration of FTSA from 20-µM to 40-µM (Figure 3-3).

Analysis indicated that *Gordonia* sp. NB4-1Y was capable of tolerating a relatively high concentration of FTSA, which bodes well for analytical analysis. It is also important to consider the large growth increase between 20 and 40-µM, as utilizing a concentration of 40-µM or greater will not only help benefit the NB4-1Y population, but will increase the quantity of metabolites produced over a shorter period of time. Past studies have utilized concentrations of FTSA ranging from 1.3-mg L^{-1} (~3.0-µM) in their analysis of aerobic/anaerobic degradation of FTSA in sediment (Zhang et al. 2016) to 40-µM of FTSA in monitoring degradation using pure culture (Van Hamme et al. 2013). However, it should be noted that the latter concentration may not be relevant to environmental conditions.

4.1.3 Optimum FTAB Concentration

NB4-1Y did not appear to exude any significant toxicological characteristics from increased FTAB exposure, however this cannot be definitively stated without comparison to a $MgSO_4$ control (not conducted). Again, much like what was seen with FTSA, NB4-1Y seemed to benefit from increased FTAB concentration in terms of overall growth of the cultures, with the one exception being a slight decrease in growth rate during the exponential phase and overall growth when increasing concentration from 80-µM to 100-µM (Figure 3-4). Furthermore, as the concentration of FTAB was increased from 20-µM to 40-µM an increase in growth rate and a 52.8 % increase in overall growth was seen, with $OD_{600\ nm}$ readings of 0.126 and 0.218, respectively.

Analysis demonstrated that *Gordonia* sp. NB4-1Y can tolerate a relatively high concentration of FTAB. Much like FTSA, it is important to consider the large growth increase between 20 and 40-µM, as utilizing a concentration of 40-µM or greater will not only help benefit the NB4-1Y population, but will increase the quantity of metabolites produced over a shorter period of time. However, concentrations 40-µM or greater may not be representative of environmental conditions.

Gordonia sp. NB4-1Y exhibited fairly similar growth curves when FTSA or FTAB were provided as the sole-sulfur source. It is not overly surprising due to the resiliency of microorganisms and to the fact that both compounds are extremely similar themselves (Appendix: Table 7-2). It is important to note however, that NB4-1Y was not able to utilize FTAB as well as it was able to exploit FTSA. While both sets of cultures exhibited similar growth early on (e.g. the 24-hr time point), NB4-1Y experienced decreased overall growth after 168-hrs at all concentrations. Overall growth for 100-µM FTSA cultures plateaued around 0.550 (Figure 3-3), while 100-µM FTAB cultures plateaued around 0.300 (Figure 3-4).

4.2 FTSA Liquid Cultures and C18 Eluates

In this study, *Gordonia* sp. NB4-1Y were grown in sulfur-limiting conditions with 60-µM of FTSA, FTAB or $MgSO_4$ as the sole-sulfur source. A total of 168 cultures and 168 C18 cartridges under seven different conditions were incubated over a 168-hr time period and were analyzed using an ultra-high pressure liquid chromatography chain coupled to an Orbitrap mass spectrometer. Analysis of FTSA/FTAB cultures and their respective C18 eluate identified and quantified a total of eleven metabolites: PFBA, PFPeA, PFHxA, PFHpA, 4:3-FTCA, 5:3-FTCA, 6:2-FTCA, 6:2-FTUA, 5:2-sFTOH, 5:2-FT-ketone, and 6:2-FTOH. However, PFHpA was not identified in FTAB

analysis and was not found to increase in concentration over FTSA degradation. It is believed that PFHpA is an impurity in the FTSA stock solution, as it was seen in sterile FTSA cultures. Analysis of sterile FTSA/FTAB cultures indicates that very little, if any, degradation of FTSA/FTAB occurs naturally (Figure 3-8; Figure 3-11). In combination with the analysis done on inoculated FTSA/FTAB cultures, sterile FTSA/FTAB cultures indicate that FTSA/FTAB degradation is occurring due to microbial activity. With all culture purity checks coming back positive for only *Gordonia* sp. NB4-1Y, it is evident that NB4-1Y is responsible for the degradation of FTSA, as previously determined by Van Hamme et al. (2013), and responsible for the degradation of FTAB.

4.2.1 Analysis of FTSA Liquid Cultures and C18 Eluates

With FTSA as their sole-sulfur source, *Gordonia* sp. NB4-1Y degraded 99.97 % of the environmental contaminant after 168-hrs (Figure 3-8). Significant degradation of FTSA did not appear to occur until after 72-hrs of incubation. This may be attributed to NB4-1Y adjusting to its new environment (lag phase) or there is enough sulfur already present in the media for NB4-1Y to survive for the first 72-hrs (therefore, has no need to use energy to extract the sulfur from FTSA), or a combination of both. However, with the appearance of the first metabolites after 24-hrs, it is clear that FTSA desulfonation is occurring.

The first analytes detected were 6:2-FTUA and 6:2-FTCA in the aqueous phase, after 24-hrs (Figure 3-8), 5:3-FTCA in the aqueous phase, after 48-hrs (Figure 3-10), and 5:2-FT-ketone the volatile phase, after 24-hrs (Figure 3-9). The early production of 6:2-FTCA and 6:2-FTUA were expected based upon the previously proposed degradation pathways (Wang et al. 2011; Van Hamme et al. 2013) (Figure 1-2*;* Figure 1-3). Production of 6:2-FTCA and 6:2-FTUA increases relatively slow, with the most significant increase occurring between the 96 and 168-hr time points (Figure 3-8). This is supportive of NB4-1Y utilizing contaminating sulfate already present in the

media combined with minor degradation of FTSA before sulfur must be extracted and significant degradation occurs by NB4-1Y.

The majority of metabolites produced after 168-hrs were identified in the volatile phase (Table 3-3), with those analytes being 5:2-FT-ketone (43.68 mol %), 5:2-sFTOH (8.97 mol %) (Figure 3-9), and 6:2-FTOH (4.14 mol %) (Figure 3-8). It is important to note, however, that although 5:2-FT-ketone was the most abundant metabolite, further degradation of this analyte is strongly dependent upon its solubility into the aqueous phase. If 5:2-FT-ketone has low solubility, most of the analyte would exist in the gaseous phase above the liquid culture, as opposed to being dissolved in the liquid phase and within 'reach' of NB4-1Y. Therefore, the analyte would not be in close enough proximity for NB4-1Y cells to further degrade the metabolite. Additionally, once the cultures are purged, all volatile compounds become unavailable for further biotransformation.

Based on Liu and Avendaño's (2013) proposed degradation pathway, the identification of 5:3-FTCA and 5:2-FT-ketone indicates the use of two potential degradation pathways by NB4-1Y. These two analytes have been identified as two separate degradation products arising from 6:2-FTUA. Additionally, with 5:2-FT-ketone comprising the vast majority of metabolites produced (Figure 3-9), there is strong evidence that NB4-1Y preferentially utilizes one pathway over the other. In this case, conversion of 6:2-FTUA into 5:2-FT-ketone is preferred opposed to conversion of 6:2-FTUA into 5:3-FTCA (**Error! Reference source not found.**). Greater than 50 mol % of the metabolites produced after 168-hrs belong to the 5:2-FT-ketone pathway, termed the 'major' degradation pathway in this report. Less than 1.0 mol % of the metabolites produced belong to the 5:3-FTCA pathway, here termed the 'minor' degradation pathway (**Error! Reference source not found.**).

4.2.2 Postulated FTSA Degradation Pathway by NB4-1Y

Regarding past hypothesized degradation pathways for FTSA (Wang et al. 2011; Van Hamme et al. 2013), 6:2-FTOH (Liu and Avendaño 2013), and with the eleven metabolites detected in NB4-1Y cultures, two metabolic pathways can be proposed (***Error! Reference source not found.***).

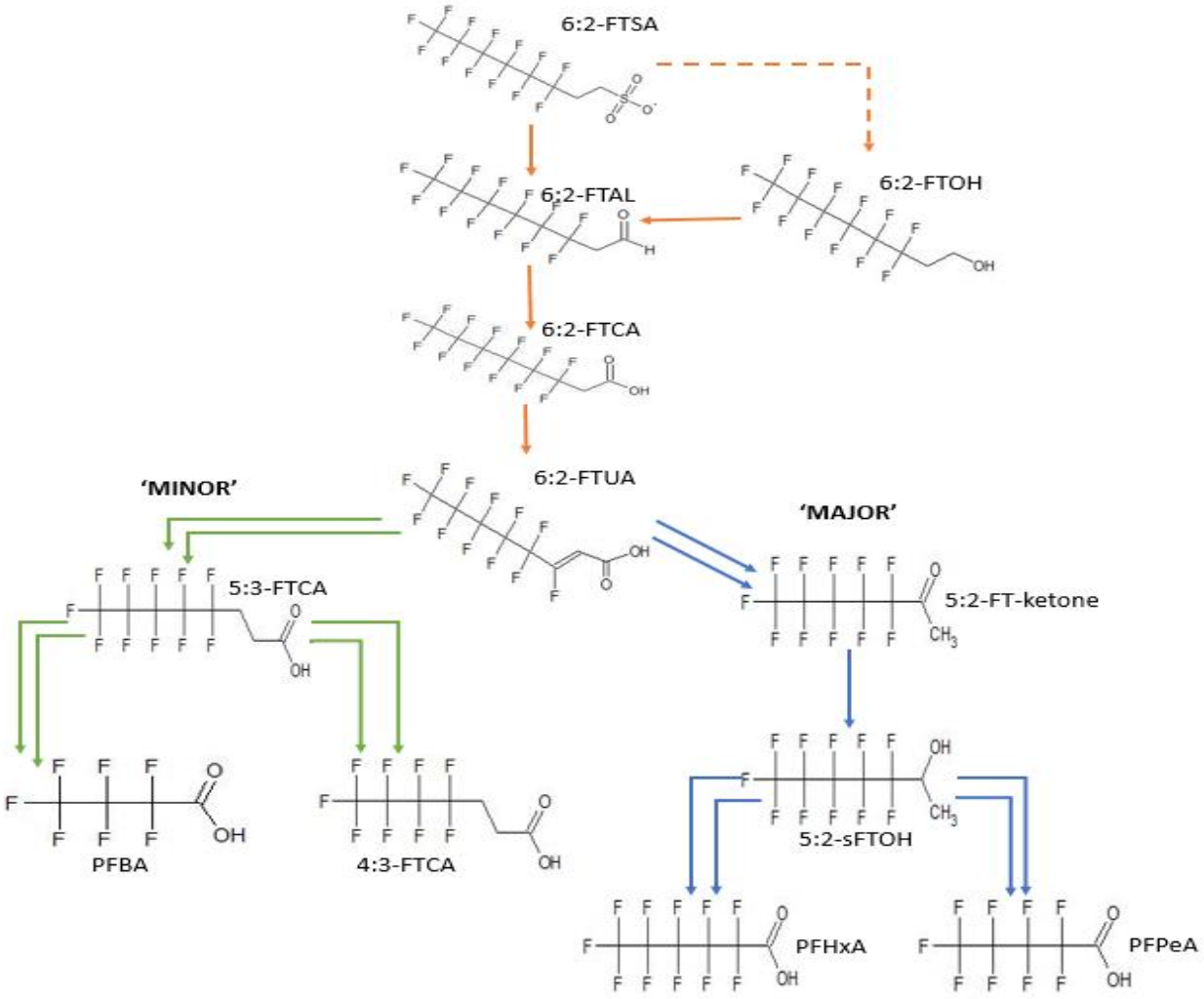


Figure 4-1: Proposed metabolic pathway utilized by *Gordonia* sp. NB4-1Y for the desulfonation of FTSA.
Orange arrows are processes involved in the initial desulfonation of FTSA. The dotted orange line indicates the identification of an alternative pathway not previously suggested by Zhang et al. (2016). Blue arrows indicate the 'major' pathway utilized by NB4-1Y and the green arrows

indicate the 'minor' pathway. Steps with two arrows indicate that two or more steps occur between analytes.

It is important to note that previous experiments had not implicated 6:2-FTOH as part of the degradation pathway utilized by *Gordonia* sp. NB4-1Y (Van Hamme et al. 2013) or mixed bacterial cultures (Wang et al. 2011). However, a more recent study has detected 6:2-FTOH in the degradation of 6:2-FTSA in aerobic sediment mixture (Zhang et al. 2016). With the generation 0.173 mol % of 6:2-FTOH at 96-hrs and an increase to 4.144 mol % at 168-hrs (Figure 3-8), it is more apparent that NB4-1Y may not generate 6:2-fluortelomer aldehyde (6:2-FTAL) by skipping the conversion of FTSA to 6:2-FTOH (***Error! Reference source not found.***). Alkane metabolism by bacteria typically converts alkanes to alcohols, then aldehydes, followed by carboxylic acids (Van Hamme et al. 2003). NB4-1Y may follow a similar process during FTSA degradation by converting FTSA to 6:2-FTOH (alcohol), then 6:2-FTAL (aldehyde), followed by 6:2-FTCA (carboxylic acid). Furthermore, the biotransformation of FTSA to 6:2-FTOH to 6:2-FTAL may be a rapid process such that detectable amounts of 6:2-FTOH may not accumulate until a high level of FTSA degradation has occurred.

4.3 FTAB Liquid Cultures and C18 Eluates

4.3.1 Analysis of FTAB Liquid Cultures and C18 Eluates

Analysis of inoculated FTAB cultures identified the same ten metabolites produced in NB4-1Y cultures during FTSA degradation, but in much lower quantities. For example, in comparison to the 43.63 mol % of 5:2-FT-ketone produced in FTSA degradation (Figure 3-9), FTAB degradation only produced 18.35 mol % over 168-hrs (Figure 3-12). This may be a result

of NB4-1Y taking longer to utilize FTAB or NB4-1Y is incapable of utilizing FTAB to the same extent as it utilizes FTSA. However, the latter may not be the case, as NB4-1Y degraded over 50 % of the FTAB provided (Figure 3-11). An alternative explanation is the degradation pathway that NB4-1Y must utilize for FTAB metabolism requires additional steps prior to releasing the sulfonate from the molecule, at minimum one if the organism is able to cleave the sulfur-nitrogen bond directly.

Significant degradation of FTAB did not occur until after 96-hrs of incubation, where approximately 80 mol % of FTAB was removed to a concentration of 29.58 mol % at 168-hrs (Figure 3-11). It is interesting to note that upon the increased degradation of FTAB, there appeared to be no production of FTSA. Previous research has postulated that initial degradation of FTAB primarily results in the production of FTSA (Zhang et al. 2016), however, these observations were made in groundwater samples from regions where AFFFs were used (Schultz et al. 2004). There are two possibilities that may account for this result. First, there was a small amount of FTSA (< 1.0 mol %) already present in in the cultures which was completely degraded within 72-hrs. Given this, the enzymes responsible for FTSA desulfonation may already be present before FTAB degradation started. Upon the degradation of FTAB, rapid utilization of FTSA could occur, effectively masking the biotransformation of FTAB into FTSA. Otherwise, NB4-1Y may convert FTAB into an alternative intermediate, which can then be transformed into FTSA or it may bypass FTSA production completely (**Error! Reference source not found.**). Understanding the initial degradation process will require the production of mutants, isolation and *in vitro* analysis of the enzymes responsible, or the use of isotopically-labelled compounds to better track intermediates.

It is clear that FTAB biotransformation does follow the same lower degradation pathway as FTSA. This is evident in the quantity (mol %) of major and minor pathway metabolites

produced, as they far surpass the amount of FTSA originally present in the culture. For example, the initial concentration of FTSA in FTAB cultures peaked below 1.0 mol % (Figure 3-11), while FTSA metabolites such as 5:2-FT-ketone peaked around 18.0 mol % (Figure 3-12).

4.3.2 Postulated FTAB Degradation Pathway by NB4-1Y

With the identification of the same ten metabolites produced during FTSA degradation, a modified version of the previously postulated metabolic pathway for FTSA can be proposed for FTAB degradation. The modifications accommodate the unknown initial degradation processes of FTAB (**Error! Reference source not found.**). As mentioned previously (Section 1.2), it has also been reported that 6:2 fluorotelomer sulfonamide [F(CF2)6CH2CH2SO2NH2] (6:2-FTSAOA) is initially produced in FTAB degradation by blue mussels (Moe et al. 2012). They also report that four other metabolites were produced in FTAB degradation (Moe et al. 2012). Furthermore, their analysis of soil samples known to contain AFFF products identified the same metabolites (Moe et al. 2012). These metabolites may be part of the degradation pathway NB4-1Y utilizes when

degrading FTAB.

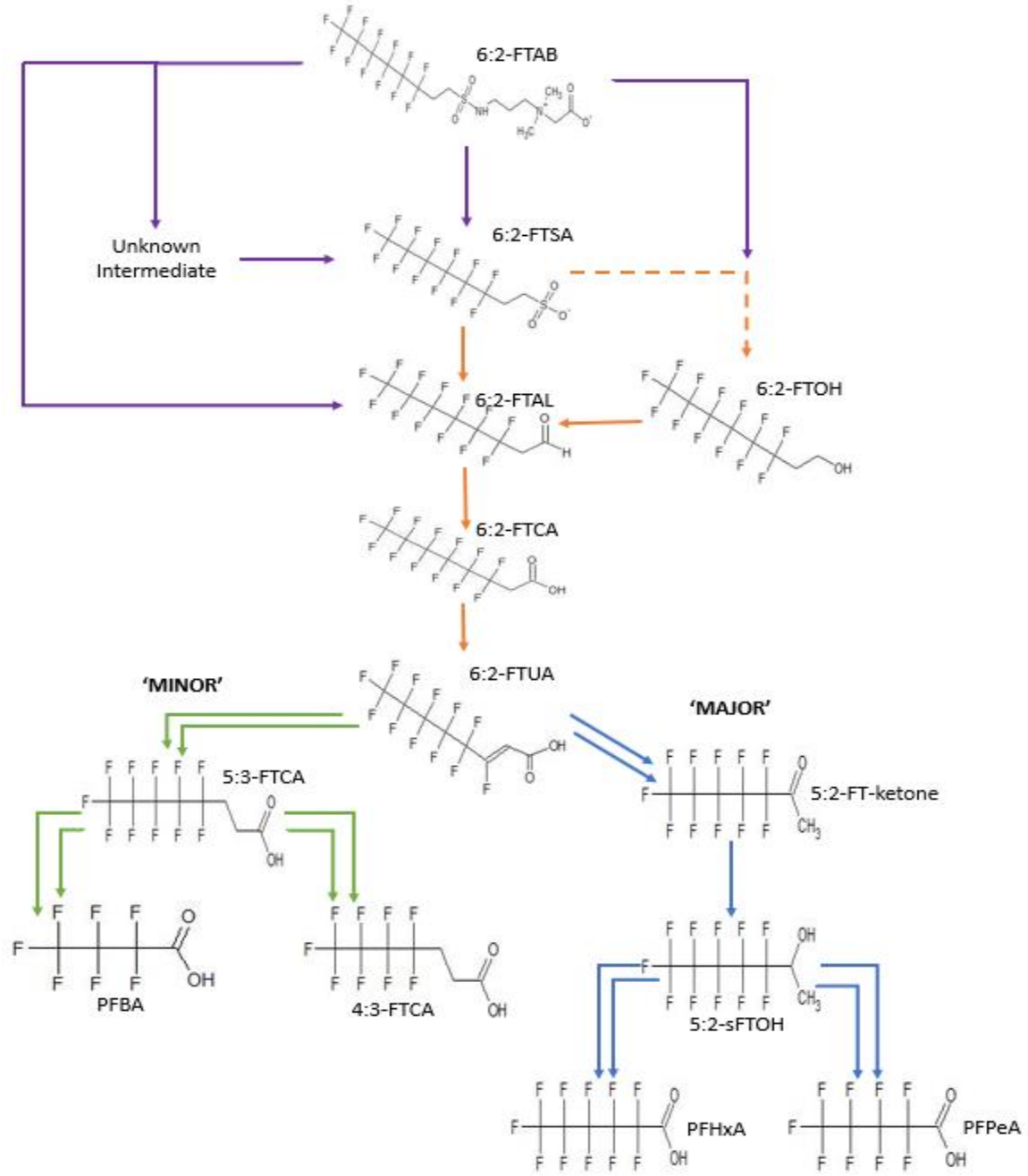


Figure 4-2: Proposed metabolic pathway utilized by *Gordonia* sp. NB4-1Y for the degradation of FTAB.
Orange arrows are processes involved in the initial desulfonation of FTSA. The dotted orange line indicates the identification of an alternative pathway previously suggested by Zhang et al (2016). Blue arrows indicate the 'major' pathway utilized by NB4-1Y and the green arrows indicate the 'minor' pathway. Steps with two arrows indicate that two or more steps occur between analytes. Purple arrows are proposed pathways that *Gordonia* sp. NB4-1Y may use to degrade FTAB.

While our experiment demonstrated that both FTSA and FTAB are utilized by NB4-1Y, it is not clear which steps are biologically or chemically-mediated. Referring to Figure 1-2, Van Hamme et al. (2013) report that the transformation of 6:2-FTS to 6:2-FTAL is biologically-mediated through desulfonation genes ISGA 1218 & 1222, while the conversion of 6:2-FTCA to 6:2-FTUCA may be chemically-mediated through the spontaneous loss of hydrogen fluoride. In other words, each transformation step throughout degradation may be a result of an enzymatic reaction or natural chemical degradation. For example, 6:2-FTCA may be relatively unstable, resulting in the spontaneous loss of HF, producing 6:2-FTUCA.

5 CONCLUSION AND FUTURE WORK

Gordonia sp. NB4-1Y is capable of complete desulfonation of FTSA, degradation of FTAB, and use of both compounds as the primary sulfur source under sulfur-limiting conditions. A total of eleven metabolites for FTSA and ten metabolites for FTAB have been identified and quantified, with additional analyses for new intermediates currently in progress. NB4-1Y appears to utilize two degradation pathways –major (5:2-FT-ketone) & minor (5:3-FTCA)– diverging from 6:2-FTUA. Conversion of 6:2-FTUA into 5:2-FT-ketone was the metabolic pathway primarily used by NB4-1Y with more than 50 % of metabolites being 5:2-FT-ketone, 5:2-sFTOH, PFPeA, and PFHxA. Although FTAB degradation did not reach the same extent as FTSA after 168-hrs, results indicate that the lower degradation pathway is similar to what is seen with FTSA, and that both major and minor metabolic routes are present.

The metabolite 6:2-FTOH was identified as a biotransformation product of FTSA degradation. Previous studies had not detected 6:2-FTOH and hypothesized that FTSA transformation bypassed 6:2-FTOH production, producing 6:2-FTAL directly (Wang et al. 2011; Van Hamme et al. 2013). However, recently Zhang et al. (2016) detected 6:2-FTOH (<2.5 mol %) in an aerobic sediment mixture over 90 days, suggesting that 6:2-FTOH was an initial degradation product of 6:2-FTSA (Zhang et al. 2016). The initial degradation of FTAB may not involve the production of FTSA. NB4-1Y may bypass production of FTSA by conversion to an alternative intermediate, otherwise FTSA is rapidly utilized keeping levels below detectable limits. Lastly, it would be interesting to determine how the sulfonamide alkyl betaine portion of FTAB is metabolized. Additional analysis scanning for this portion of FTAB may provide some insight into this process. Isolating and utilizing this segment of FTAB as a supply of sulfur, nitrogen, and carbon while repeating the experiment is an intriguing aspect.

Attempts to extract mRNA from *Gordonia* sp. NB4-1Y cultures under the same sulfur-limiting conditions is underway. Once sufficient mRNA is recovered, the transcriptome can be sequenced in order to better understand gene expression changes when NB4-1Y metabolizes FTSA and FTAB. These experiments will facilitate future research isolating enzymes involved in FTAB and FTSA metabolism, verifying their function *in vitro*, and designing molecular tools for monitoring their metabolism by diverse microbial communities in the environment. Additionally, further analysis of stored samples is being conducted in hopes of identifying new intermediates and degradation products, as it is not completely clear which degradation steps are biologically-mediated, and which are chemically-mediated.

6 LITERATURE CITED

Arenskotter M, Broker D, Steinbuchel A. 2004. Biology of the metabolically diverse genus *Gordonia*. Appl. Environ. Microbiol. 70:3195–3204.

Backe WJ, Day TC, Field JA. 2013. Zwitterionic, cationic, and anionic fluorinated chemicals in aqueous film forming foam formulations and groundwater from US military bases by nonaqueous large-volume injection HPLC-MS/MS. Environ. Sci. Technol. 47:5226–5234.

Boiteux V, Dauchy X, Bach C, Colin A, Hemard J, Sagres V, Rosin C, Munoz J-F. 2017. Concentrations and patterns of perfluoroalkyl and polyfluoroalkyl substances in a river and three drinking water treatment plants near and far from a major production source. Sci. Total Environ. 583:393–400.

Butt CM, Muir DC, Mabury SA. 2014. Biotransformation pathways of fluorotelomer-based polyfluoroalkyl substances: A review. Environ. Toxicol. Chem. 33:243–267.

Clarke BO, Smith SR. 2011. Review of "emerging"organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. Environ. Int. 37:226–247.

Dinglasan MJA, Ye Y, Edwards EA, Mabury SA. 2004. Fluorotelomer alcohol biodegradation yields poly- and perfluorinated acids. Environ. Sci. Technol. 38:2857–2864.

Drzyzga O. 2012. The strengths and weaknesses of *Gordonia*: a review of an emerging genus with increasing biotechnological potential. Crit. Rev. Microbiol. 38:300–316.

Higgins C, Field J, Criddle C, Luthy R. 2005. Quantitative determination of perfluorochemicals in sediments and domestic sludge. Environ. Sci. Technol. 39:3946–3956.

Houtz EF, Sutton R, Park J-S, Sedlak M. 2016. Poly-and perfluoroalkyl substances in wastewater: Significance of unknown precursors, manufacturing shifts, and likely AFFF impacts. Water Res. 95:142–149.

Krafft MP, Riess JG. 2015. Per-and polyfluorinated substances (PFASs): Environmental challenges. Curr. Opin. Colloid Interface Sci. 20:192–212.

Liu J, Avendaño SM. 2013. Microbial degradation of polyfluoroalkyl chemicals in the environment: a review. Environ. Int. 61:98–114.

Liu J, Wang N, Szostek B, Buck RC, Panciroli PK, Folsom PW, Sulecki LM, Bellin CA. 2010. 6-2 Fluorotelomer alcohol aerobic biodegradation in soil and mixed bacterial culture. Chemosphere 78:437–444.

Loganathan BG, Sajwan KS, Sinclair E, Senthil Kumar K, Kannan K. 2007. Perfluoroalkyl sulfonates and perfluorocarboxylates in two wastewater treatment facilities in Kentucky and Georgia. Water Res. 41:4611–4620.

Moe MK, Huber S, Svenson J, Hagenaars A, Pabon M, Trümper M, Berger U, Knapen D, Herzke D. 2012. The structure of the fire fighting foam surfactant Forafac® 1157 and its biological and photolytic transformation products. Chemosphere 89:869–875.

Munoz G, Duy SV, Labadie P, Botta F, Budzinski H, Lestremau F, Liu J, Sauvé S. 2016. Analysis of zwitterionic, cationic, and anionic poly-and perfluoroalkyl surfactants in sediments by liquid chromatography polarity-switching electrospray ionization coupled to high resolution mass spectrometry. Talanta 152:447–456.

Murphy CD. 2010. Biodegradation and biotransformation of organofluorine compounds. Biotechnol. Lett. 32:351–359.

Newsted J, Beach S, Gallagher S, Giesy J. 2008. Acute and chronic effects of perfluorobutane sulfonate (PFBS) on the mallard and northern bobwhite quail. Arch. Environ. Contam. Toxicol. 54:535–545.

Park S, Lee LS, Medina VF, Zull A, Waisner S. 2016. Heat-activated persulfate oxidation of PFOA, 6: 2 fluorotelomer sulfonate, and PFOS under conditions suitable for in-situ groundwater remediation. Chemosphere 145:376–383.

Phillips M, Dinglasan-Panlilio M, Mabury S, Solomon K, Sibley P. 2007. Fluorotelomer acids are more toxic than perfluorinated acids. Environ. Sci. Technol. 41:7159–7163.

Rhoads KR, Janssen EM-L, Luthy RG, Criddle CS. 2008. Aerobic biotransformation and fate of N-ethyl perfluorooctane sulfonamidoethanol (N-EtFOSE) in activated sludge. Environ. Sci. Technol. 42:2873–2878.

Russell MH, Berti WR, Szostek B, Buck RC. 2008. Investigation of the biodegradation potential of a fluoroacrylate polymer product in aerobic soils. Environ. Sci. Technol. 42:800–807.

Schultz MM, Barofsky DF, Field JA. 2004. Quantitative determination of fluorotelomer sulfonates in groundwater by LC MS/MS. Environ. Sci. Technol. 38:1828–1835.

Sinclair E, Kannan K. 2006. Mass loading and fate of perfluoroalkyl surfactants in wastewater treatment plants. Environ. Sci. Technol. 40:1408–1414.

Van Hamme JD, Bottos EM, Bilbey NJ, Brewer SE. 2013. Genomic and proteomic characterization of *Gordonia* sp. NB4-1Y in relation to 6: 2 fluorotelomer sulfonate biodegradation. Microbiology 159:1618–1628.

Van Hamme JD, Fedorak PM, Foght JM, Gray MR, Dettman HD. 2004. Use of a novel fluorinated organosulfur compound to isolate bacteria capable of carbon-sulfur bond cleavage. Appl. Environ. Microbiol. 70:1487–1493.

Van Hamme JD, Singh A, Ward OP. 2003. Recent advances in petroleum microbiology. Microbiol. Mol. Biol. Rev. 67:503–549.

Wang N, Buck RC, Szostek B, Sulecki LM, Wolstenholme BW. 2012. 5: 3 Polyfluorinated acid aerobic biotransformation in activated sludge via novel “one-carbon removal pathways.” Chemosphere 87:527–534.

Wang N, Liu J, Buck RC, Korzeniowski SH, Wolstenholme BW, Folsom PW, Sulecki LM. 2011. 6:2 Fluorotelomer sulfonate aerobic biotransformation in activated sludge of waste water treatment plants. Chemosphere 82:853–858.

Zhang S, Lu X, Wang N, Buck RC. 2016. Biotransformation potential of 6: 2 fluorotelomer sulfonate (6:2 FTSA) in aerobic and anaerobic sediment. Chemosphere 154:224–230.

7 APPENDIX

7.1 LC-MS Method Parameters Summary

Table 7-1: Summary of LC-MS method parameters

Instrument	Thermo Q-Exactive Orbitrap mass spectrometer Dionex Ultimate 3000 UHPLC chain		
Ionization	Electrospray ionization source operated in fast polarity switching mode		
Acquisition mode	Full Scan MS mode		
Analytical column	Thermo Hypersil Gold aQ column (100 × 2.1 mm; 1.9 µm)		
Delay column	Thermo Hypercarb column (20 × 2.1 mm; 7 µm)		
Column Temperature	40°C		
Mobile Phases	A: 0.15% acetic acid in HPLC-water B: 0.15% acetic acid in acetonitrile Flow rate (mL/min) 0.55		
Gradient Profile	Time (min)	% B	
	0.0	10	
	6.5	72.5	
	7.0	100	
	9.5	100	
	9.6	10	
	12	10	
Injection Volume	15 µL		
Source/gas	Sheath gas flow rate 45 Aux gas flow rate 10 Sweep gas flow rate 10 Spray voltage (|kV|) 3 Capillary temperature (°C) 320 S-lens RF level 55		
Orbitrap parameters	Resolution 70,000 AGC target 5e6 Maximum Inject Time (ms) 50 Scan range (m/z) 150–700		
Quantitative analytes and correspondence with ISs	Analyte	m/z	IS
	PFBA	212.97947	MPFBA
	PFPeA	262.97669	MPFPeA
	PFHxA	312.97335	MPFHxA
	PFHpA	362.97013	MPFOA

6:2 FTAB	571.09362	M62-FTS
6:2 FTSA	426.96866	M62-FTS
3:3 FTCA	241.00995	M62-FTCA
4:3 FTCA	291.00676	M62-FTCA
5:3 FTCA	341.00356	M62-FTCA
6:2 FTCA	376.98472	M62-FTCA
6:2 FTUA	356.97849	M62-FTUA
6:2 FTOH	423.02658	MPFOA
5:2 sFTOH	373.02978	MPFOA
5:2 Ketone	310.99299	MPFOA
MPFBA	216.99177	-
MPFPeA	265.98674	-
MPFHxA	314.98039	-
MPFOA	416.97975	-
M62-FTCA	378.99142	-
M62-FTS	428.97537	-
M62-FTUA	358.98520	-

7.2 Control Charts – Absolute Area

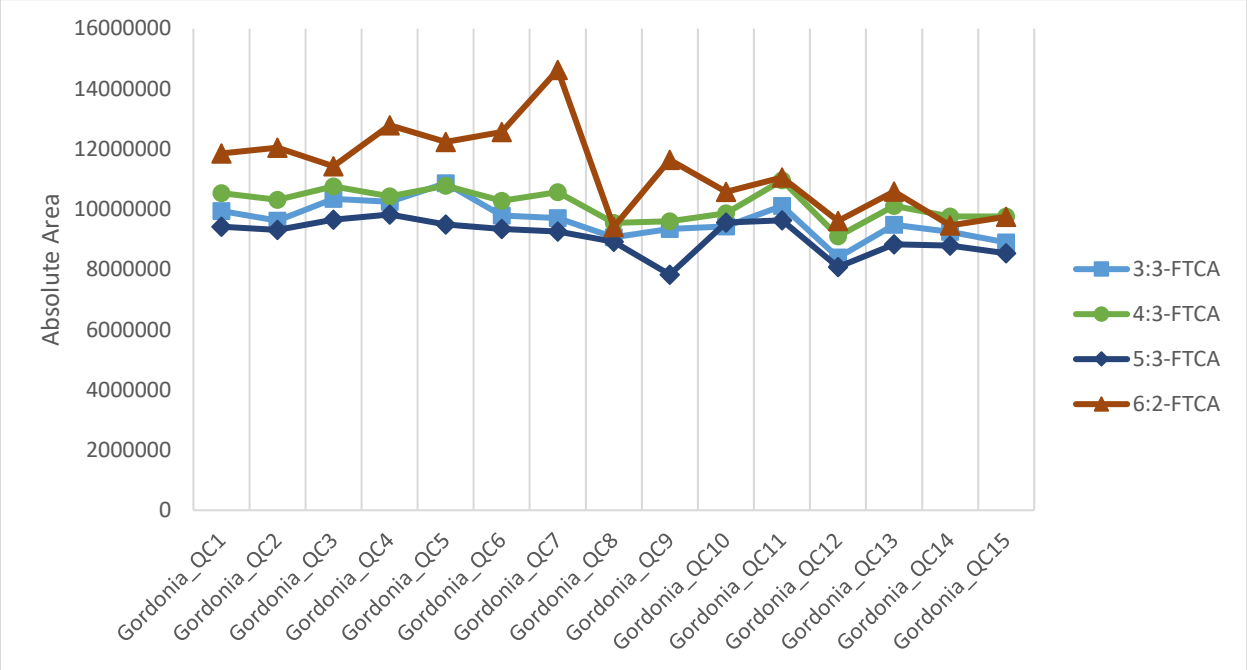


Figure 7-1: Illustration of quality control approach along the analytical sequence; control chart used to monitor the signal received upon the detection of 5-ng mL^{-1} 3:3-FTCA, 4:3-FTCA, 5:3-FTCA, and 6:2-FTCA.

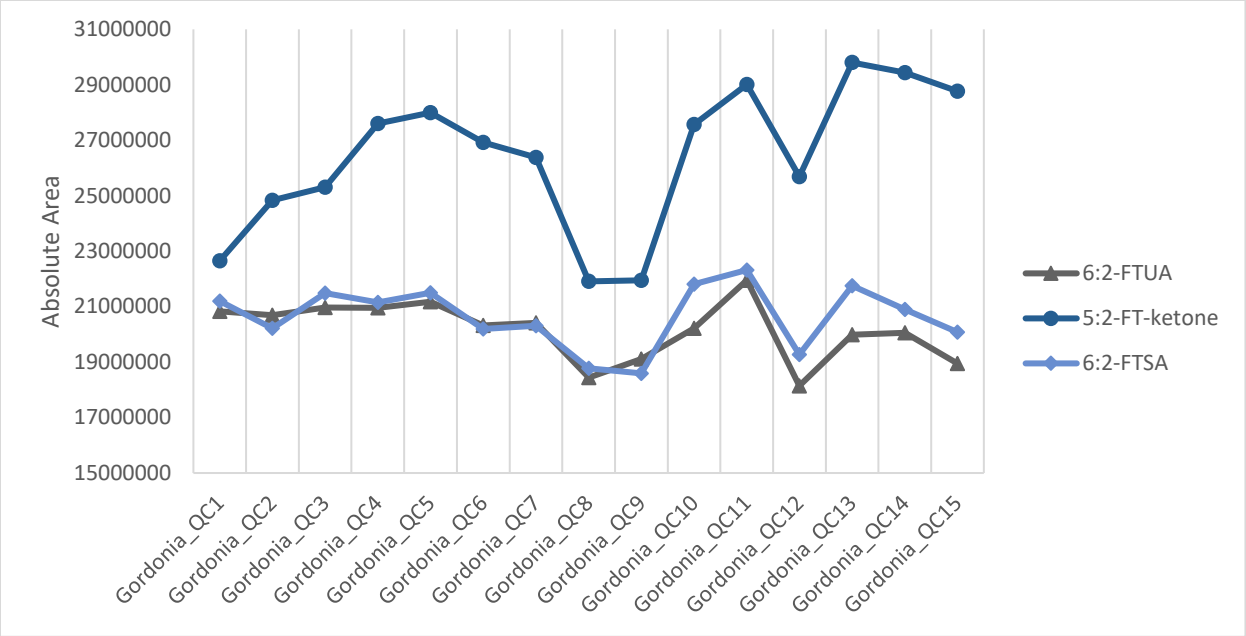


Figure 7-2: Illustration of quality control approach along the analytical sequence; control chart used to monitor the signal received upon the detection of 5-ng mL^{-1} 6:2-FTUA, 6:2-FTSA, and 87.5-ng mL^{-1} 5:2-FT-ketone.

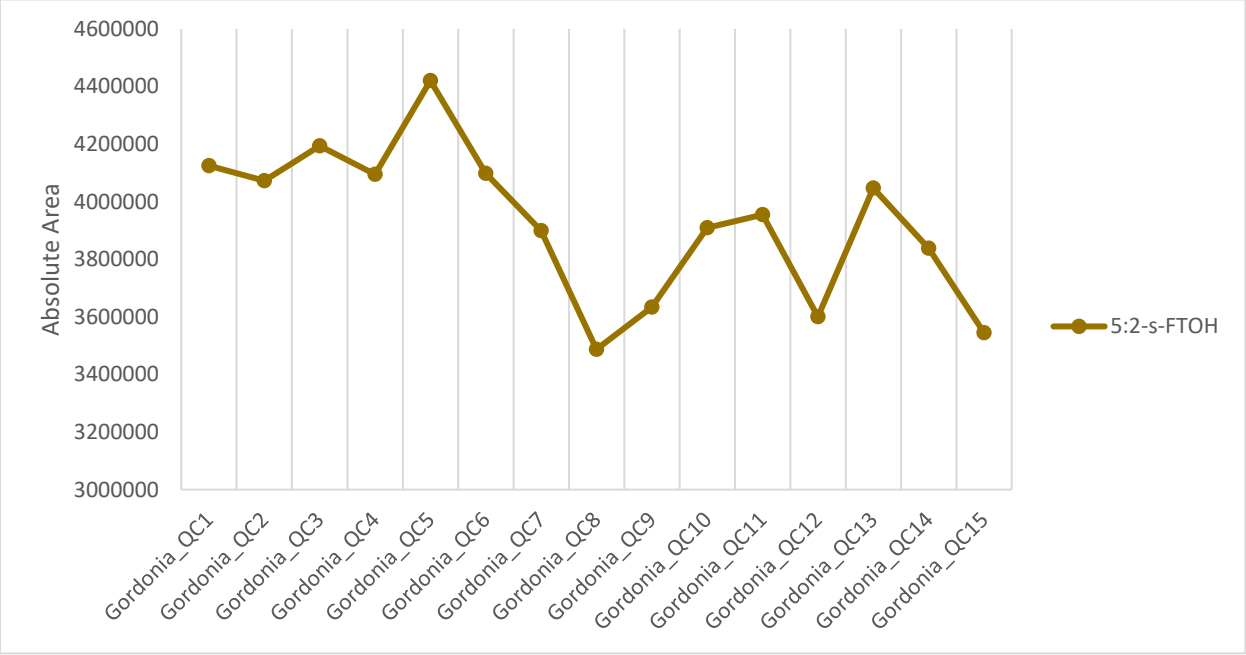


Figure 7-3: Illustration of quality control approach along the analytical sequence; control chart used to monitor the signal received upon the detection of 87.5-ng mL^{-1} 5:2-sFTOH.

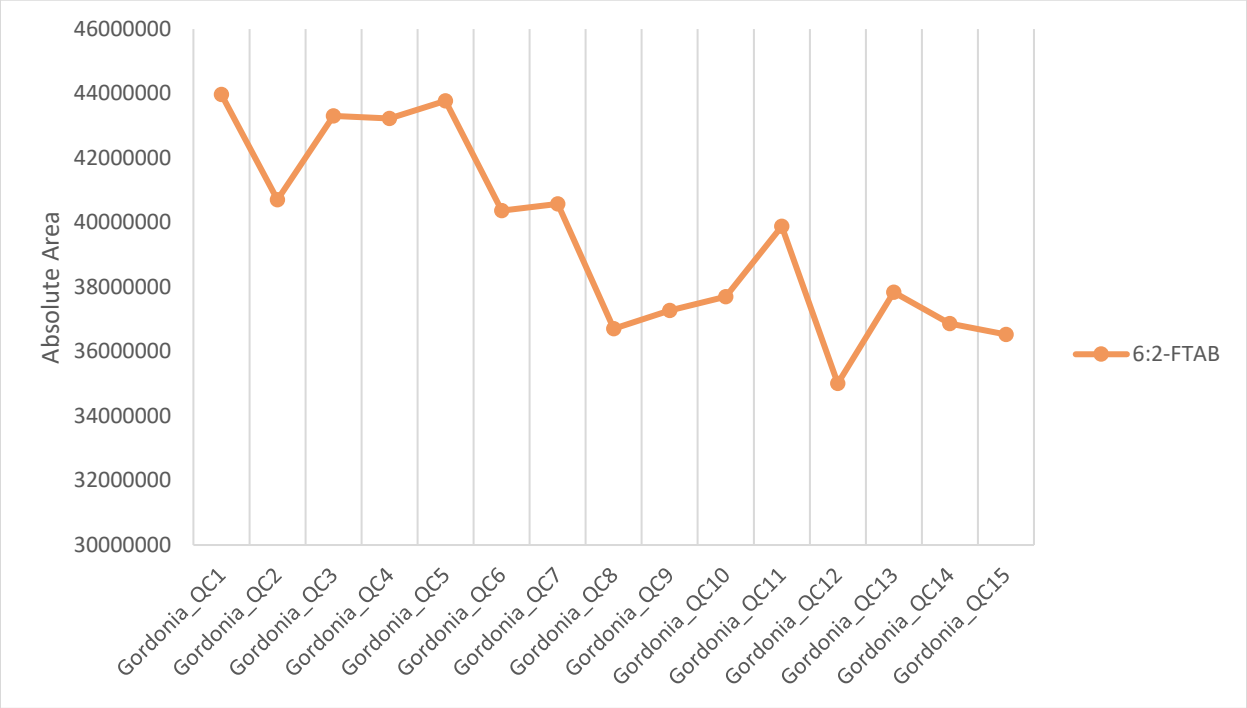


Figure 7-4: Illustration of quality control approach along the analytical sequence; control chart used to monitor the signal received upon the detection of 5-ng mL^{-1} FTAB.

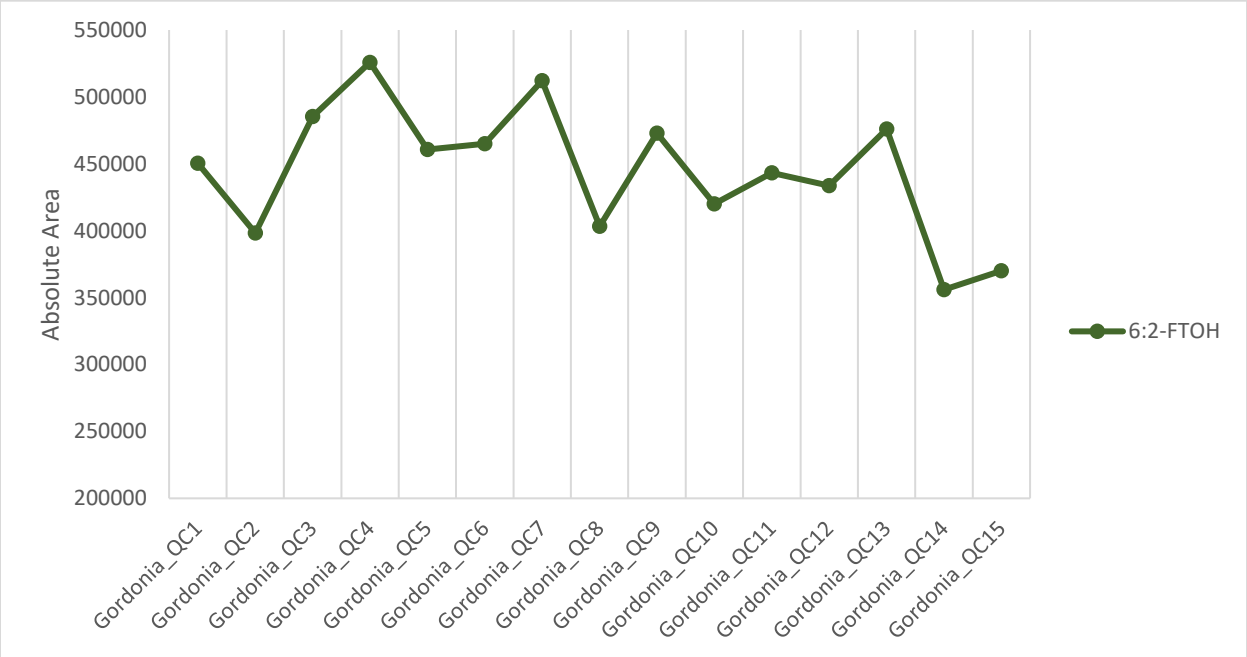


Figure 7-5: Illustration of quality control approach along the analytical sequence; control chart used to monitor the signal received upon the detection of 87.5-ng mL^{-1} 6:2-FTOH.

7.3 Control Charts – Analyte/IS Ratio

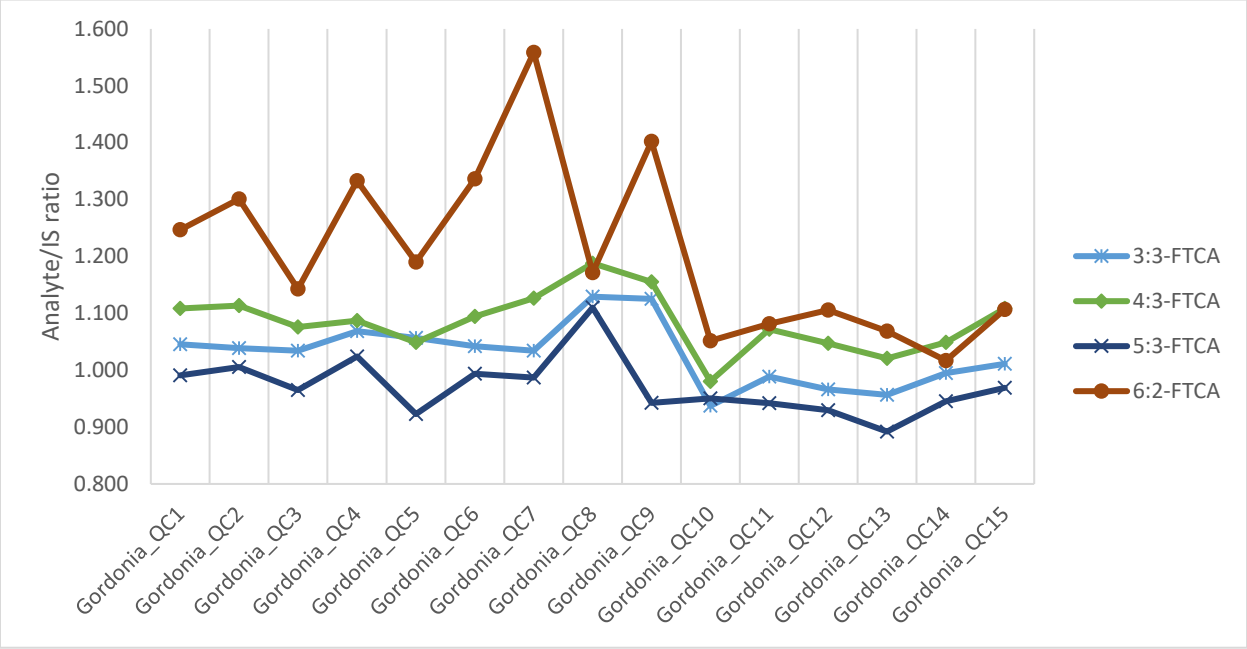


Figure 7-6: Illustration of quality control approach along the analytical sequence; control chart used to monitor the signal received upon the detection of 5-ng mL^{-1} 3:3-FTCA, 4:3-FTCA, 5:3-FTCA, and 6:2-FTCA.

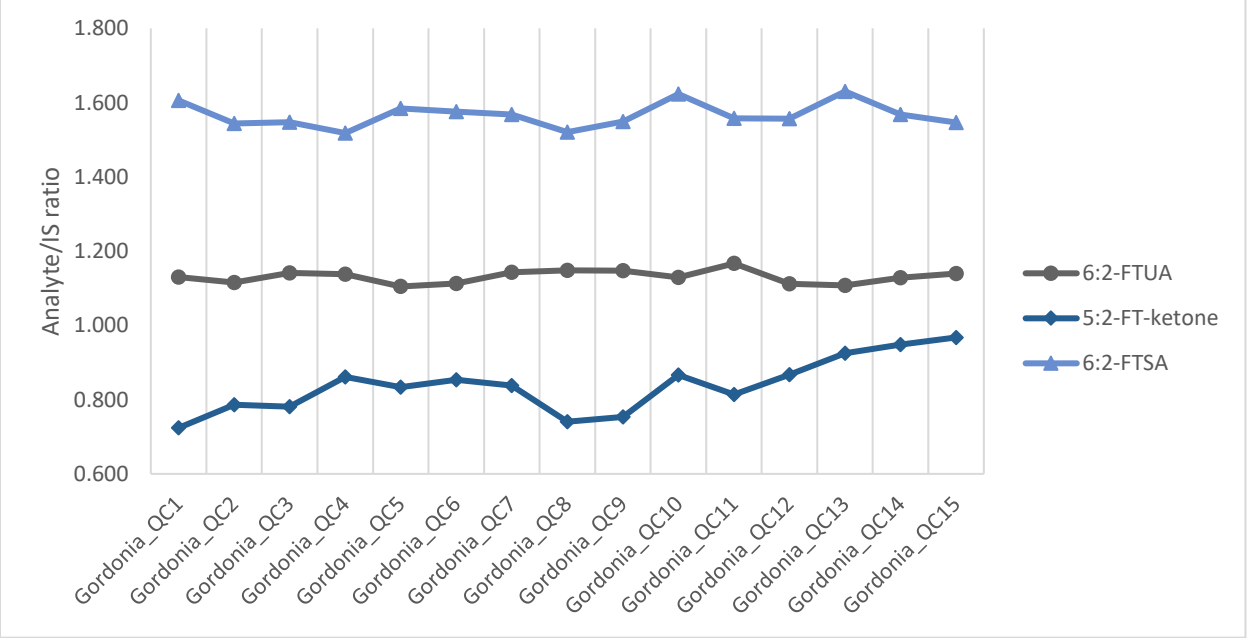


Figure 7-7: Illustration of quality control approach along the analytical sequence; control chart used to monitor the signal received upon the detection of 5-ng mL^{-1} 6:2-FTUA, 6:2-FTSA, and 87.5 ng mL^{-1} 5:2-FT-ketone.

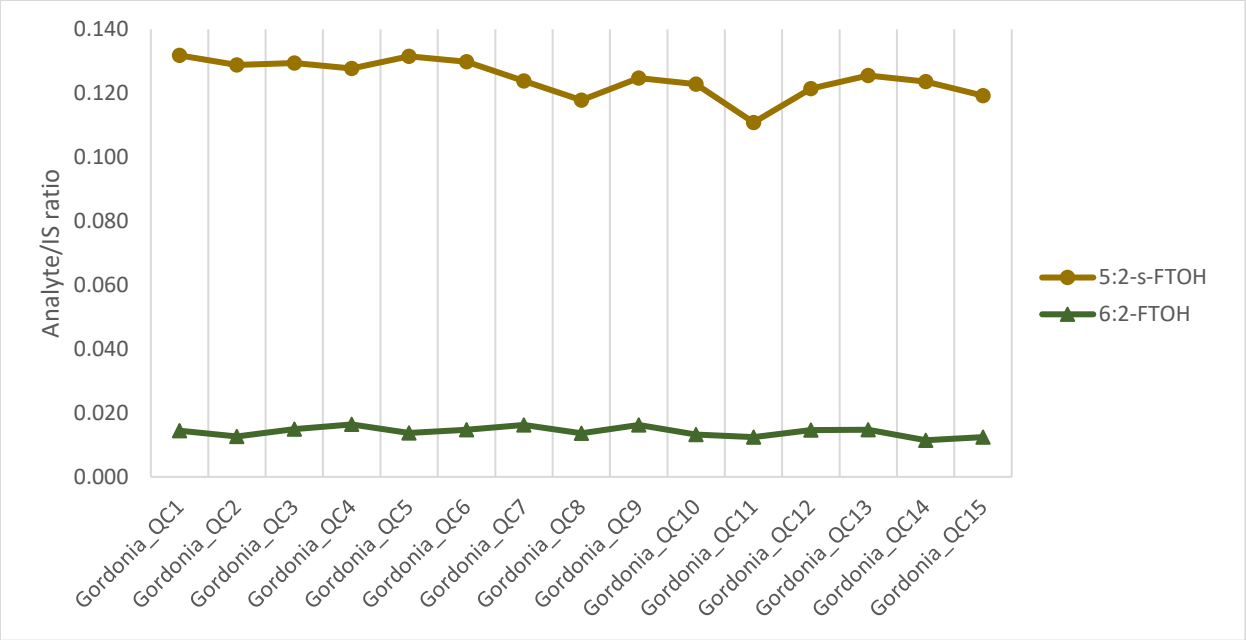


Figure 7-8: Illustration of quality control approach along the analytical sequence; control chart used to monitor the signal received upon the detection of 87.5-ng mL^{-1} 5:2-sFTOH and 6:2-FTOH.

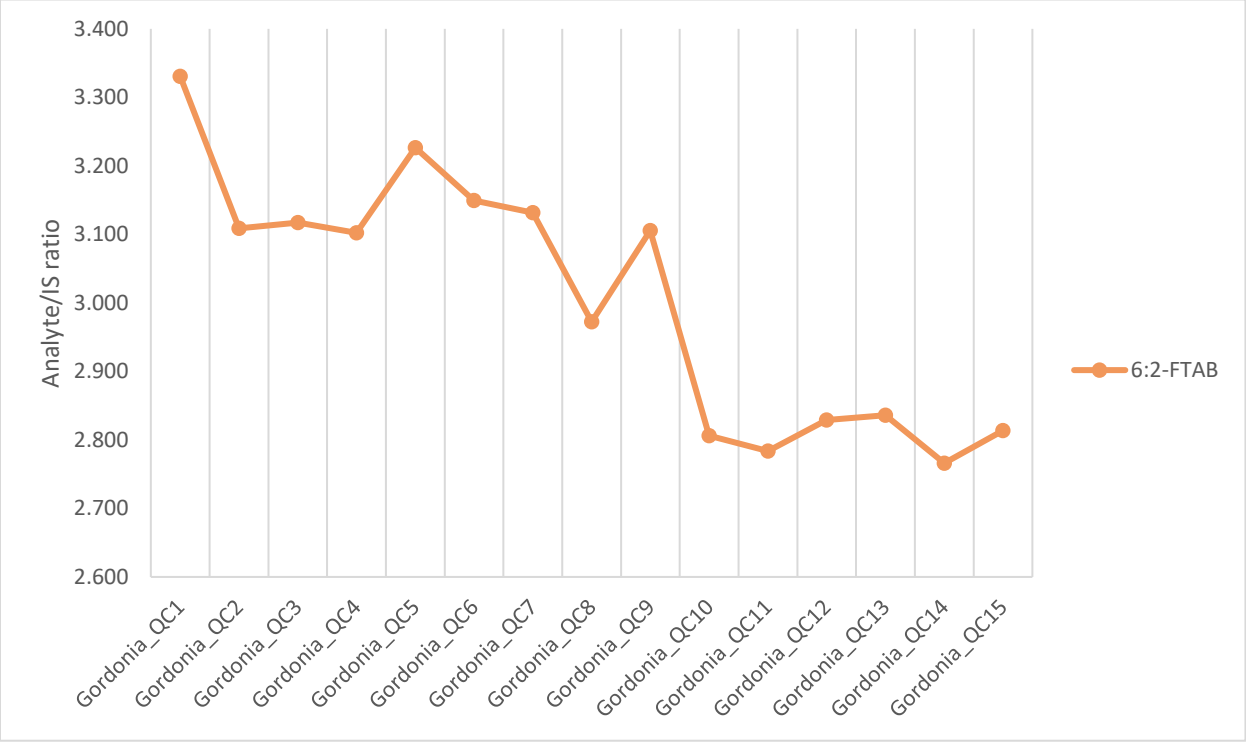
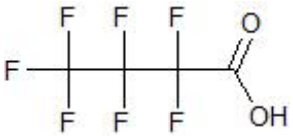
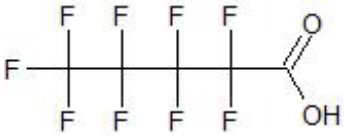
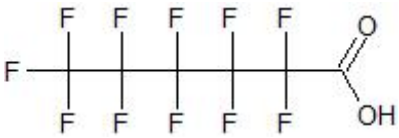
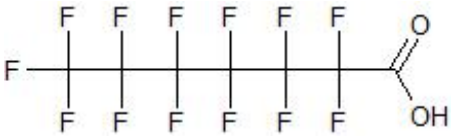
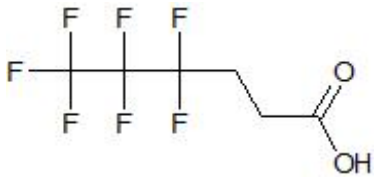
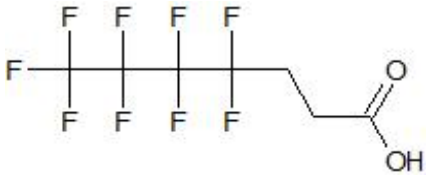
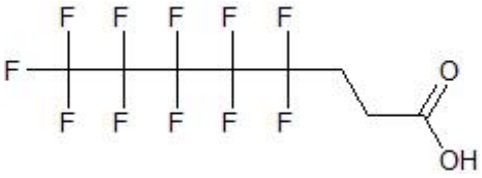
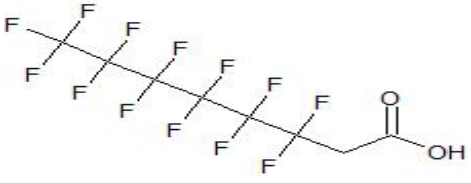
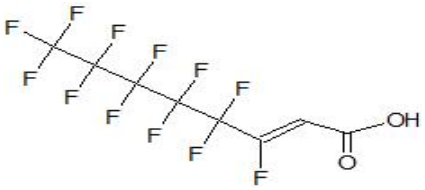
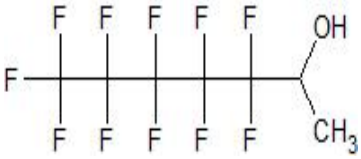
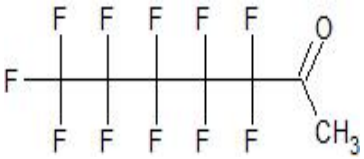
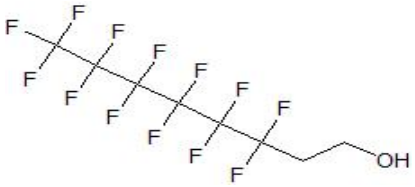
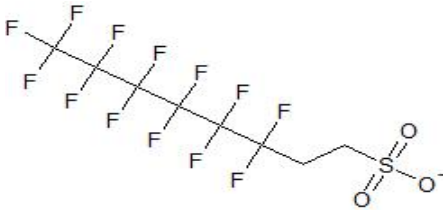


Figure 7-9: Illustration of quality control approach along the analytical sequence; control chart used to monitor the signal received upon the detection of 5-ng mL^{-1} FTAB.

7.4 PFAS Summary

Table 7-2: PFAS analytes in the study, their acronym, and molecular structure.

Chemical Name	Acronym	Molecular Structure
Perfluorobutyric acid	PFBA	
Perfluoropentanoic acid	PFPeA	
Perfluorohexanoic acid	PFHxA	
Perfluoroheptanoic acid	PFHpA	
3:3 fluorotelomer carboxylic acid	3:3-FTCA	
4:3 fluorotelomer carboxylic acid	4:3-FTCA	
5:3 fluortelomer carboxylic acid	5:3-FTCA	
6:2 fluorotelomer carboxylic acid	6:2-FTCA	

Chemical Name	Acronym	Molecular Structure
6:2 fluorotelomer unsaturated carboxylic acid	6:2-FTUA	
5:2 secondary fluorotelomer alcohol	5:2-sFTOH	
5:2 fluorotelomer ketone	5:2-FT-ketone	
6:2 fluorotelomer alcohol	6:2-FTOH	
6:2 fluortelomer sulfonate	6:2-FTSA	
6:2 fluortelomer sulfonamide alkyl betaine	6:2-FTAB