NSERC USRA Report – Summer 2025 – TRU

Developments

Proteomics & transcriptomics to identify proteins involved in 6:2 FTSA and FTAB metabolism

To quantify and observe mRNA levels over time when NB4-1Y is provided with 6:2 FTSA, 6:2 FTAB, $MgSO_4$, and 1-octanesulfonate, a non-fluorinated structural analogue of 6:2 FTSA, as sole sources of sulfur, a time course experiment was carried out in triplicate. At each time point cell pellets were harvested for RNA extraction, OD 600 nm was measured (Figure 1), and fluoride ion concentration was measured (Figure 2). Generation times were calculated for all treatments (Table 1) indicating longer generation times for samples exposed to PFAS compared to control treatments such as $MgSO_4$ and 1-octanesulfonate.

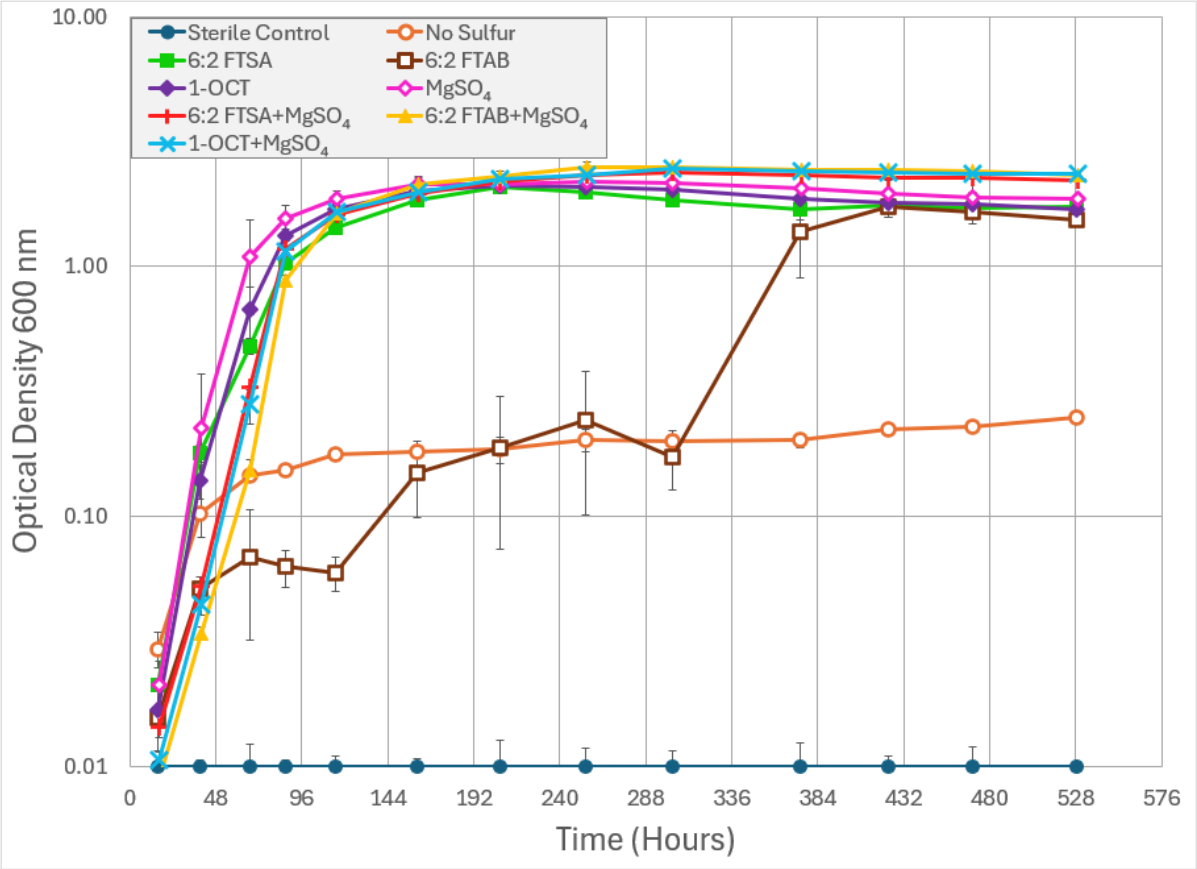

Figure 1. OD 600 nm readings, of NB4-1Y growth provided with various sulfur sources. Plotted using log scale (n = 3; error bars represent standard deviation).

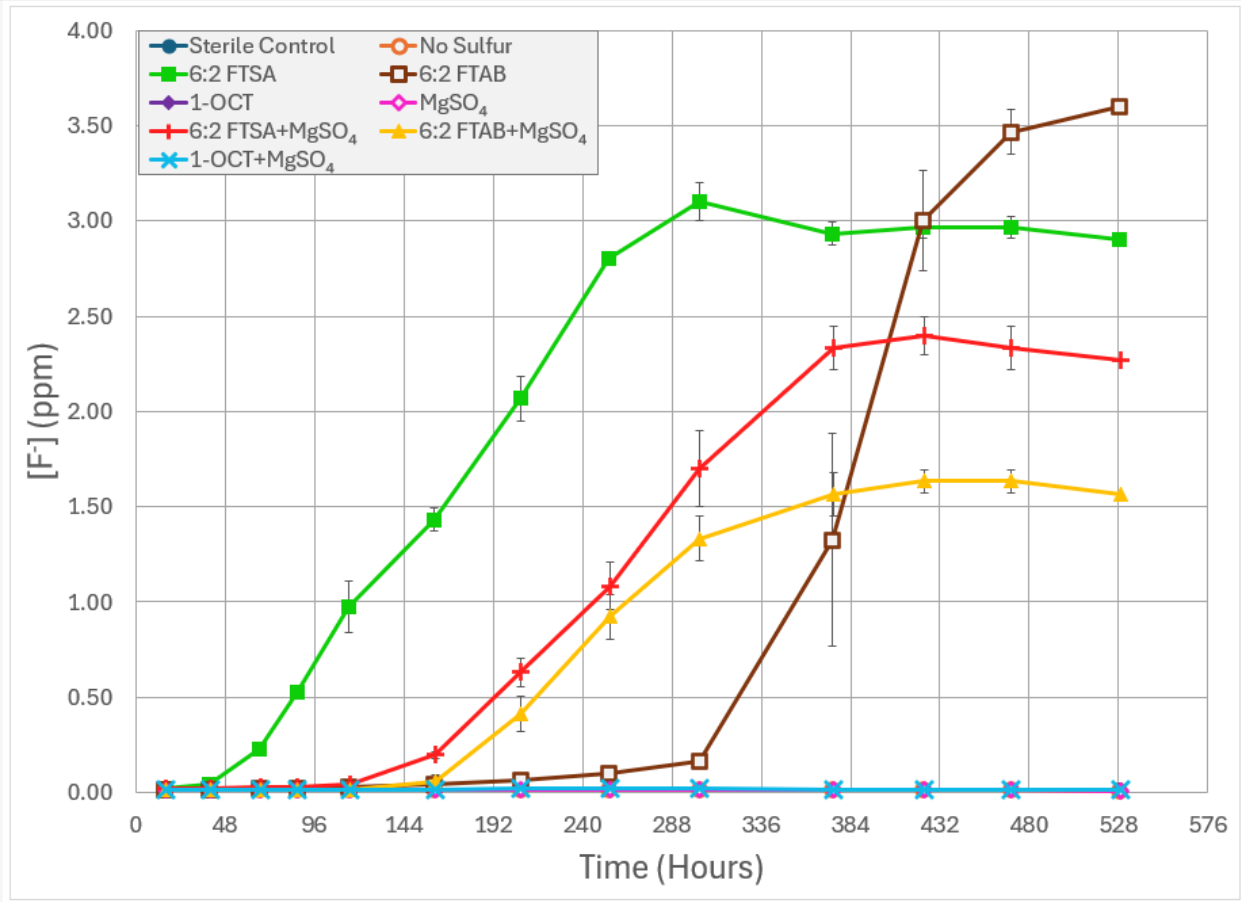

Figure 4. Fluoride release in NB4-1Y cultures provided with various sulfur sources (n = 3; error bars represent standard deviation).

Table 1. Generation time in hours for each treatment in time course experiment calculated using the exponential phases of NB4-1Y growth curves.

Treatment	Generation Time (g) (hours)
No Sulfur	13.3
6:2 FTSA	11.4
6:2 FTAB	23.9
1-OCT	9.6
$MgSO_4$	9.0
6:2 FTSA+$MgSO_4$	10.5
6:2 FTAB+$MgSO_4$	10.1
1-OCT+$MgSO_4$	10.1

Previously developed assays and reverse transcriptase quantitative digital polymerase chain reaction (dPCR) will be used to quantify mRNA levels over time. Six genes of interest, thought to be involved in the metabolism of 6:2 FTSA by NB4-1Y, and two reference genes (Table 2) were selected based on previous transcriptome and proteome data and literature respectively. Specifically, dimethylsulfone monooxygenase sfnG was selected as a gene target because the enzyme it codes for may desulfonate 6:2 FTSA. Four LLM class flavin-dependent oxidoreductase genes were selected because these versatile

enzymes are known to catalyze a variety of reactions including hydroxylations, epoxidations, and Baeyer–Villiger rearrangements. One gene coding for an acyl-CoA dehydrogenase family protein is also a target, because of our publications showing that acetyl-CoA adducts could be formed during PFAS metabolism. If the gene targets are expressed more in samples exposed to PFAS then the hypothesis that these genes of interest contribute to the ability of NB4-1Y to metabolize PFAS will be supported.

Table 2. Proteins coded for by gene targets and their corresponding accension number selected for reverse transcriptase quantitative digital polymerase chain reaction (dPCR).

Accession Number	Protein
Q9K23_RS05625	dimethylsulfone monooxygenase SfnG
Q9K23_RS08775	LLM class flavin-dependent oxidoreductase
Q9K23_RS08755	LLM class flavin-dependent oxidoreductase
Q9K23_RS21130	acyl-CoA dehydrogenase family protein
Q9K23_RS08670	LLM class flavin-dependent oxidoreductase
Q9K23_RS09265	LLM class flavin-dependent oxidoreductase
Q9K23_RS02195	Recombinase A (*recA*)
Q9K23_RS01395	type I glyceraldehyde 3-phosphate dehydrogenase

RNA has been extracted from all NB4-1Y cell pellets from two time points (time point 3 and 6). Using the extracted RNA samples normalized to 2ng/µL, cDNA has been synthesized for all samples from the two time points as well. Various qPCR experiments have been performed using both NB4-1Y genomic DNA and cDNA from the time course experiment described above, to test PCR efficiencies using single plex and duplex assays. The resulting standard curves have displayed PCR efficiencies between 60.8% – 98.5% (not shown). This data will help to further elucidate which assays are most efficient when duplexed together, thus making future quantification of target gene expression using dPCR more efficient and cost effective.

Plans for the next report:

Subtask 1-a: Proteomics & transcriptomics to identify proteins involved in 6:2 FTSA and FTAB metabolism

Work will continue to extract RNA and synthesize cDNA from cell pellets from additional time points. Once samples are prepared for chip digital PCR (dPCR) gene expression can be monitored using previously validated assays and data can be normalized to reference genes.