

# Changes in Chemical Composition of ISS Archive Water Samples from Collection to Analysis

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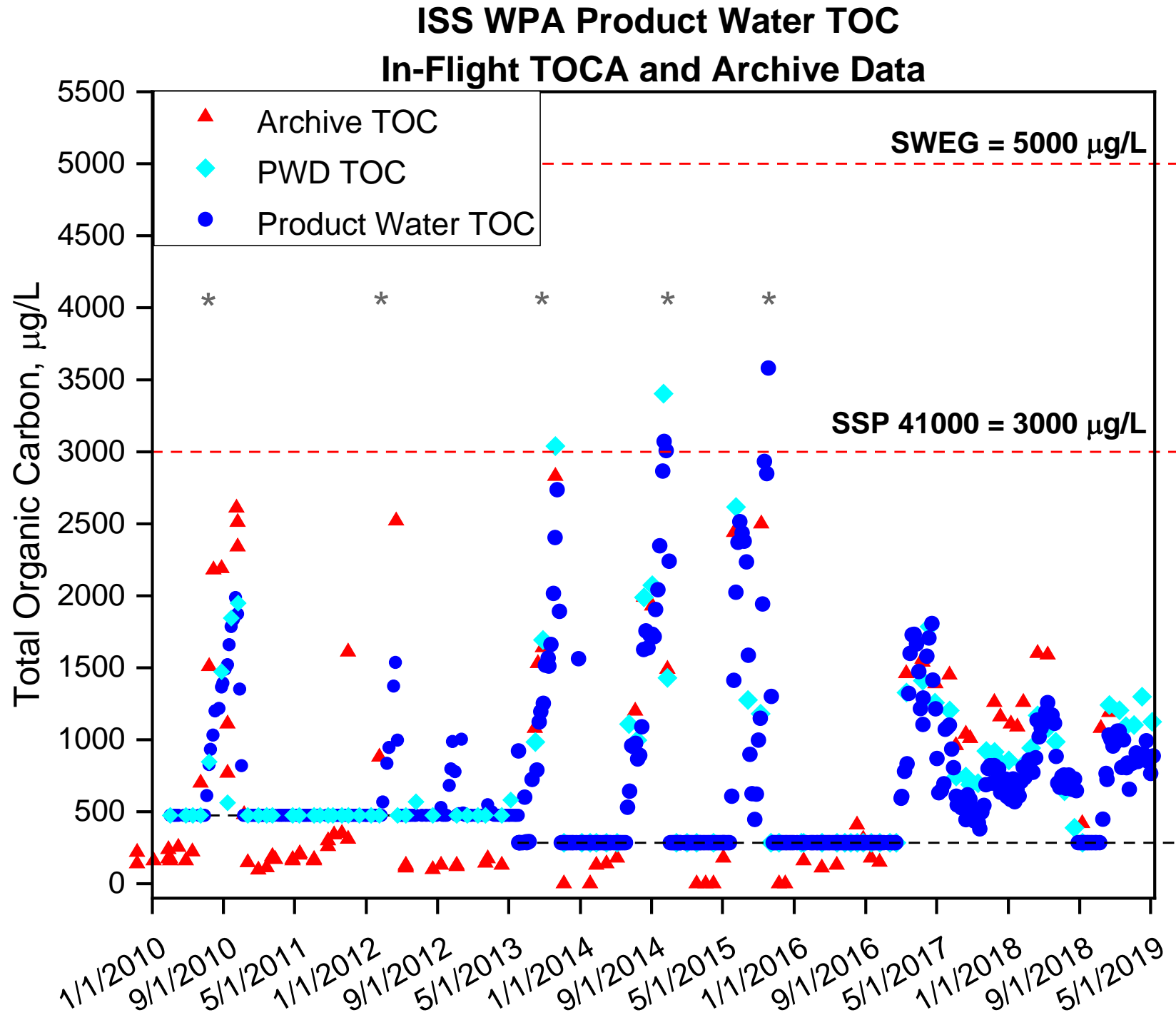
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# Outline

- Background
  - TOC increase and MF Bed replacement
  - SpaceX-12
    - Acetate and Bicarbonate?
  - SpaceX-14
    - Teflon v/s Mylar/Teflon
- Methods and Experimental Results
- Newer archival results
- Non-potable samples

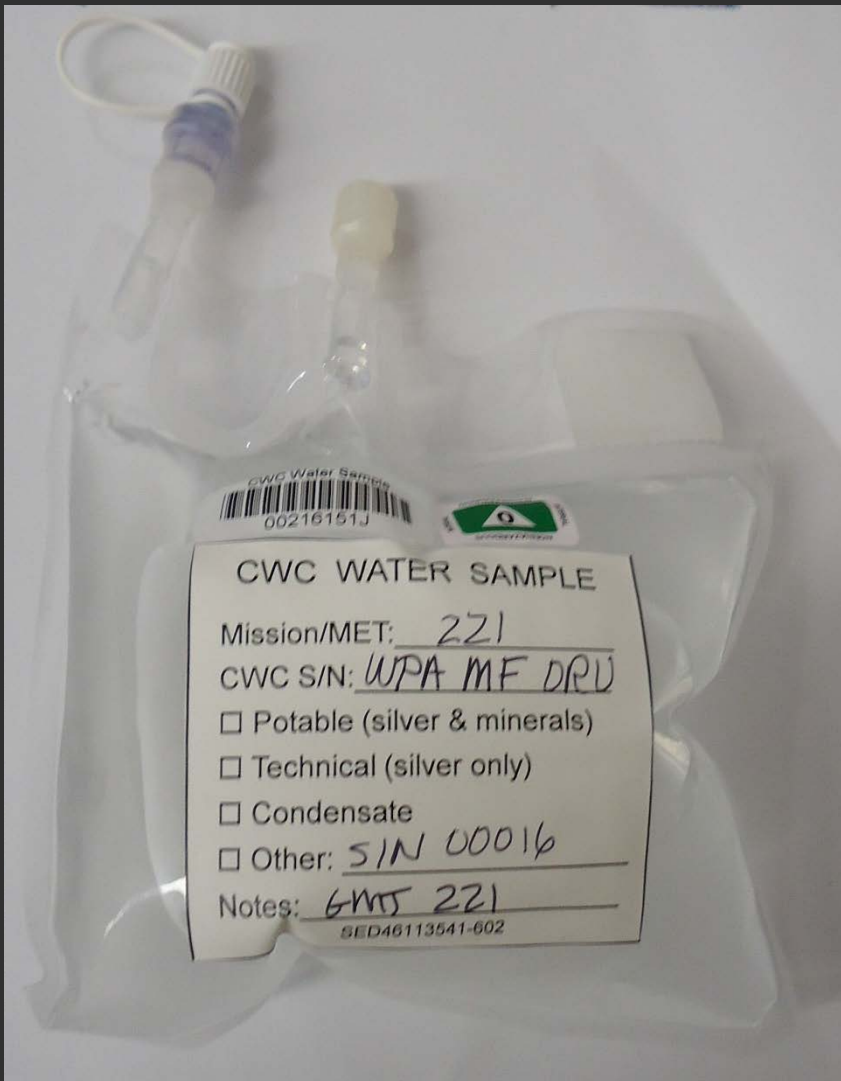
# TOC Increases



# Multifiltration Bed Extension

- Extended TOC trend provided opportunity to extend the life of the MF beds
- Ionic breakthrough of bed #2 would previously have resulted in replacement of the beds
- Ground testing showed that initial breakthrough contaminants could be processed by the Catalytic Reactor
  - 1) bicarbonate, 2) acetate, 3) ammonium
- Product water TOC has been unaffected by additional load
- Samples of the effluent from both beds collected and returned on SpaceX-12

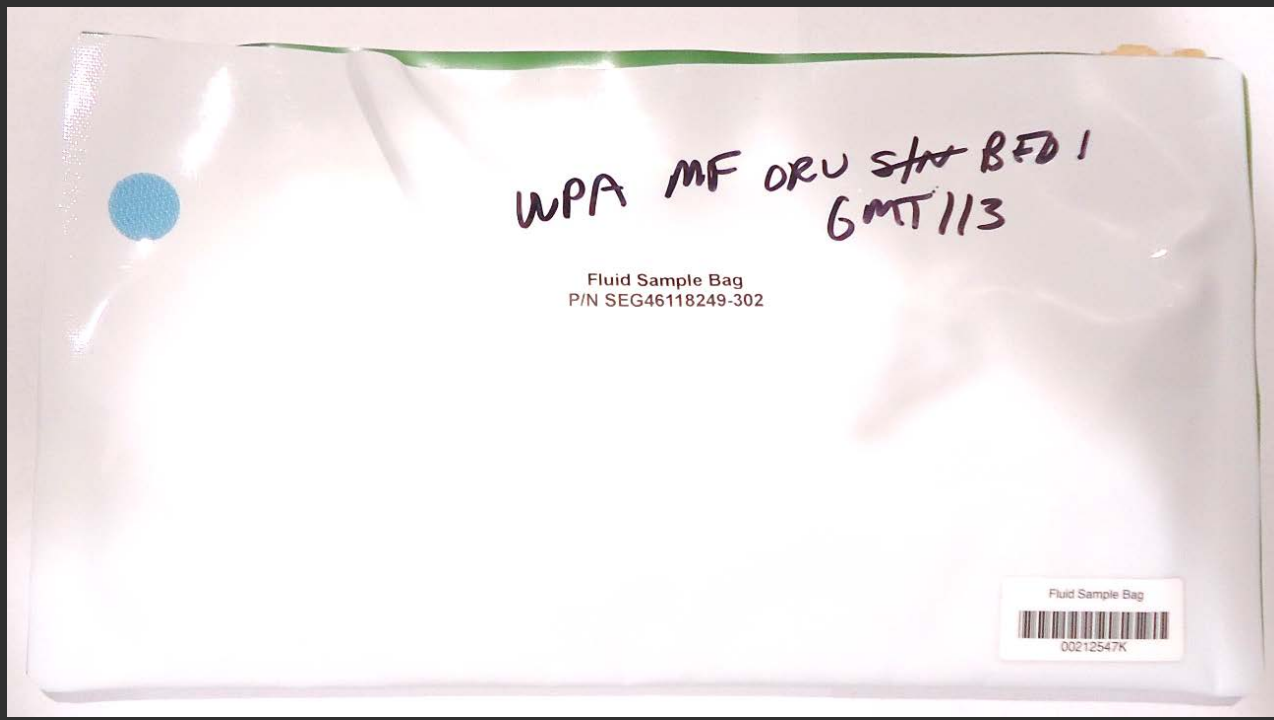
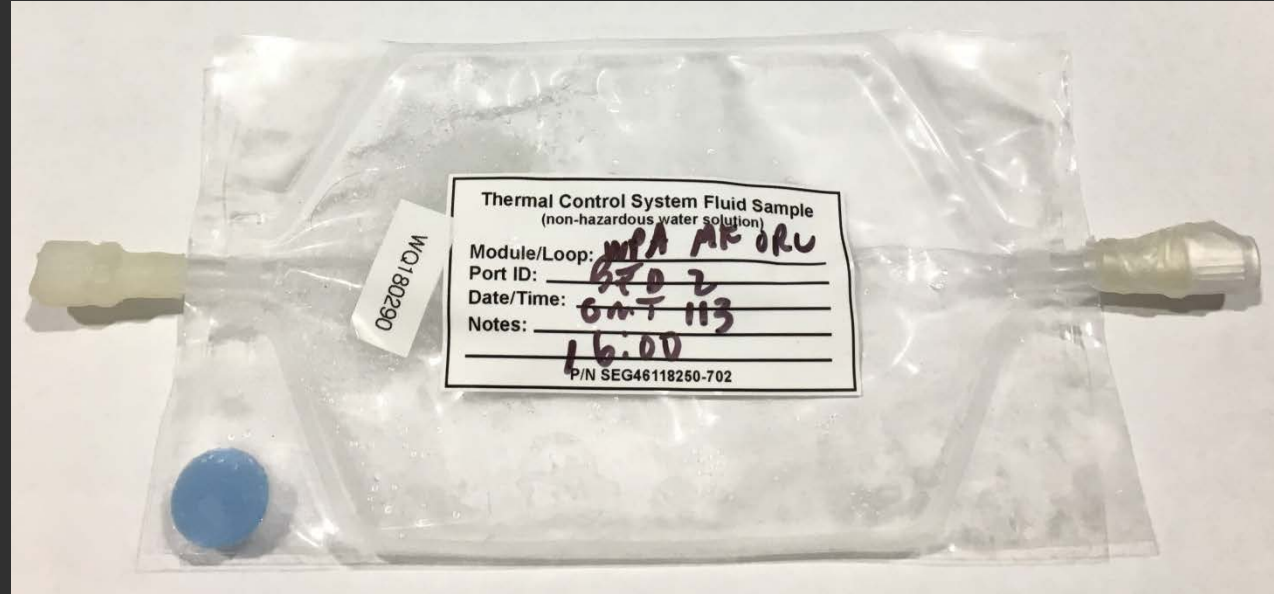
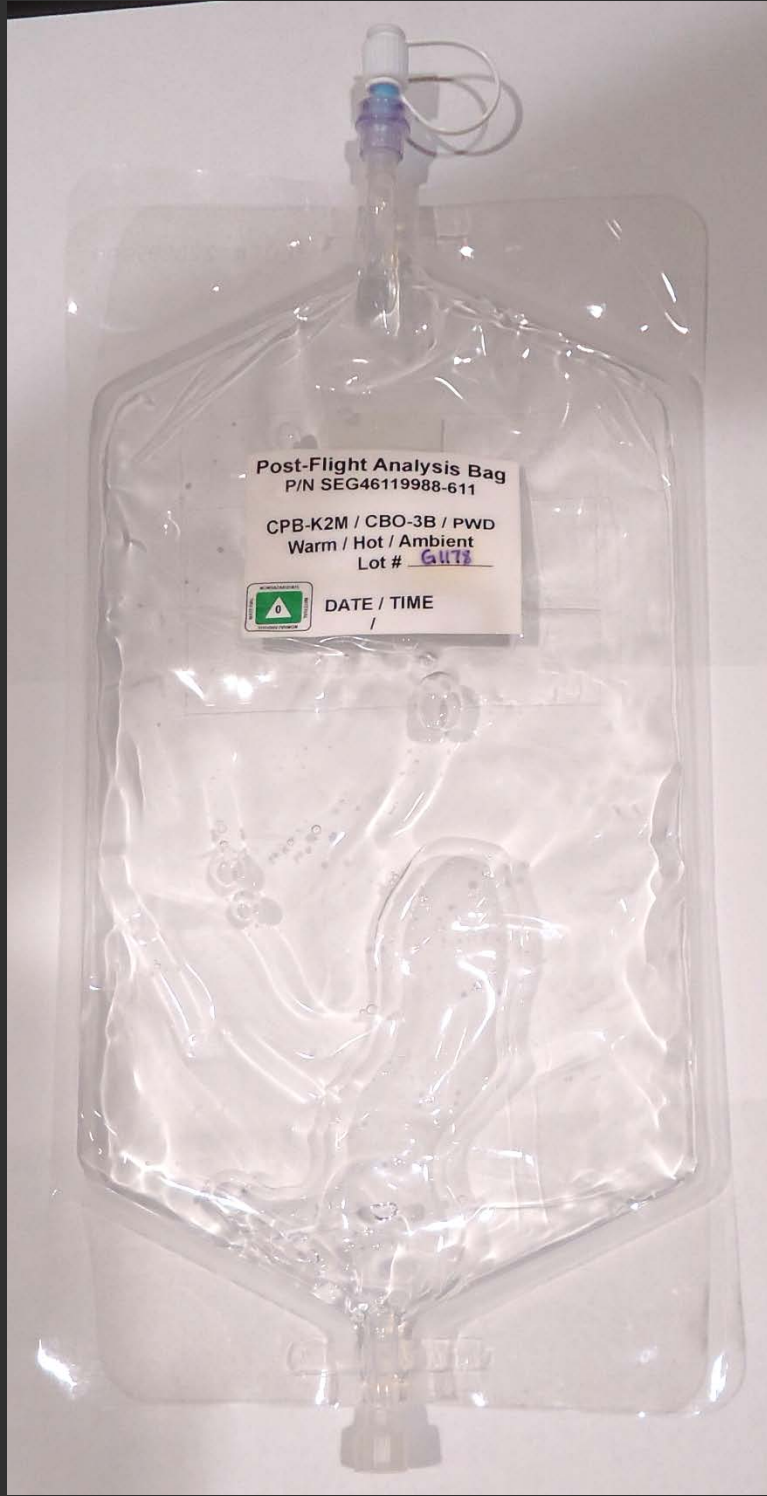
# SpaceX-12 Return



Mission	SpX-12		
Sample Location	WPA MF Bed ORU S/N 00016	WPA MF Bed ORU S/N 00017	
Sample Description	WPA MF Bed Effluent, Teflon Bag	WPA MF Bed Effluent, Teflon Bag	
Sample Date	8/9/2017	8/9/2017	
	Units		
<b>Cations</b>			
Ammonium	mg/L	<0.25	<0.25
<b>Total Organic Carbon</b>			
Inorganic Carbon	mg/L	<3.0	<3.0
Organic Carbon	mg/L	23.2	19.1
<b>Volatile Organics</b>			
Acetone	µg/L	5,300	6,020
2-Butanone (Methyl ethyl ketone)	µg/L	291	<50
<b>Semi-volatile Organics</b>			
Methyl sulfone	µg/L	148	<50
N,N-Dimethyl acetamide	µg/L	400	not found
N,N-Dimethylformamide	µg/L	440	not found
2-Ethoxyethanol	µg/L	280	not found
<b>Alcohols</b>			
Ethanol	µg/L	10,400	8,320
Methanol	µg/L	4,010	3,800
1-Propanol	µg/L	524	440
2-Propanol (Isopropanol)	µg/L	1,360	2,440
<b>Glycols</b>			
1,2-Ethanediol (Ethylene glycol)	µg/L	1,020	1,220
1,2-Propanediol (Propylene glycol)	µg/L	4,850	3,640
<b>Silanols</b>			
Dimethylsilanediol (DMSD)	µg/L	17,000	19,000
<b>Carboxylates</b>			
Acetate	µg/L	6,340	4,690
Propionate	µg/L	964	<500

# Where is the Bicarbonate?

- As expected, breakthrough of acetate was observed in both beds
- Bicarbonate levels (as TIC) were lower than expected based on the conductivity readings, even though it was expected to break through first
- Believed that CO<sub>2</sub> was diffusing through the Teflon sample bag, leading to lower-than-expected TIC concentrations
- Second set of samples collected and returned on SpaceX-14
- One sample for each bed collected in normal Teflon bag and one collected in smaller ITCS bag sealed in Mylar pouch



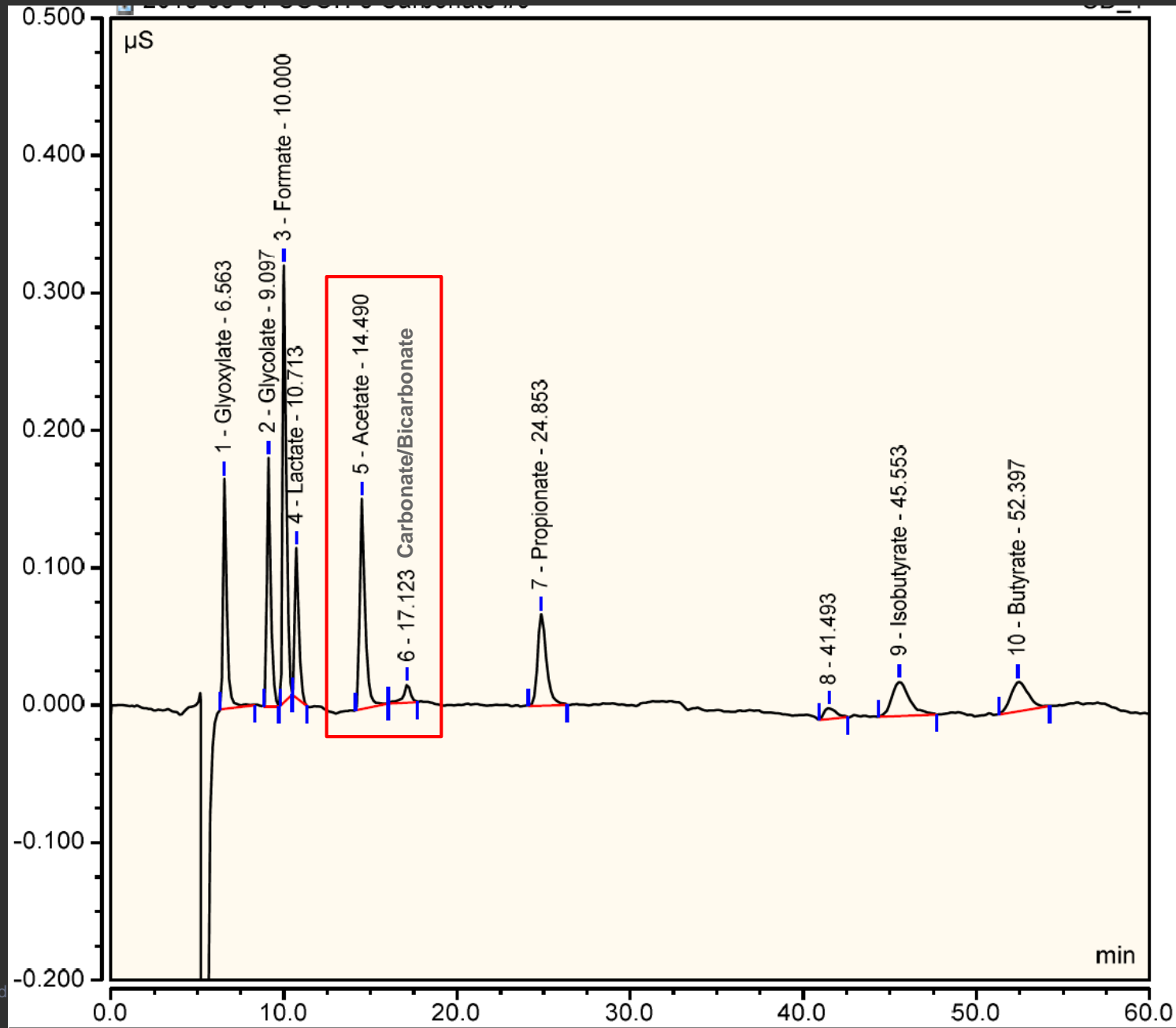
# SpaceX-14 Return

Mission	SpaceX-14				
Sample Location		WPA MF Bed #1 ORU S/N 00016	WPA MF Bed #1 ORU S/N 00016	WPA MF Bed #2 ORU S/N 00017	WPA MF Bed #2 ORU S/N 00017
Sample Description		MF Bed Effluent, Teflon Bag	MF Bed Effluent, Teflon/Mylar Bag	MF Bed Effluent, Teflon Bag	MF Bed Effluent, Teflon/Mylar Bag
Sample Date		4/23/2018	4/23/2018	4/23/2018	4/23/2018
	Units				
<b>Cations</b>					
Ammonium	mg/L	<0.25	<0.25	<0.25	<0.25
<b>Total Organic Carbon</b>					
Inorganic Carbon	mg/L	2.51	3.23	0.70	0.63
Organic Carbon	mg/L	50.1	57.2	35.5	35.1
<b>Volatile Organics</b>					
Acetone	µg/L	5,550	6,610	6,160	8,010
2-Butanone (Methyl ethyl ketone)	µg/L	578	630	< 50	50
Acetaldehyde	µg/L	not found	not found	770	not found
Trimethylsilanol	µg/L	not found	60.0	not found	not found
Dimethyl sulfide	µg/L	92	110	not found	35
<b>Semi-volatile Organics</b>					
Methyl sulfone	µg/L	172	NA	118	NA
2-Ethoxyethanol	µg/L	230	NA	370	NA
N,N-Dimethyl acetamide	µg/L	490	NA	not found	NA
N,N-Dimethylformamide	µg/L	370	NA	390	NA
<b>Alcohols</b>					
Ethanol	µg/L	50,700	55,900	42,900	43,700
Methanol	µg/L	4,730	5,490	3,710	3,820
1-Propanol	µg/L	761	599	578	434
2-Propanol (Isopropanol)	µg/L	4,530	4,010	3,920	2,800
<b>Glycols</b>					
1,2-Propanediol (Propylene glycol)	µg/L	2,050	NA	< 1000	NA
<b>Silanols</b>					
Dimethylsilanediol (DMSD)	µg/L	18,000	NA	19,000	NA
<b>Carboxylates</b>					
Acetate	µg/L	1,970	10,200	< 500	< 500
Butyrate	µg/L	< 500	968	< 500	< 500
Propionate	µg/L	< 500	4,030	< 500	< 500



# Where Did The Carboxylates Come From?

Initial concerns focused on potential sampling issues or interference from carbonate/bicarbonate

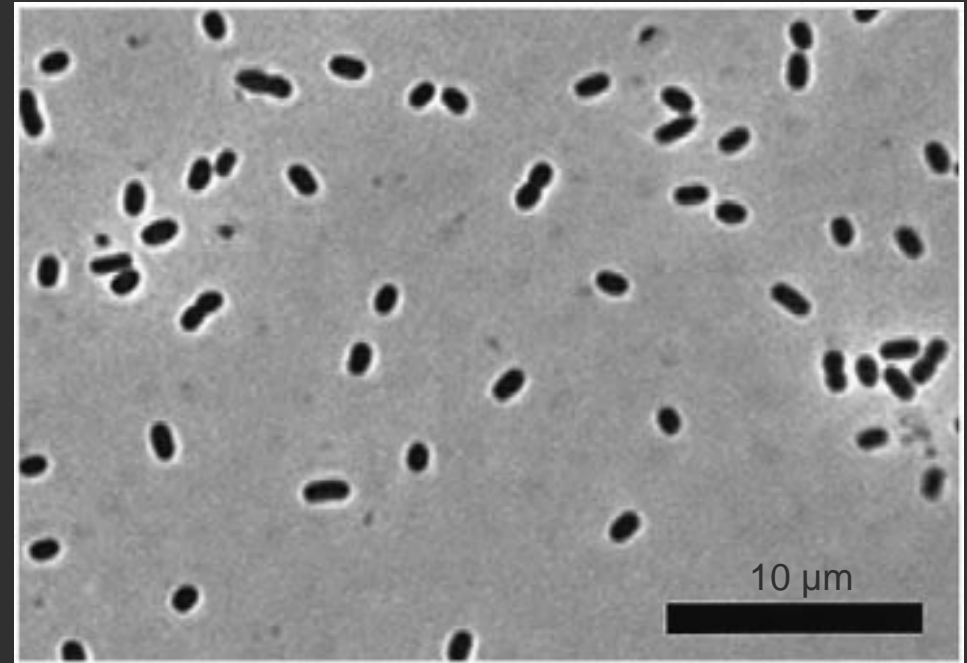
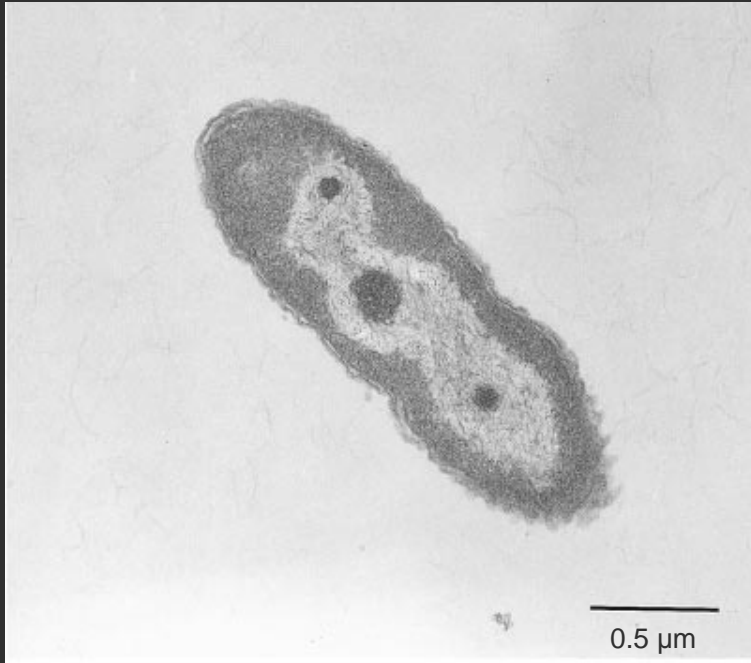


# Further Testing on Teflon/Mylar Sample

	<b>Analysis Date</b>	5/16/2018	6/21/2018	6/21/2018	6/25/2018
		Initial aliquot	5/16/2018 aliquot, room temp.	Archived sample (4 °C, Mylar)	6/21/2018 archived aliquot, room temp.
<b>Compound</b>	<b>Units</b>				
Acetate	µg/L	10,200	< 500	8,500	< 500
Butyrate	µg/L	968	< 500	867	< 500
Propionate	µg/L	4,030	< 500	4,740	< 500

# What is Causing the Changes?

- Gas diffusion and/or temperature-dependent mechanism



Zhang et al. (2000) *Int. J. Syst. Evol. Microbiol.* 50:743-749

- Microbial analysis of the MF bed 00016 (bed #1) sample collected in the normal Teflon bag showed the presence of *Burkholderia* species and *Burkholderia kururiensis* at a total count of  $4.1 \times 10^6$  CFU/mL
- Can we confirm that these bacteria were responsible for the losses?

# Methods

- **Isolation**

- Bacteria from archive water samples cultured on Reasoner's 2A (R2A) media for 48 hours
- Identified via 16S rDNA sequencing (ABI 3500 Genetic Analyzer)
- Individual isolates frozen at  $-80\text{ }^{\circ}\text{C}$  for future use

- **Current Testing**

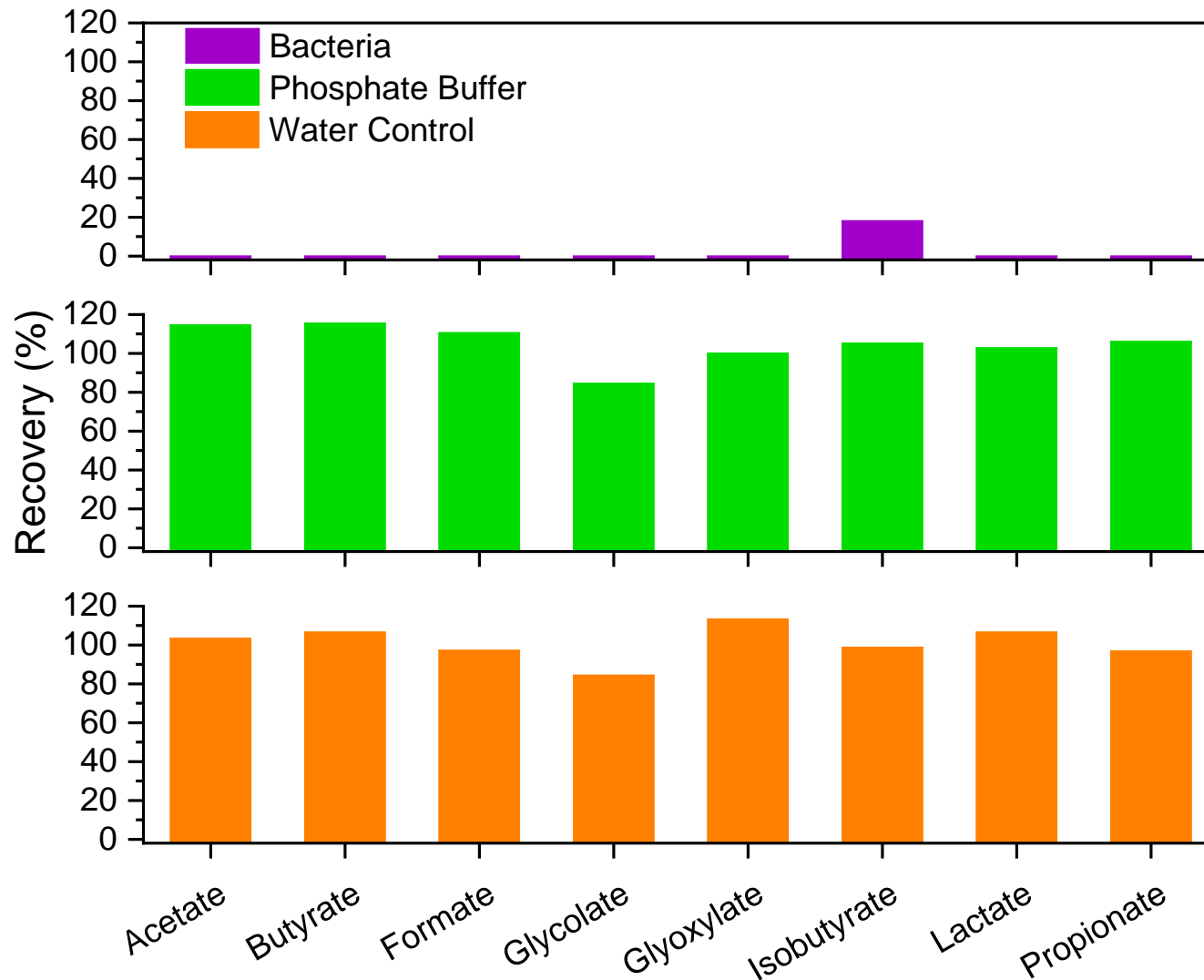
- 10  $\mu\text{L}$  loop of each isolate obtained from frozen stock, inoculated in R2A broth, and grown at  $35\text{ }^{\circ}\text{C}$  overnight
- Concentrations determined using serial dilution and plating methods followed by overnight culture at  $35\text{ }^{\circ}\text{C}$
- Individual isolates centrifuged, R2A removed, and re-suspended in phosphate buffer for testing

- **Chemical**

- Carboxylate analysis performed using ion chromatography (Dionex ICS-5000+)

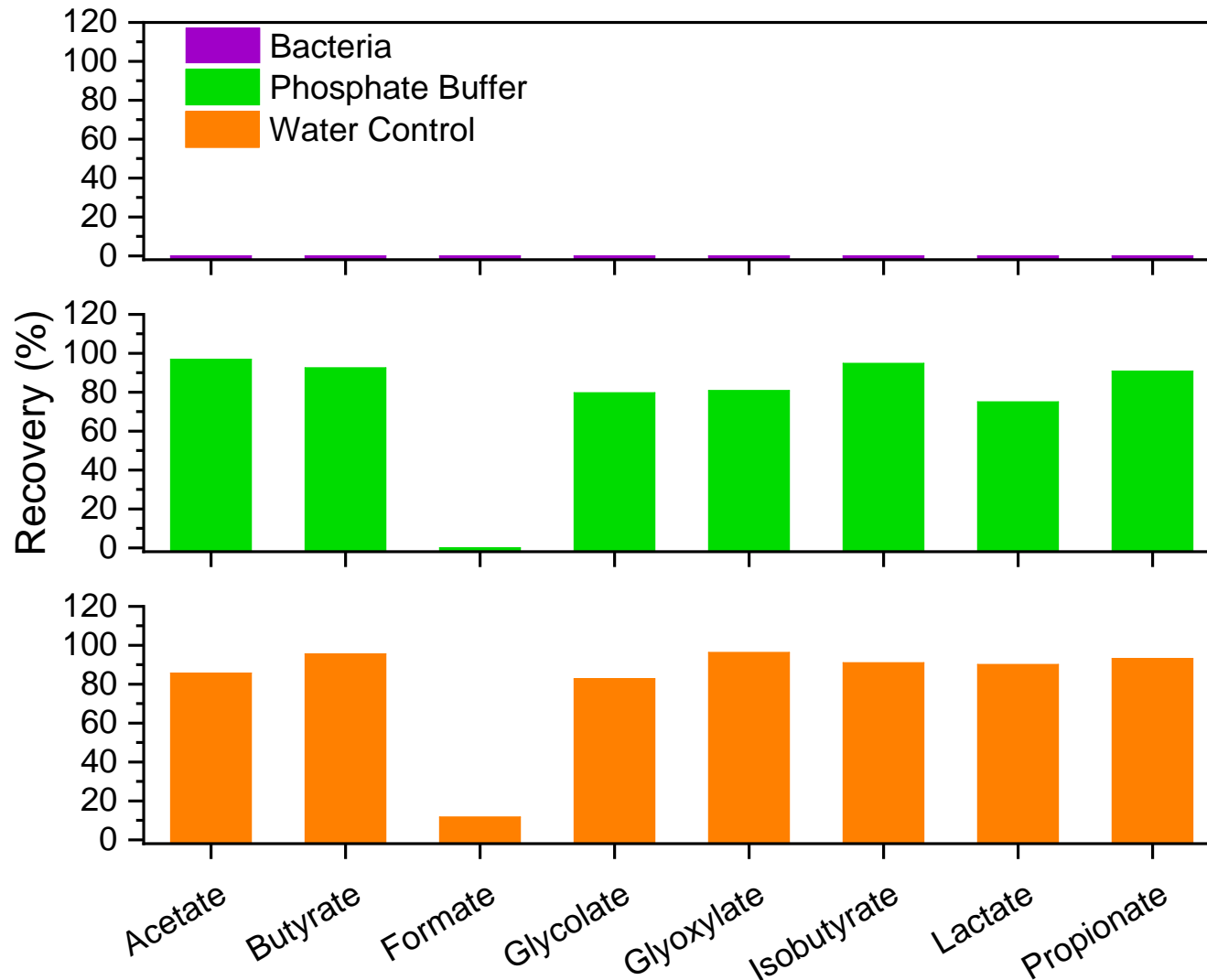
# Results of 24-hour Exposure

- 2500 µg/L carboxylate standard spiked with *B. sp.* and *B. kururiensis* at a total concentration of  $2.44 \times 10^7$  CFU/mL and analyzed after 24 hours



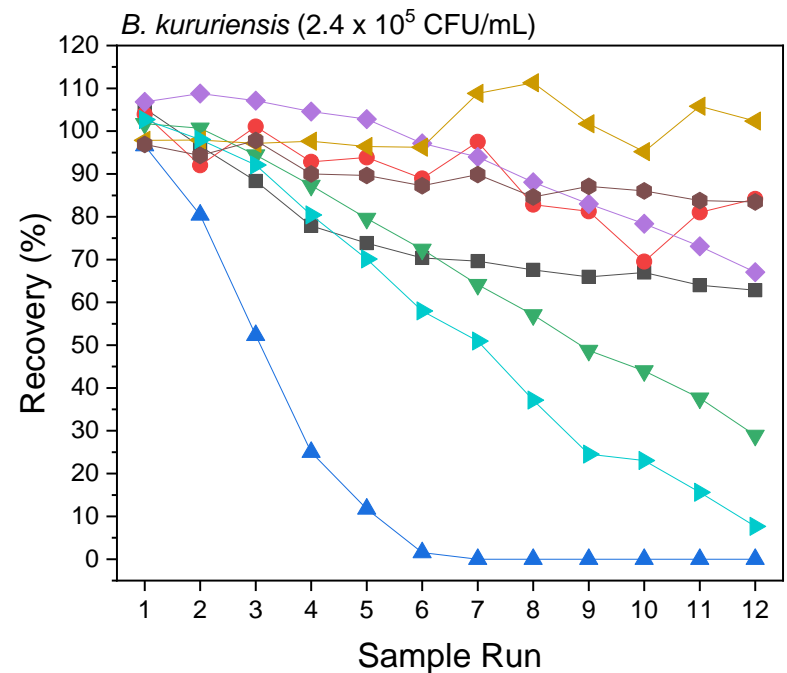
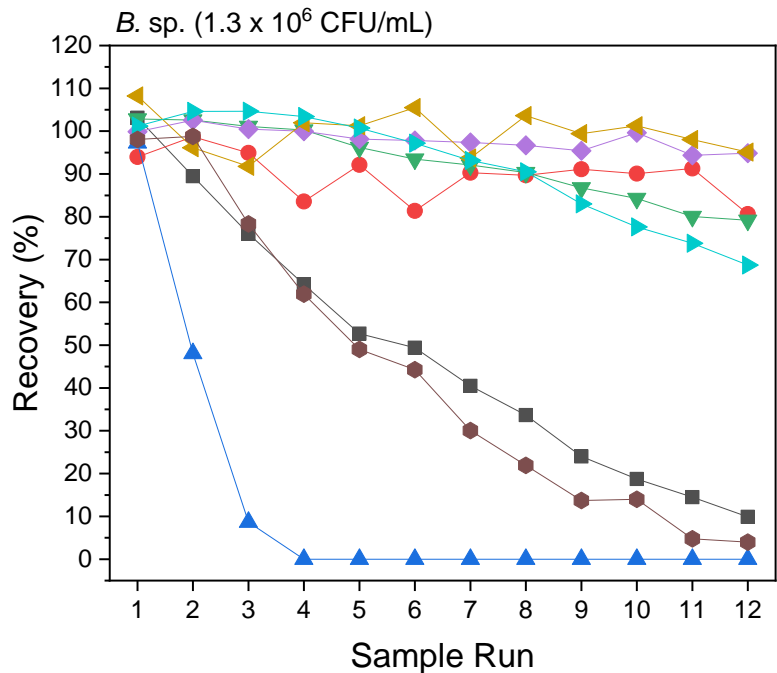
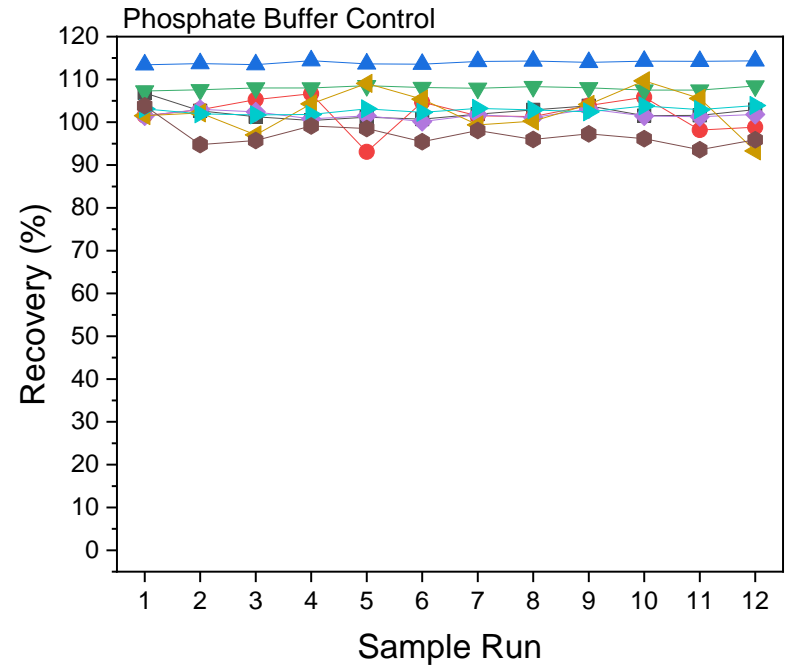
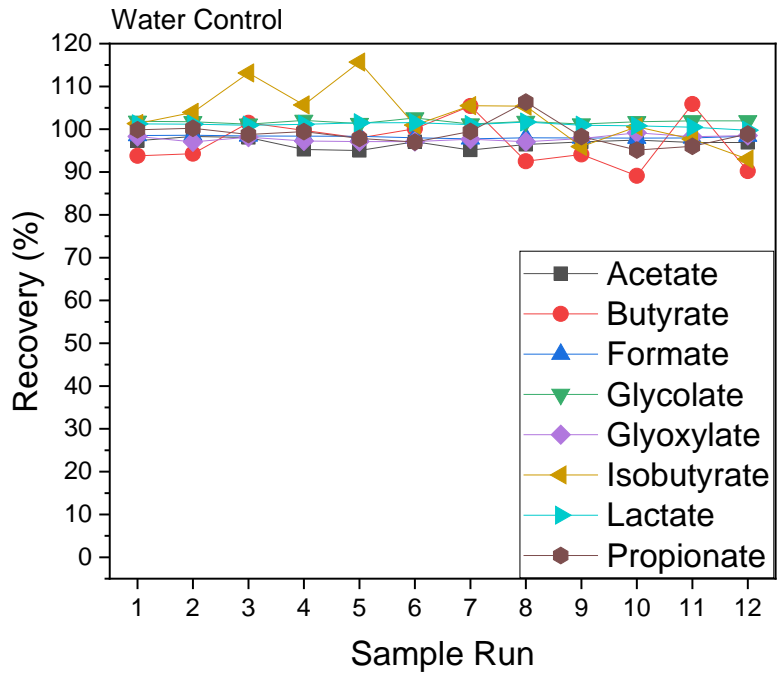
# Results of 8-day Exposure

- Remaining aliquot from 24-hour test spiked with carboxylate standard to final concentration of 2500  $\mu\text{g/L}$  and allowed to sit at room temperature for 8 days.



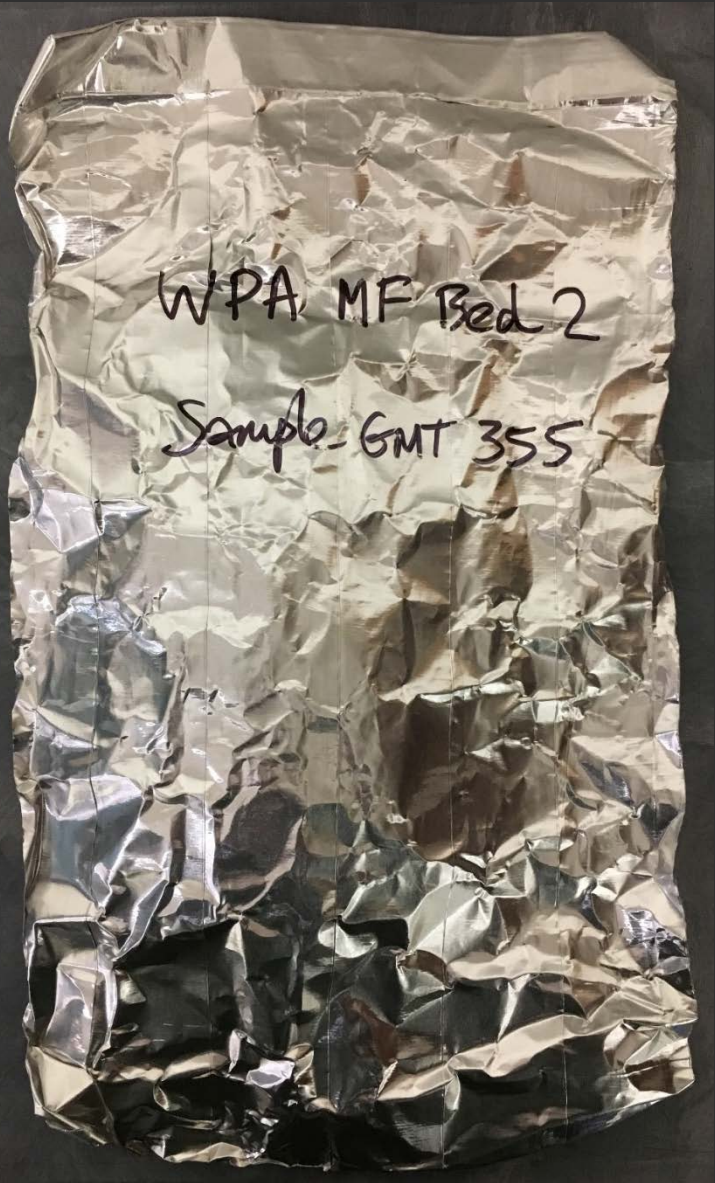
# Can We Tell *Exactly* Which Bacterium Is Responsible?

# Individual Isolate Testing





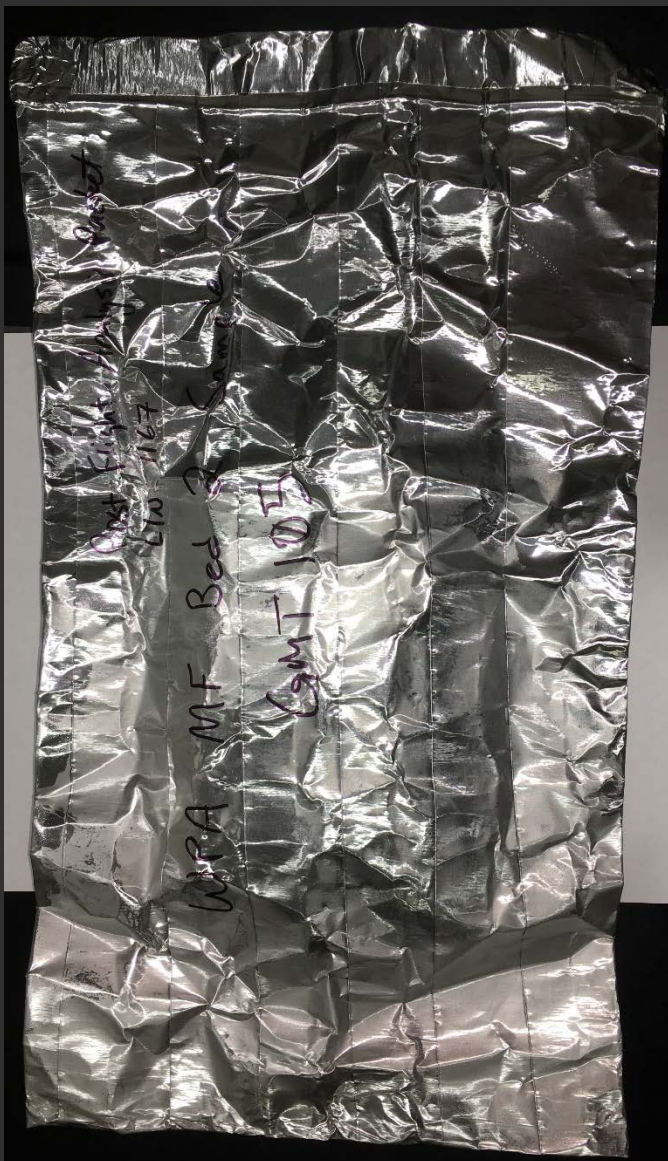
# SpaceX-16 Return



Mission	SpX-16		
Sample Location	WPA MF Bed ORU S/N 00016	WPA MF Bed ORU S/N 00017	
Sample Description	WPA MF Bed Effluent, Aluminum Tape-sealed Teflon Bag	WPA MF Bed Effluent, Aluminum Tape-sealed Teflon Bag	
Sample Date	12/21/2018	12/21/2018	
	Units		
<b>Cations</b>			
Ammonium	mg/L	11.1	<0.25
<b>Total Organic Carbon</b>			
Inorganic Carbon	mg/L	6.00	0.60
Organic Carbon	mg/L	44.2	28.9
<b>Volatile Organics</b>			
Acetone	µg/L	5,560	4,060
2-Butanone	µg/L	438	400
Trimethylsilanol	µg/L	330	not found
<b>Semi-volatile Organics</b>			
Methyl sulfone	µg/L	103	113
1-Methyl-2-pyrrolidinone	µg/L	420	not found
N,N-Dimethyl acetamide	µg/L	310	310
N,N-Dimethylformamide	µg/L	not found	200
<b>Alcohols</b>			
Ethanol	µg/L	36,900	44,700
Methanol	µg/L	3,970	5,210
1-Propanol	µg/L	583	402
1-Butanol	µg/L	<400	544
2-Butanol	µg/L	407	<400
2-Propanol (Isopropanol)	µg/L	3,480	4,280
<b>Glycols</b>			
Propylene glycol	µg/L	1,630	1,120
<b>Silanol</b>			
Dimethylsilanediol (DMSD)	µg/L	20,000	21,000
<b>Carboxylates</b>			
Acetate	µg/L	19,000	1,360
Butyrate	µg/L	1,250	<500
Propionate	µg/L	2,180	<500
<b>Non-volatiles</b>			
Urea	µg/L	<800	1,360

Bacterial Results	
WPA MF Bed ORU S/N 00016	WPA MF Bed ORU S/N 00017
5.2 x 10 <sup>5</sup> CFU/mL	8.4 x 10 <sup>4</sup> CFU/mL
<i>Burkholderia</i> sp.	<i>Burkholderia</i> sp.
<i>Curvibacter lanceolatus</i>	<i>Ralstonia insidiosa</i>
Unidentified Gram-negative rod	

# SpaceX-17 Return



Mission	SpX-17	
Sample Location	WPA MF Bed ORU S/N 00017	
Sample Description	WPA MF Bed Effluent, Aluminum Tape- sealed Teflon Bag	
Sample Date	04/15/2019	
	Units	
<b>Cations</b>		
Ammonium	mg/L	<0.25
<b>Total Organic Carbon</b>		
Inorganic Carbon	mg/L	1.20
Organic Carbon	mg/L	38.2
<b>Volatile Organics</b>		
Acetone	µg/L	3,250
<b>Alcohols</b>		
Ethanol	µg/L	12,300
Methanol	µg/L	2,880
1-Propanol	µg/L	492
2-Butanol	µg/L	456
2-Propanol (Isopropanol)	µg/L	2,870
<b>Silanols</b>		
Dimethylsilanediol (DMSD)	µg/L	15,400
<b>Carboxylates</b>		
Acetate	µg/L	38,200
Propionate	µg/L	8,450

<b>Bacterial Results</b>
WPA MF Bed ORU S/N 00017
5.5 x 10 <sup>4</sup> CFU/mL
<i>Burkholderia sp.</i>
<i>B. kururiensis</i>

# Other Questions

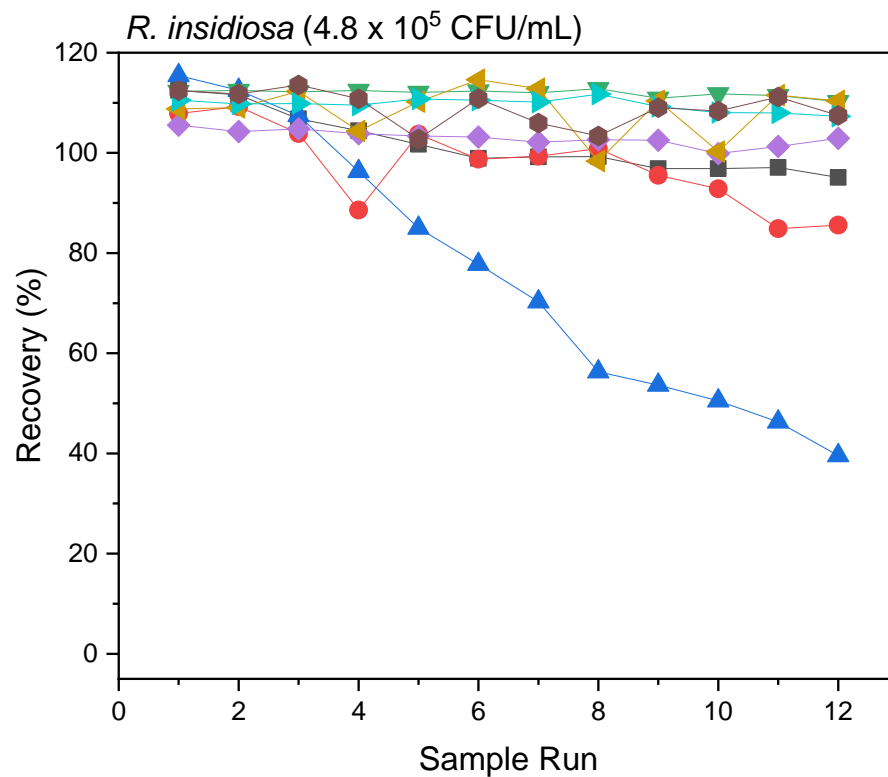
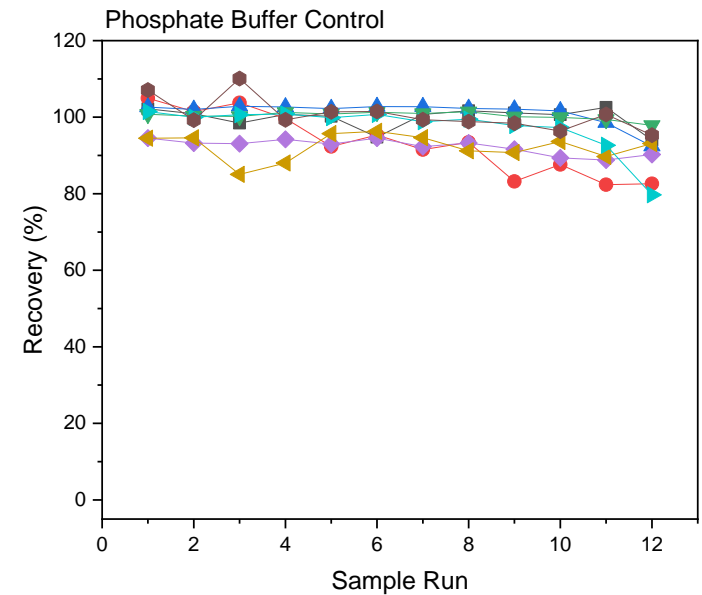
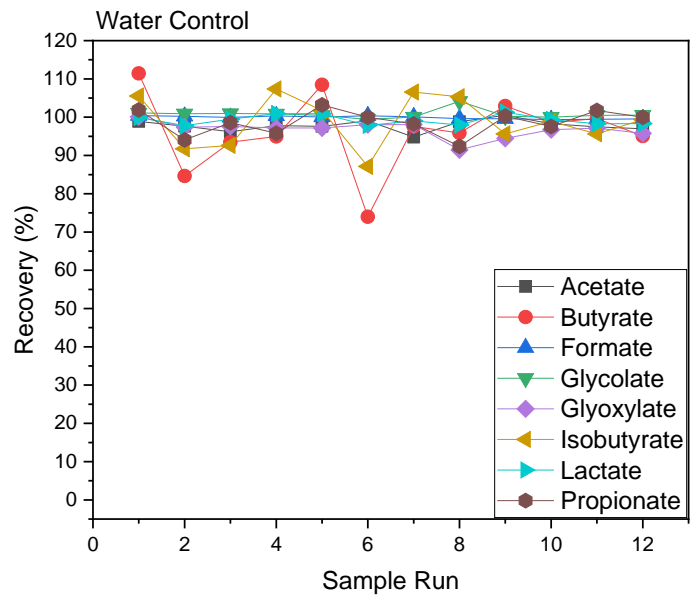
- How might this affect some other non-potable samples?
  - Condensate
  - Wastewater
- Is this behavior specific to *Burkholderia*?
- What is the concentration dependence?

# Condensate

	Increment	43	45	46	47			48	50		51	52	54	54	55	58
	Return Flight	SpX-6	Soyuz 43	Soyuz 44	SpX-8			SpX-9	SpX-10		SpX-11	SpX-12	SpX-13	SpX-14	SpX-14	SpX-16
Compound																
Acetate		634	13500	42900	64300	54100	46300	60400	<500	31600	<500	9170	<500	2670	41200	39100
Butyrate		NA	<500	<500	<500	593	565	<500	<500	<500	<500	<500	<500	<500	<500	<500
Formate		<625	547	<500	2860	11400	9730	6650	<500	<500	<500	<500	<500	<500	<500	<500
Glycolate		<625	<500	<500	<5000	<5000	<5000	<1000	<1000	<1000	<1000	<5000	4840	6400	<1000	<1000
Glyoxylate		<625	<500	<500	<500	<500	<500	<500	<500	<500	<500	729	813	<500	<500	<500
Isobutyrate		NA	<500	<500	<500	<500	<500	<500	<500	<500	<500	<500	<500	<500	<500	1170
Lactate		<625	<500	<500	44300	2920	2130	<500	<500	<500	<500	<500	<500	<500	23700	<500
Propionate		<625	<500	<500	1400	1730	1200	1220	<500	<500	<500	<500	<500	<500	<500	<500
Bacterial Counts		1.8x10 <sup>5</sup>	4.6x10 <sup>4</sup>	2.9x10 <sup>5</sup>	1.8x10 <sup>5</sup>			2x10 <sup>4</sup>	1.5x10 <sup>4</sup>	4.3x10 <sup>4</sup>	3.5x10 <sup>5</sup>	3.6x10 <sup>4</sup>	1.7x10 <sup>5</sup>	5.2x10 <sup>4</sup>	3.9x10 <sup>5</sup>	1.2x10 <sup>6</sup>
Bacterial ID		<i>Ralstonia insidiosa</i>	<i>Cupriavidus metallidurans</i>	<i>Ralstonia pickettii</i>	<i>Ralstonia insidiosa</i>			<i>Caulobacter vibrioide</i>	<i>Flexibacter</i> sp.	<i>Ralstonia insidiosa</i>	<i>Ralstonia insidiosa</i>	<i>Ralstonia insidiosa</i>	<i>Ralstonia insidiosa</i>	<i>Ralstonia insidiosa</i>	<i>Ralstonia insidiosa</i>	<i>Ralstonia insidiosa</i>
		<i>Cupriavidus metallidurans</i>	<i>Ralstonia insidiosa</i>	<i>Sphingobium xenophagum</i>	<i>Cupriavidus basilensis</i>			<i>Ralstonia insidiosa</i>	<i>Cupriavidus basilensis</i>	<i>Flexibacter</i> sp.	Unidentified Gram-negative rod	Unidentified Gram-negative rod	Unidentified Gram-negative rod	Unidentified Gram-negative rod	Unidentified Gram-negative rod	Unidentified Gram-negative rod
				Unidentified Gram-negative rod					<i>Cupriavidus metallidurans</i>							



# Can We Account for Losses in the Wastewater?



# Clearly, *R. insidiosus* isn't responsible for the lack of most carboxylates in the SpaceX-13 wastewater

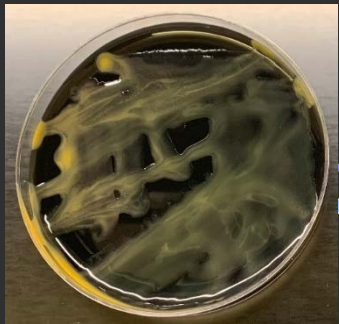
## What about the Unidentified Gram-negative Rods?

- 2 morphologies actually present in the SpX-13 sample
- Identification not possible under nominal JSC Microbiology Lab procedures

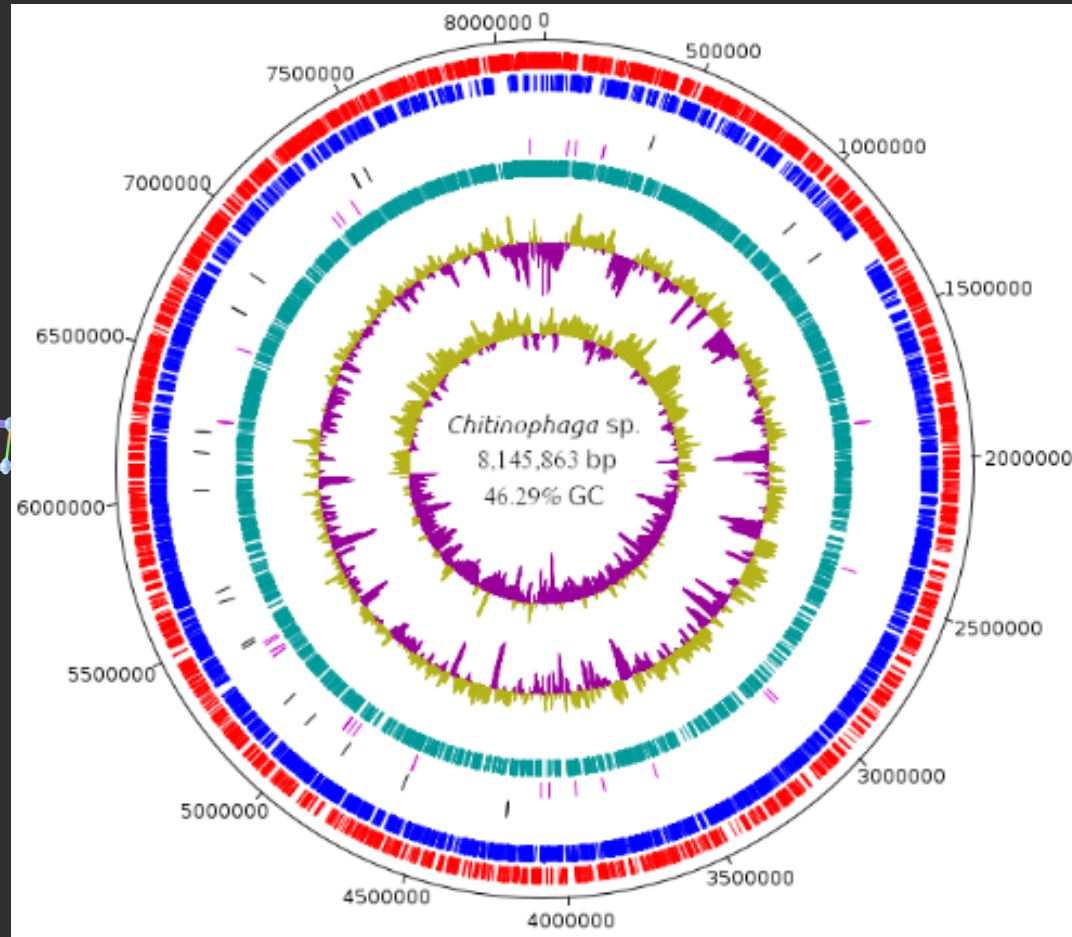


# Whole Genome Sequencing

## Full genome sequencing with the MinION



*Chitinophaga* sp.





# Summary

- Microbial growth can affect the chemical results obtained from ISS archival water samples
- Utilization of carbon sources for bacterial growth can be minimized by limiting the oxygen present and/or maintaining the samples at reduced temperature
- On-orbit sample processing (i.e. packaging) is one method to limit the oxygen, but this may not be realistic for all samples
- Need for this on-orbit processing depends on what information is hoped to be gained from archival sample analysis

# Acknowledgements

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