

Survival and Adaptation of Bacteria in Ultrapure Water Systems

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The Challenge

- **To produce water of quality:**
 - TOC < 1 ppb
 - Particles (>0.05 μ m) < 500 / L
 - Oxygen < 10 ppb
 - **Bacteria \leq 1 CFU / L**
 - Resistivity = 18.2 M- Ω cm

Bacterial Contamination of UPW

– The Main Issues

- Compromises the quality of the final product
 - Semiconductor industry
 - Pharmaceutical industry
- Decreased efficiency in heat exchangers (<10%)
- Decreases life-time of ultrafilters, RO membranes etc.
- Overall increase in expenditure

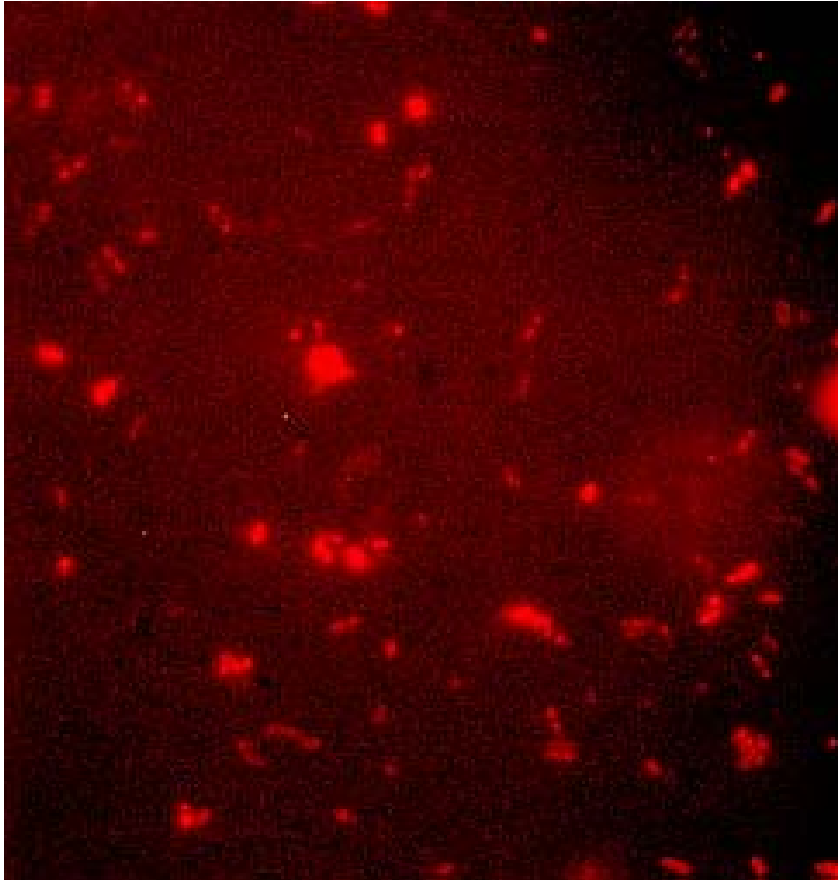
Survival Strategies of Oligotrophs

- Cells are very small ($<0.2 \mu\text{M}$)
- Production of EPS
- Broad substrate range
- Growth on $<1 \text{ mg carbon / L}$
- Increased adhesion to surfaces
- Reduced rate of metabolism

Plate Counts (Quantitative)

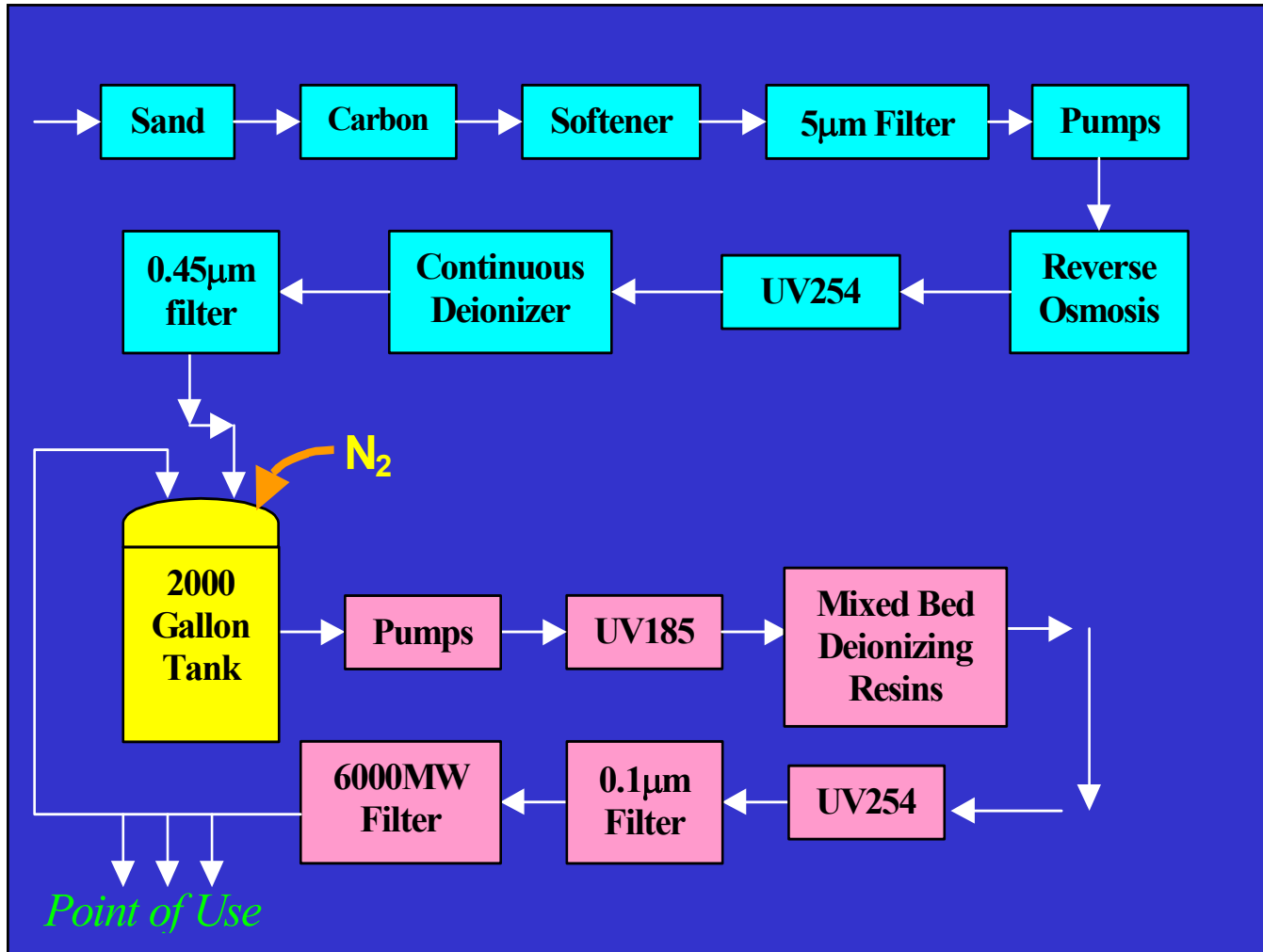
- ASTM Standards
 - R2A media
 - Incubation at 28°C for 48-72 hours
- Data from Our research suggests:
 - Use of diluted R2A media advantageous
 - Oligotrophs require up to 4 weeks incubation
 - Underestimates viable population by ≥ 20 fold

Direct Staining - Quantitative

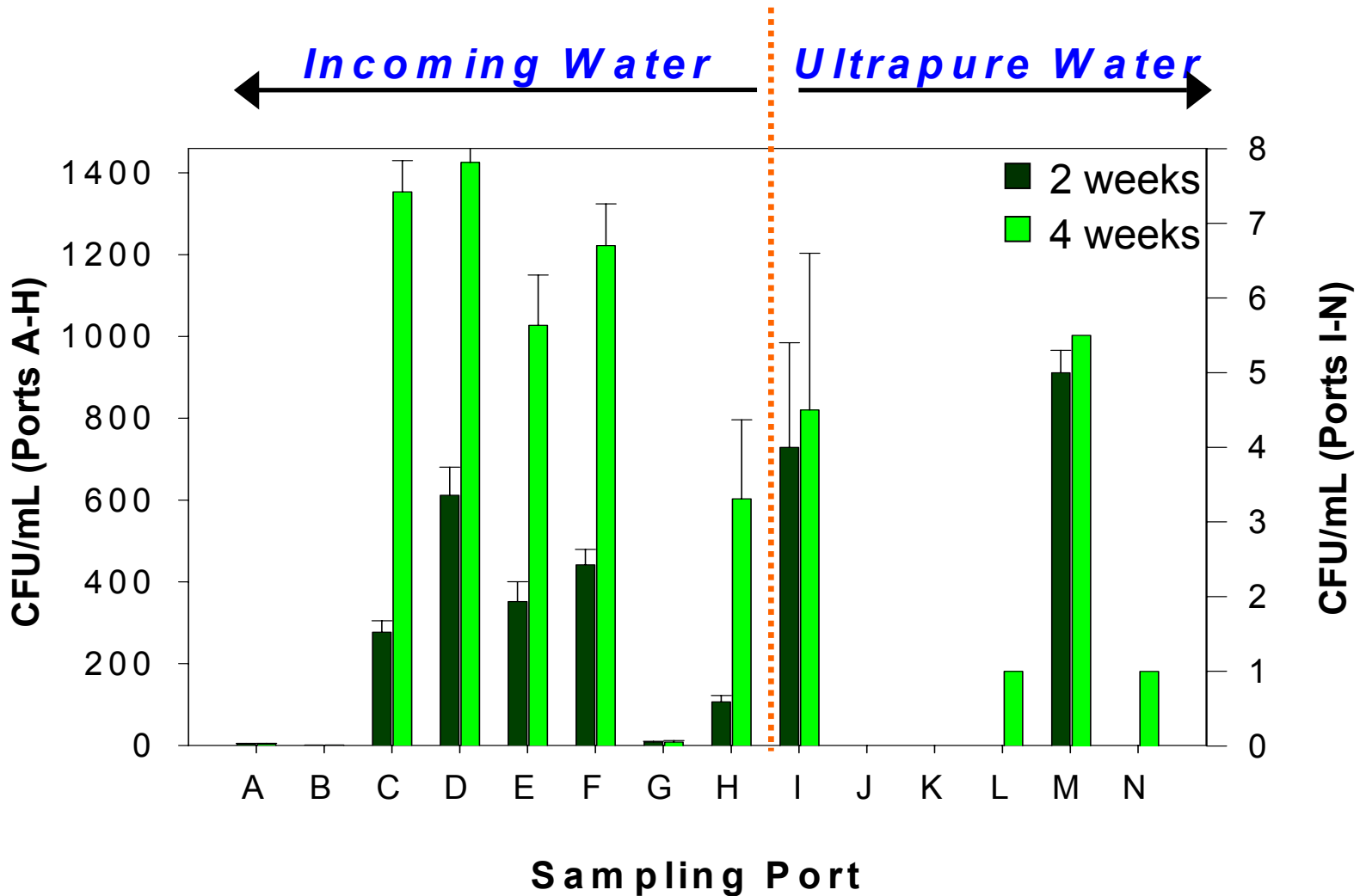


- Viable cells
 - **Cyanotolyl tetrazolium chloride (CTC)**
 - Artificial electron acceptor → reduced within electron transport chain
 - Intracellular formation of red colored formazans
- Total cells
 - **4',6'-diamidino-2-phenylindole (DAPI)**
 - Binds to bacterial DNA
 - Stained cells fluoresce blue

Schematic of Typical UPW System

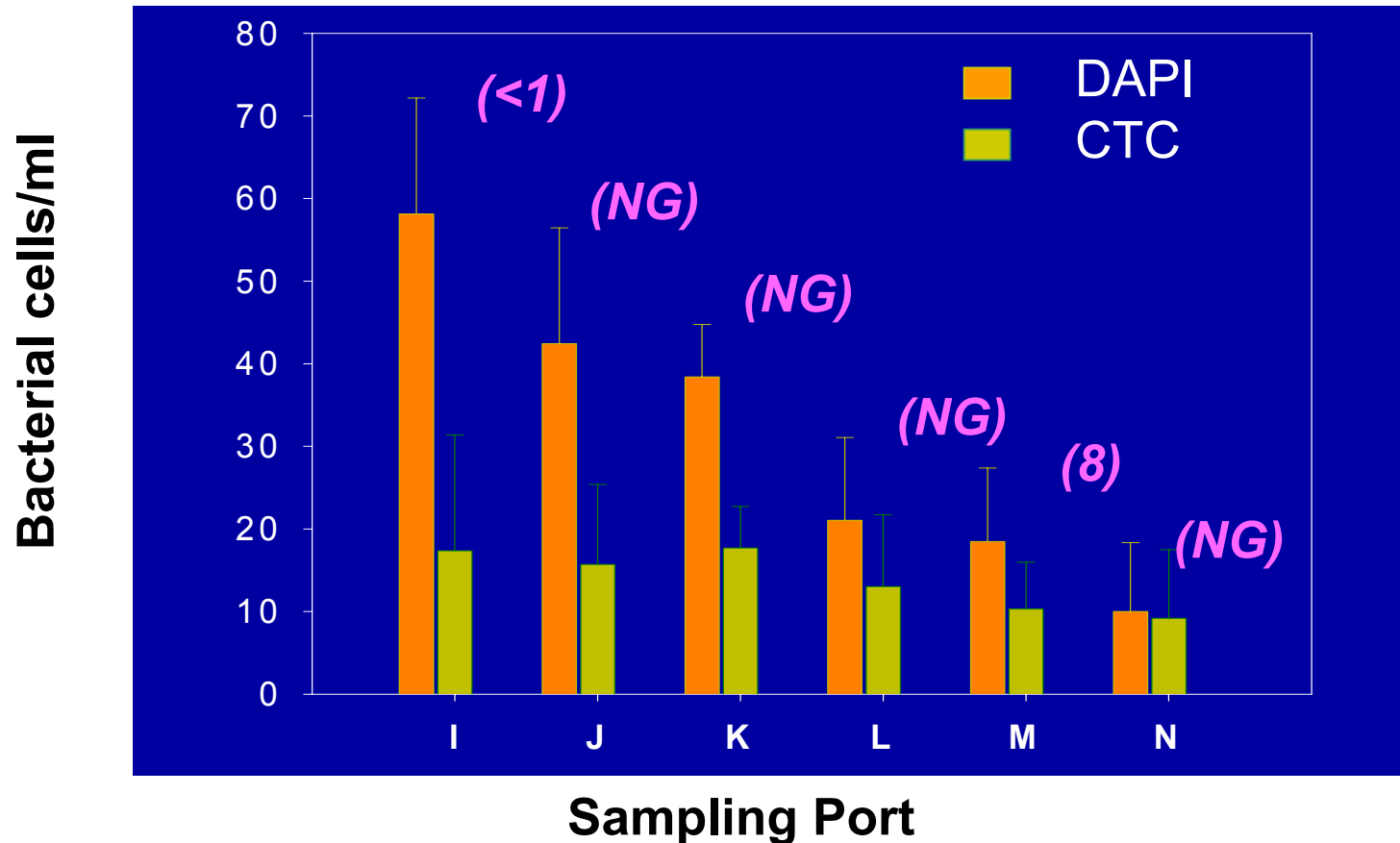


Effect of Incubation Time on Bacterial Enumeration

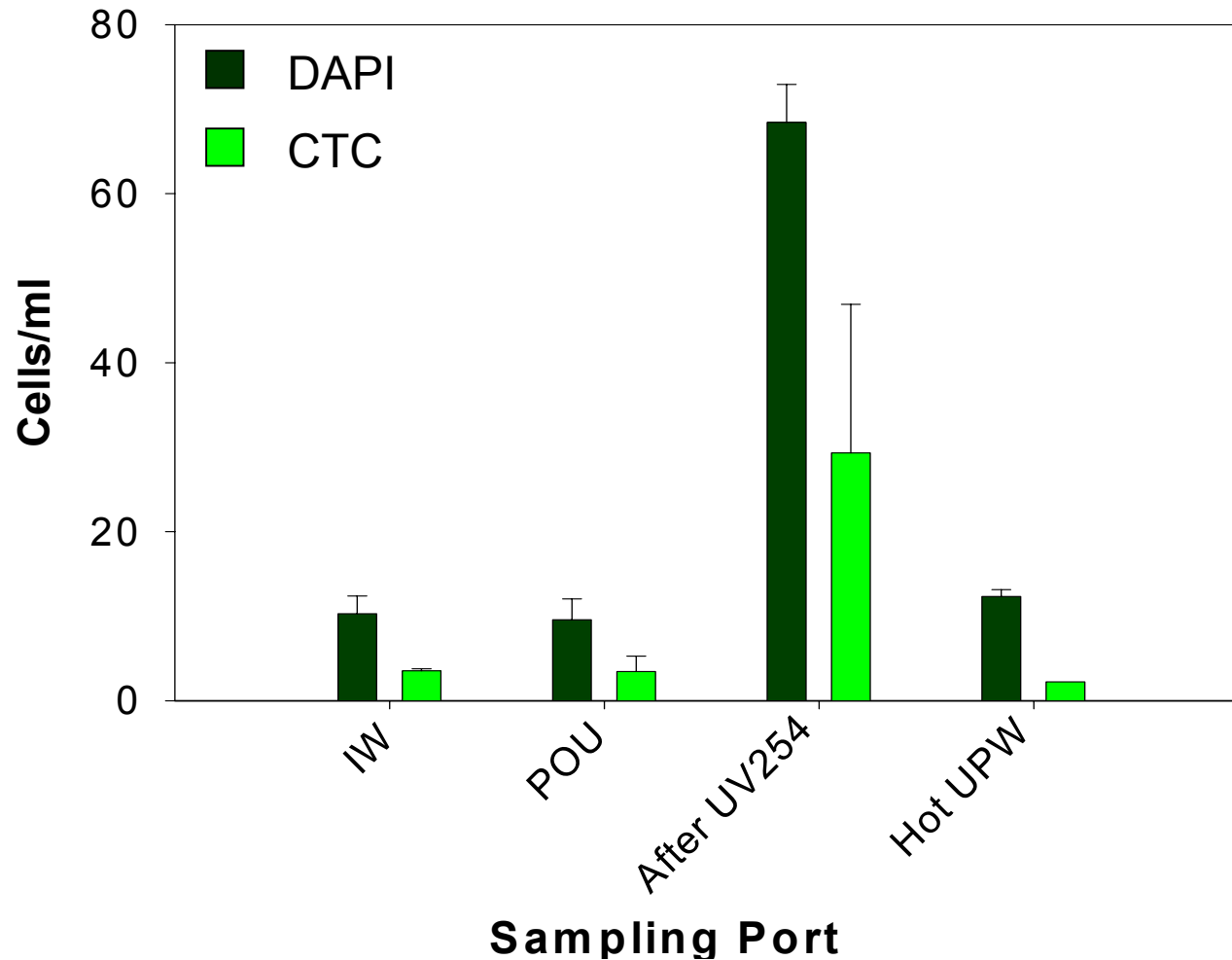


Enumeration of Bacteria in UPW

- Comparison of Plating and Direct Staining



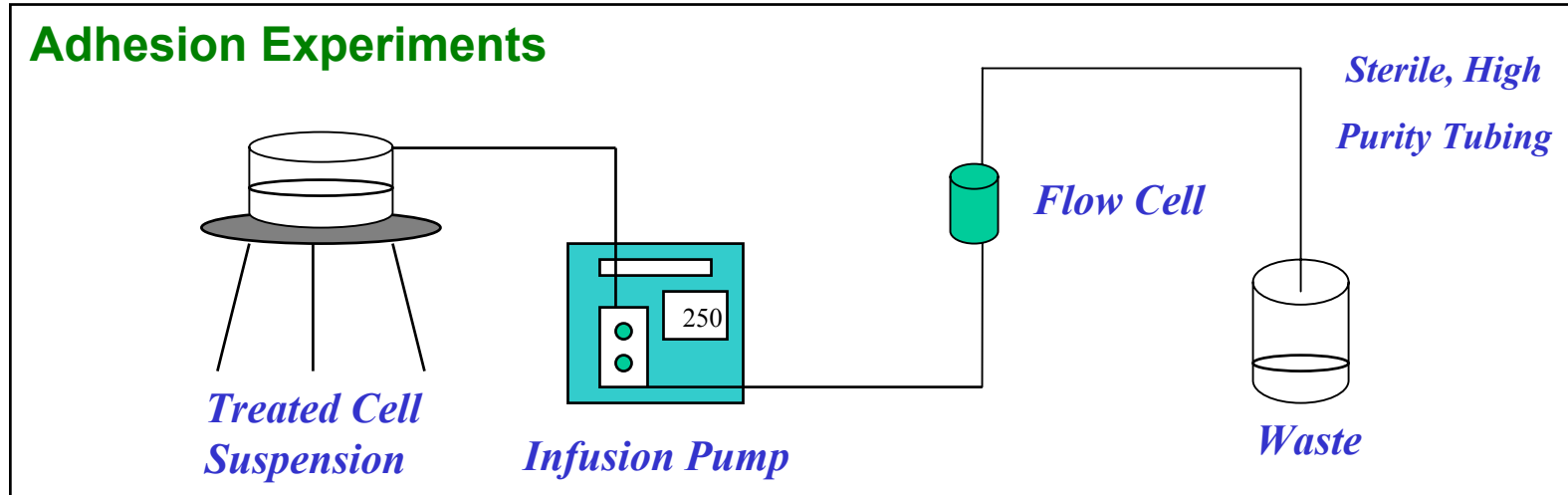
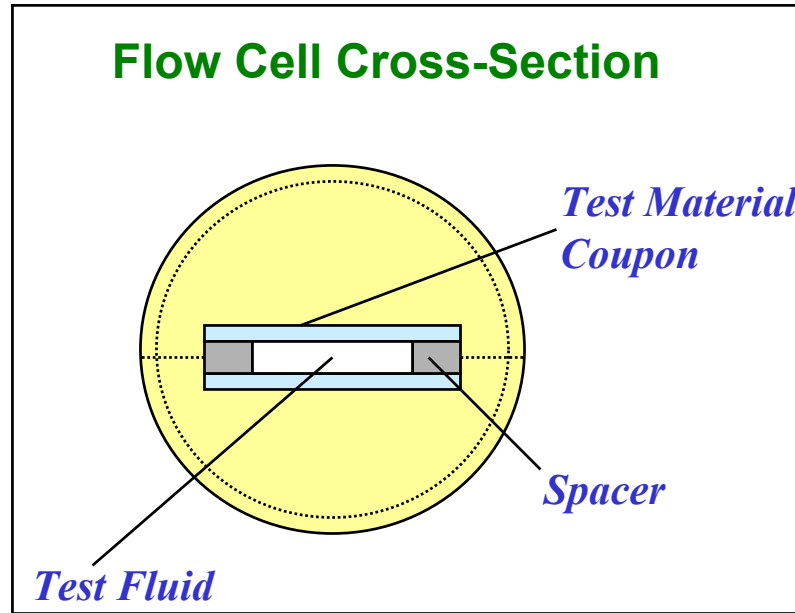
Enumeration of Bacterial Contamination – Industrial System 1



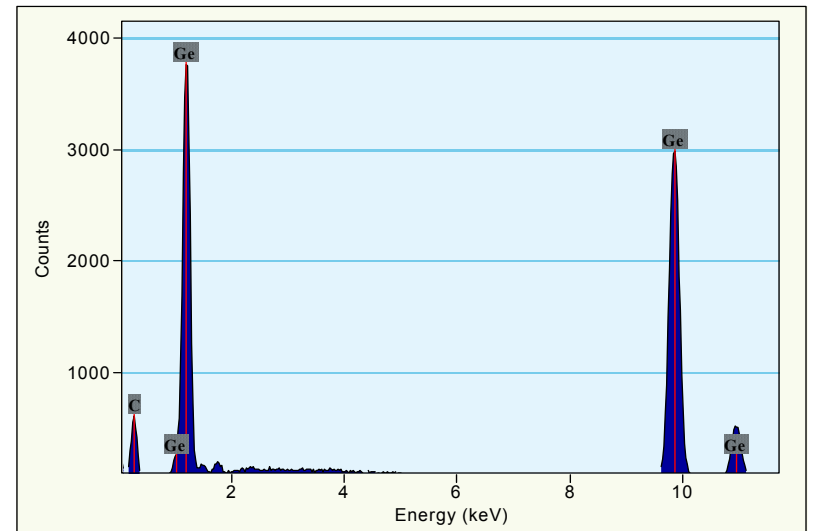
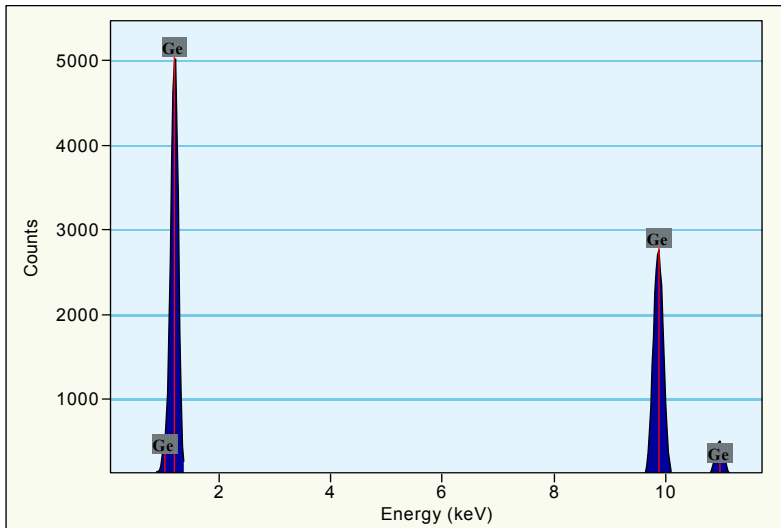
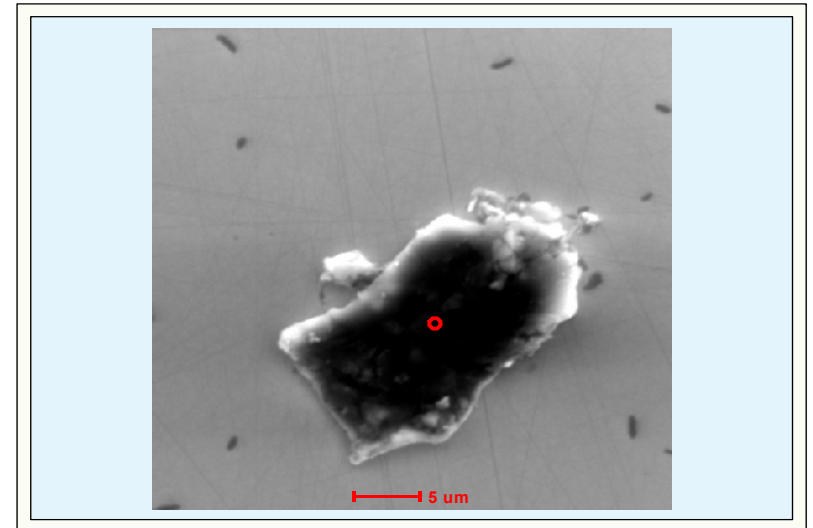
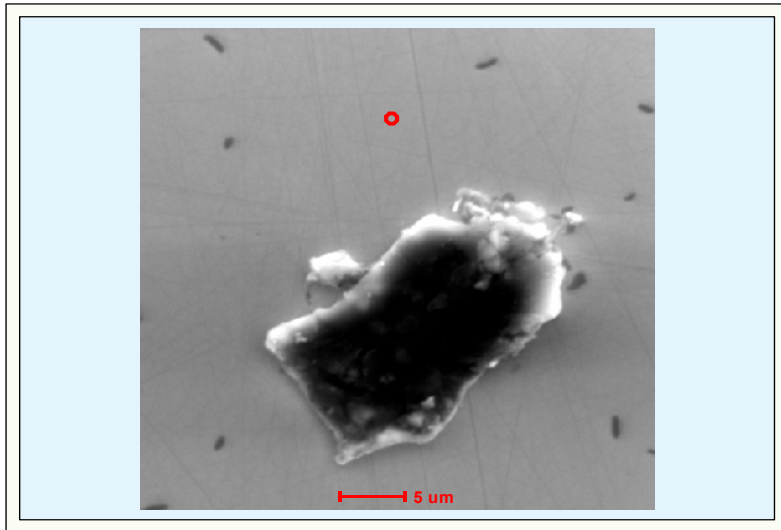
POU in Fabrication Facility Bacteria

- Etch Tanks
 - Mixing Chemicals
 - Background flow rate of water
- Sprayers
- Piping to tanks

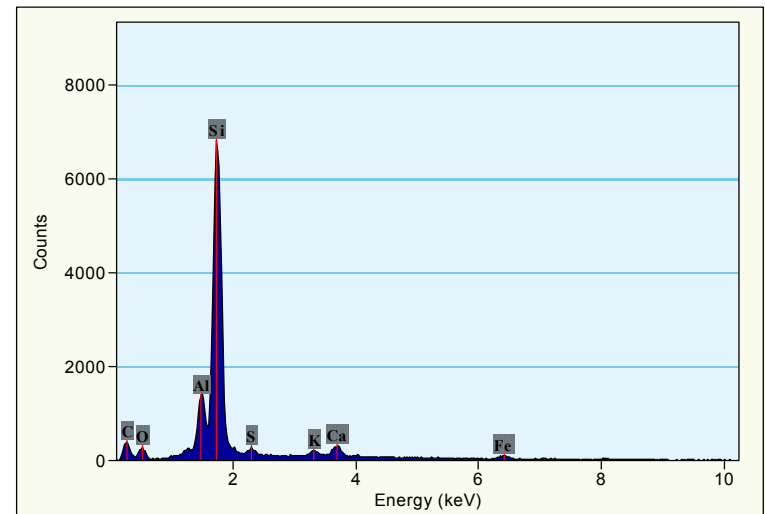
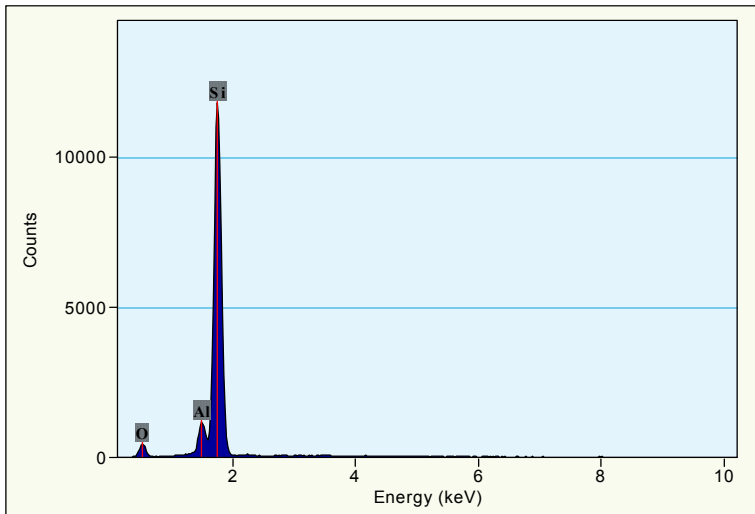
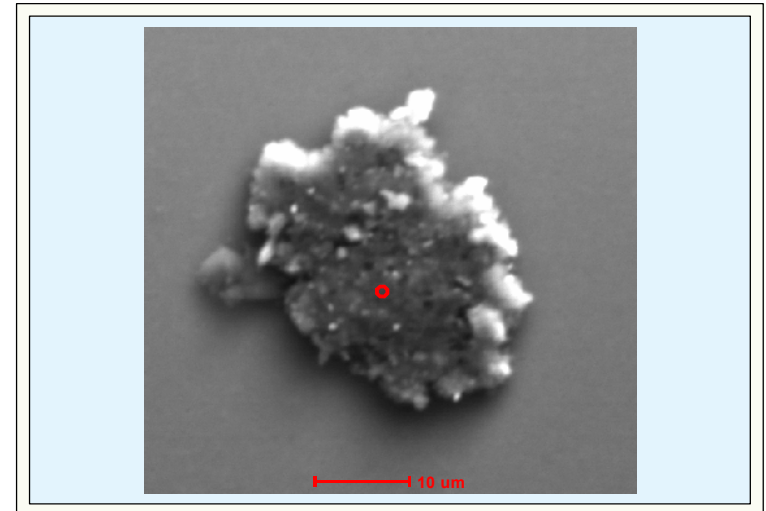
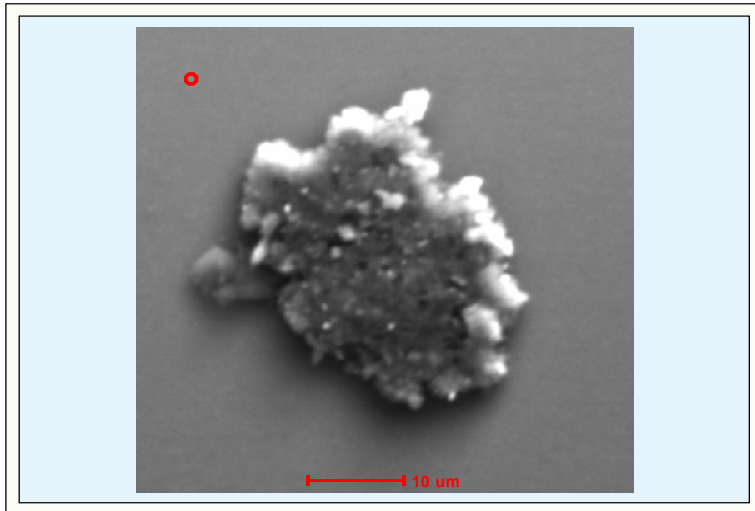
Flow Cell and Apparatus Design



Survival in Ge- Crystals



Survival on Al- Wafer Surfaces



Main Bacteria Found in UPW System

ISOLATED FROM MAKE-UP LOOP:

Mycobacterium
Flavobacterium
Alcaligenes
Acinetobacter
Burkholderia
Rhodobacter
Flavobacterium

Microbacterium
Arrthrobacter
Bacillus
Caulobacter
Pseudomonas
Aquaspirillum
Rhodococcus

Kocuria
Bradyrhizibium
Luteibacter
Deinococcus
Stenotrophomonas
Ideonella/Leptothix
Rhodopseudomonas

Sphingomonas
Xylena
Ribrivivax
Agromyces
Aeromicrobium
Xantomonas
Ralstonia

ISOLATED FROM POLISHING LOOP:

Pseudomonas
Burkholderia

Sphingomonas
Blastobacter

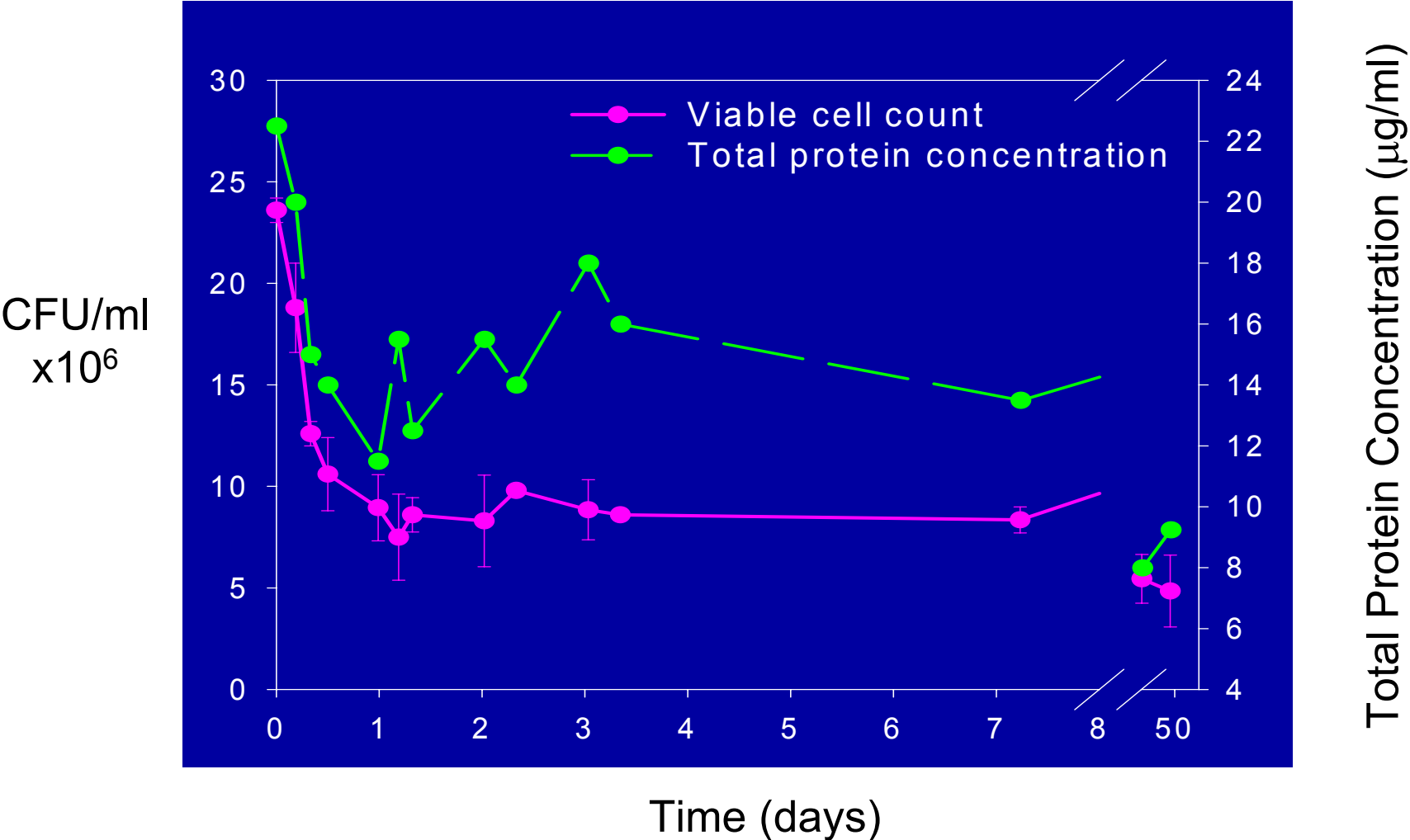
Bradyrhizibium
Flavobacterium

Microbacterium

Characterization of Key Strains

	<i>Ralstonia sp.</i>	<i>Bradyrhizobium sp.</i>
Physiology	Gram –ve rods	Gram –ve rods
Area of Isolation	After UV254	After 0.1µm filter
Growth under microaerophilic conditions	+	+
Growth substrates	33 out of 44 C-sources tested (mainly amino acids and carbohydrates)	29 out of 44 C-sources tested (mainly carbohydrates)

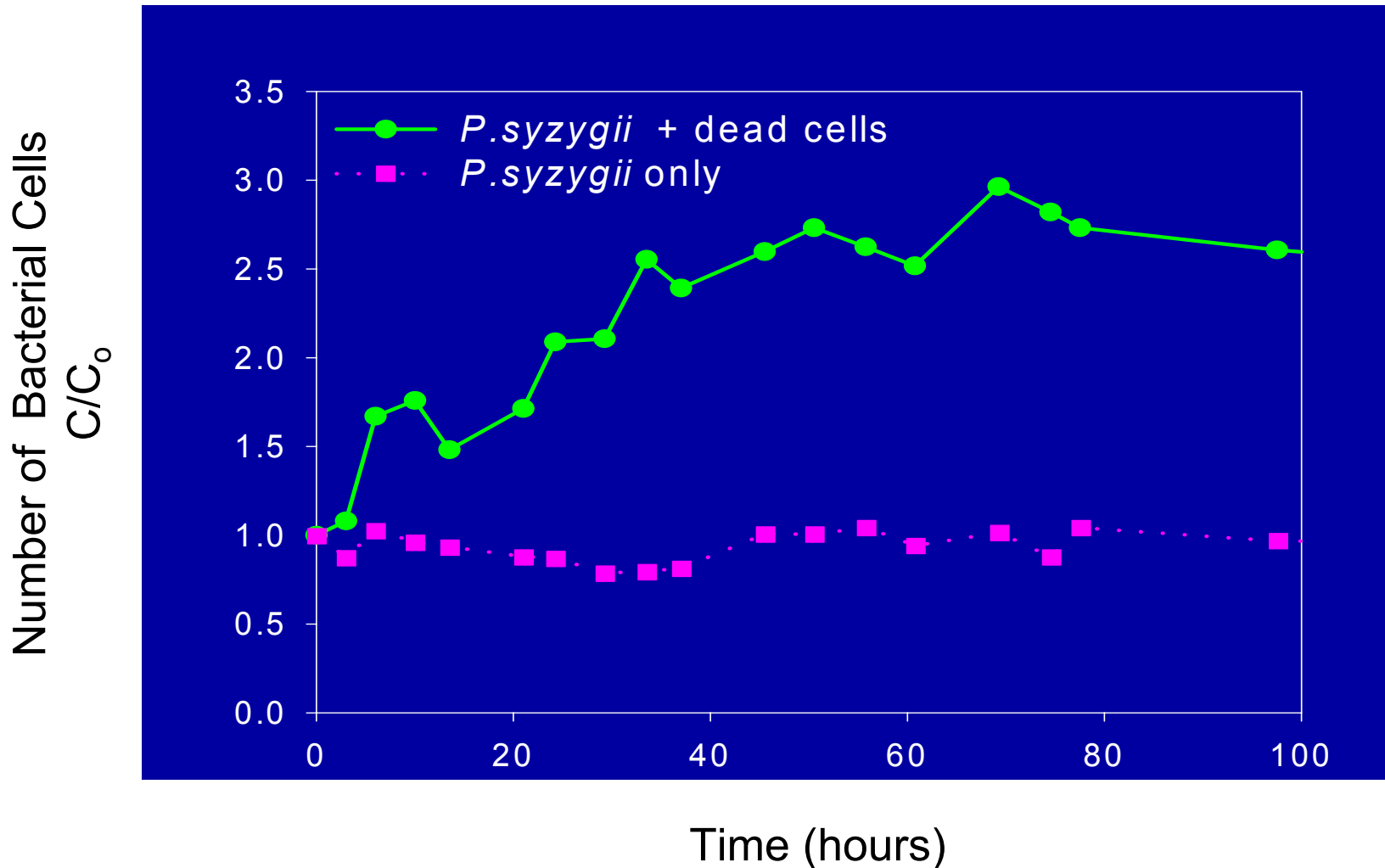
Survival of *Bradyrhizobium* sp. in UPW



Survival of Bacteria in UPW

- Decrease in cell size
- Stabilization of bacterial cell numbers
- Indefinite periods of survival following stabilization of numbers
- Fluctuations in cells numbers
 - Influence of cryptic growth?

Influence of Dead Bacterial Cells on UPW Quality - Polishing Loop

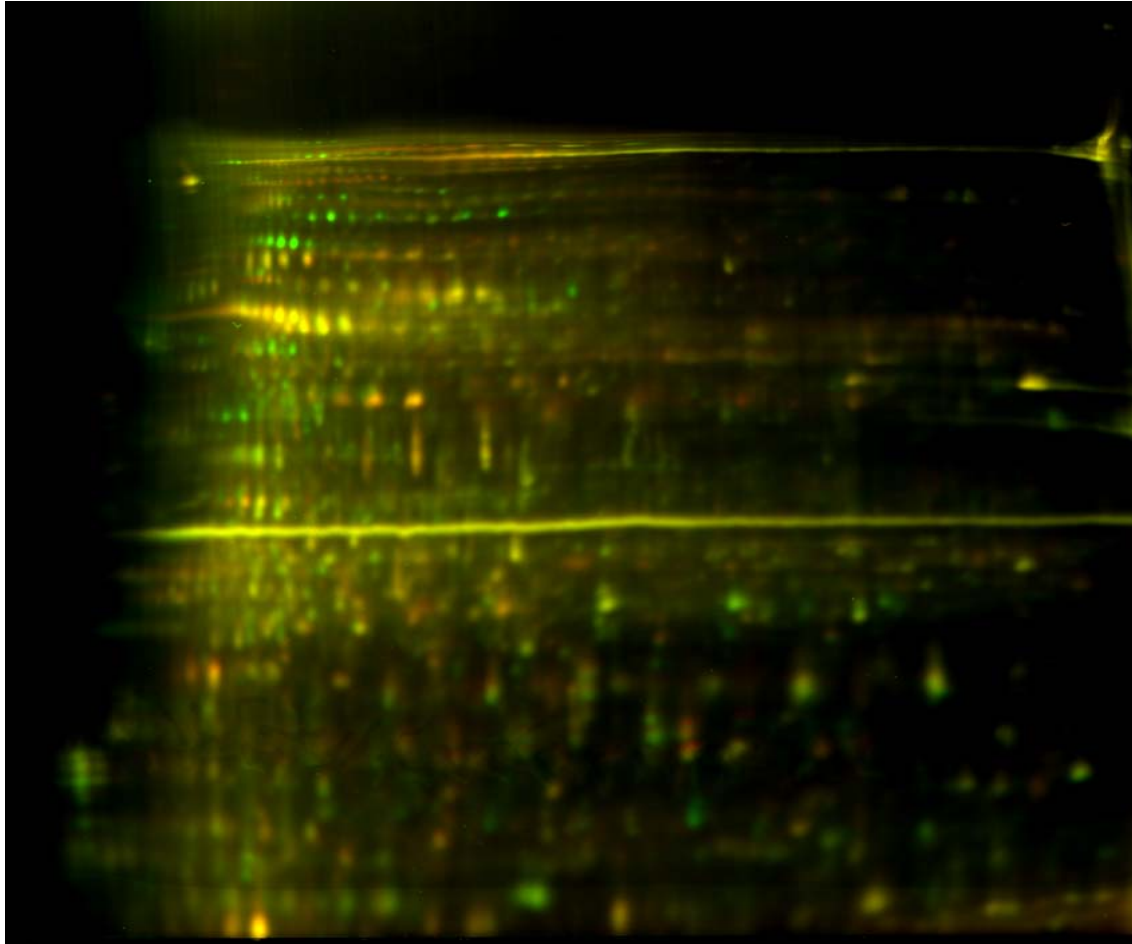


2-D Gel Protein Analysis

- Question to be answered
 - Determine if different proteins are expressed when grown in UPW and rich media

- Procedure
 - Grow bacteria in rich media for 1 week
 - Freeze half the cells for analysis (rich media sample)
 - Place other half in UPW for 2 months (UPW sample)
 - Send both samples in for analysis

Protein Analysis – Cells in UPW vs media

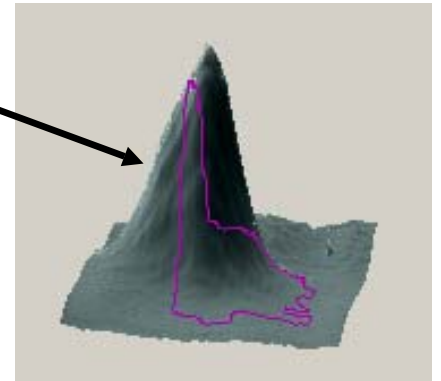
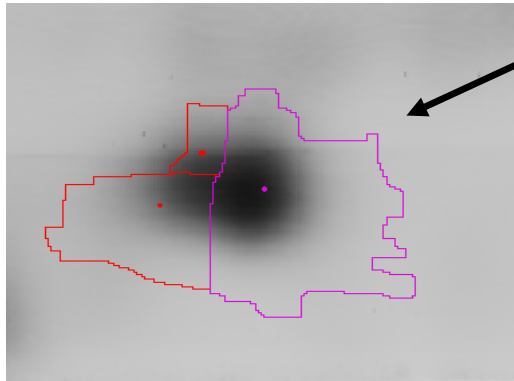
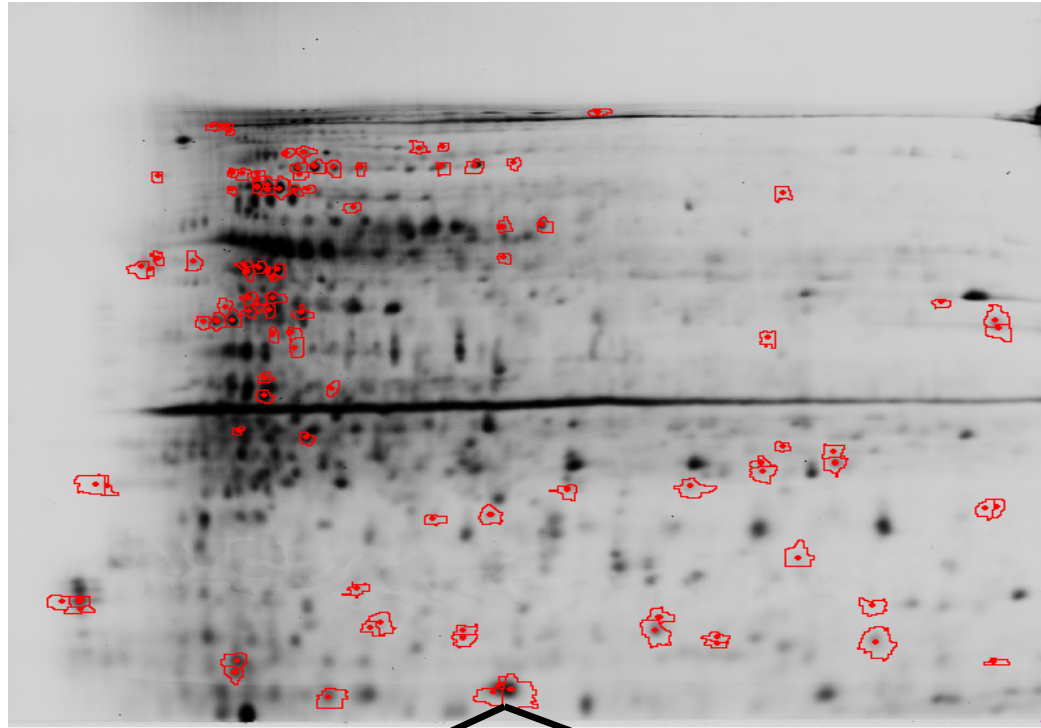


**Ultra Pure Water
sample labeled
with Cy3 in green**

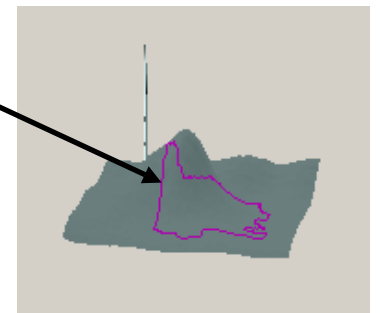
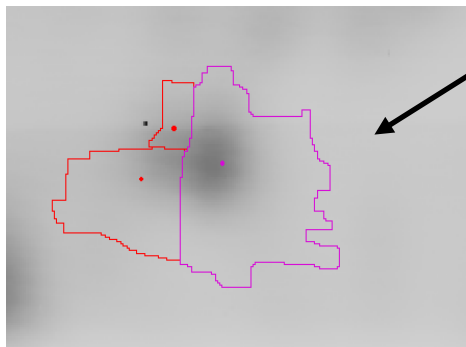
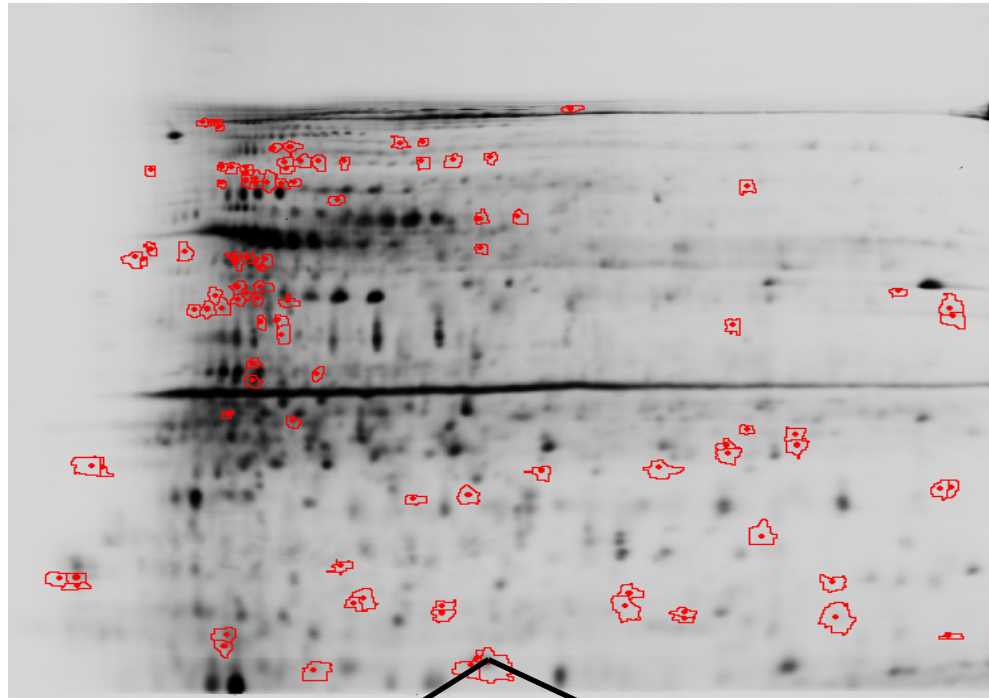
**Rich Media
sample labeled
with Cy5 in red**

**Yellow/ Orange
from overlapping
cy-dye
fluorescence,
indicating
proteins present
in both samples**

UPW



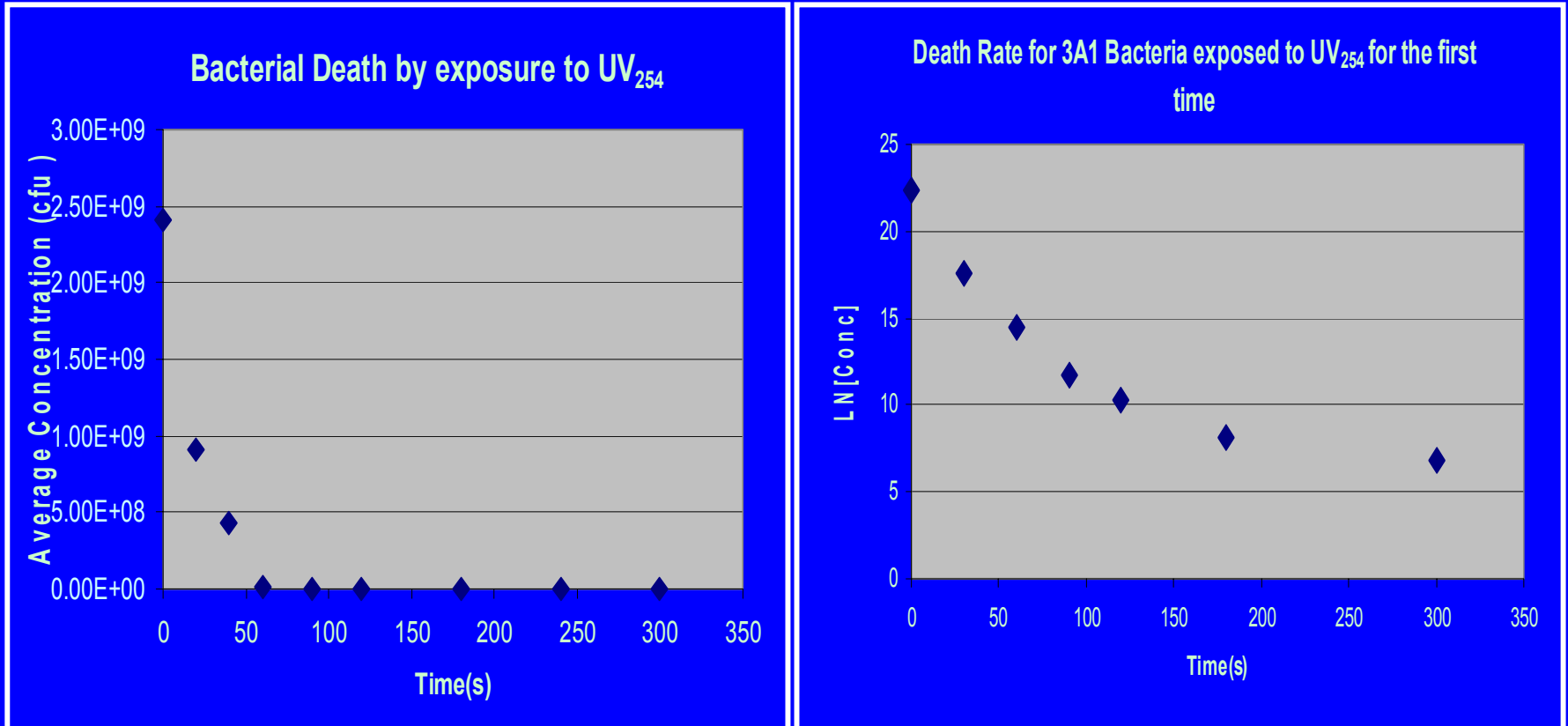
Rich Media



UV254 – The Issues

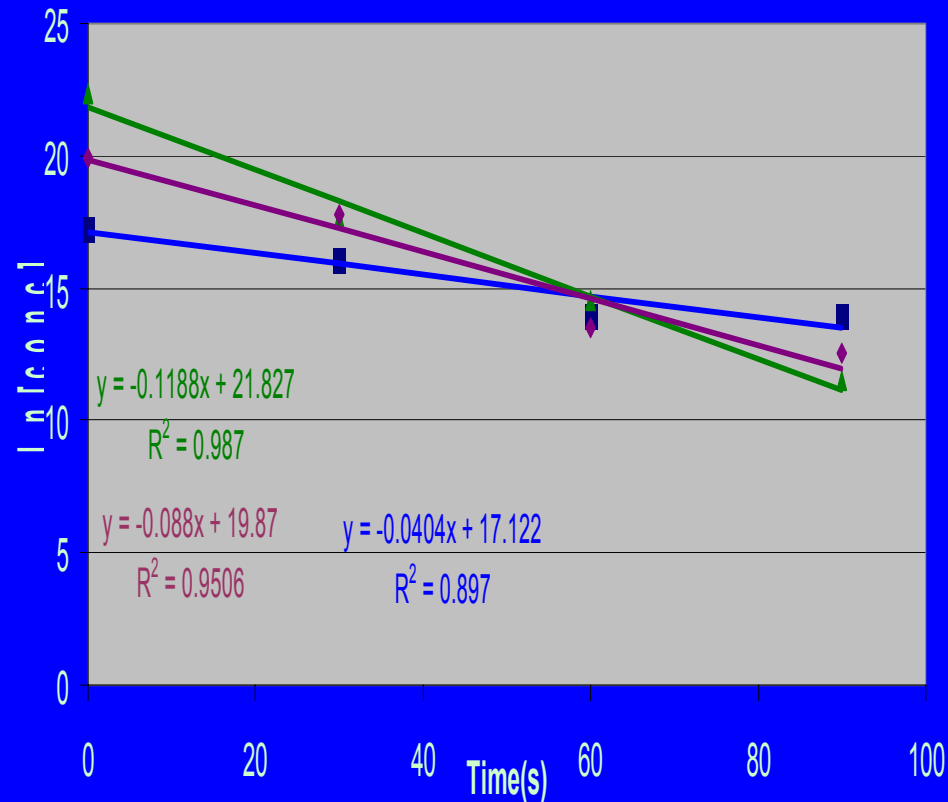
- UV254 used for bacterial control
 - Damages bacterial DNA
- UA Research indicates:
 - Area immediately proceeding UV254 very prone to biofouling → Cryptic growth prevalent?
 - Rate of cell death by UV irradiation is affected by the presence and nature of organics in water
 - Impenetrable to EPS/biofilm
 - Some strains are more adhesive after exposed to UV254

Experimental Results

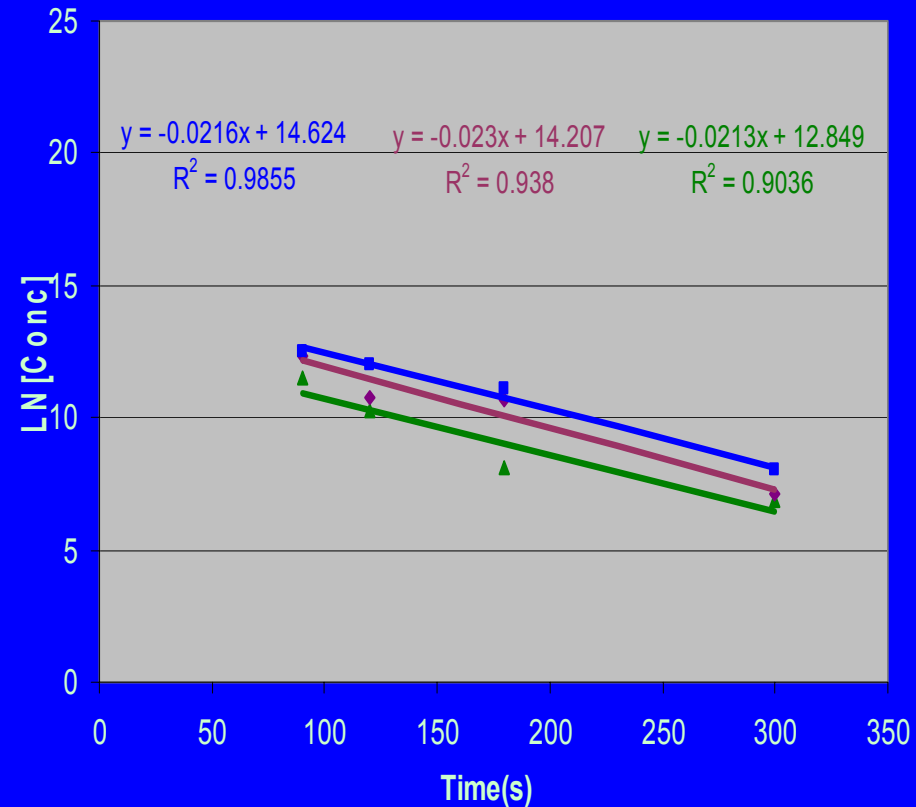


First-exposed Bacteria

LN[conc] vs Time for 3A1 Bacteria First Exposed to UV₂₅₄ for the First 90 Seconds



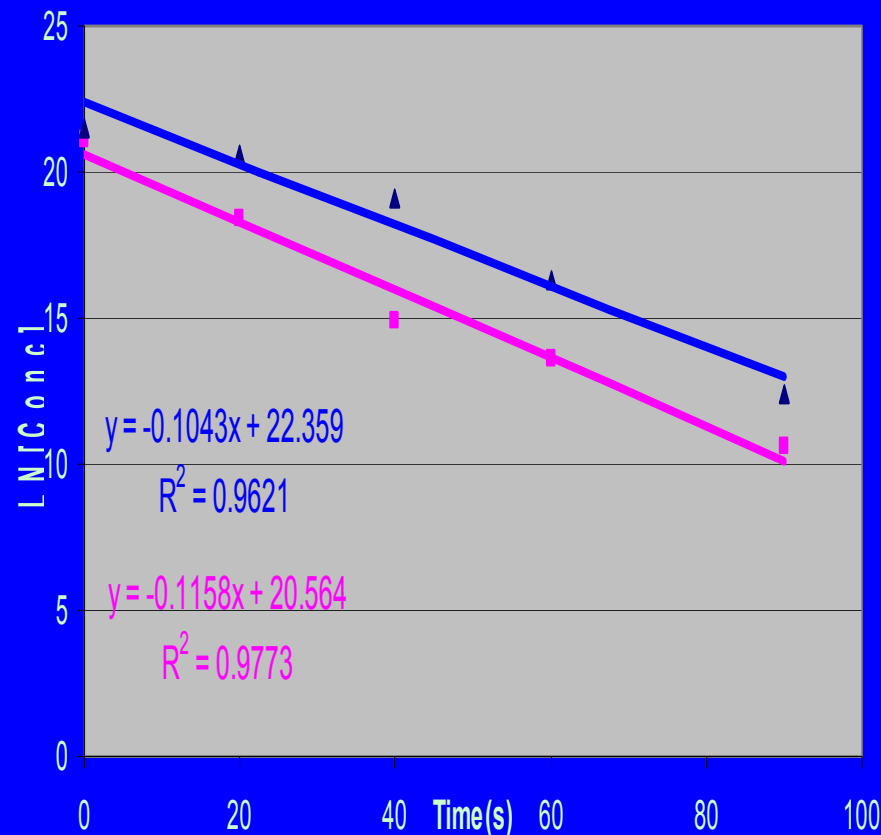
LN[conc] vs Time for 3A1 Bacteria First Exposed to UV₂₅₄ after 90 seconds



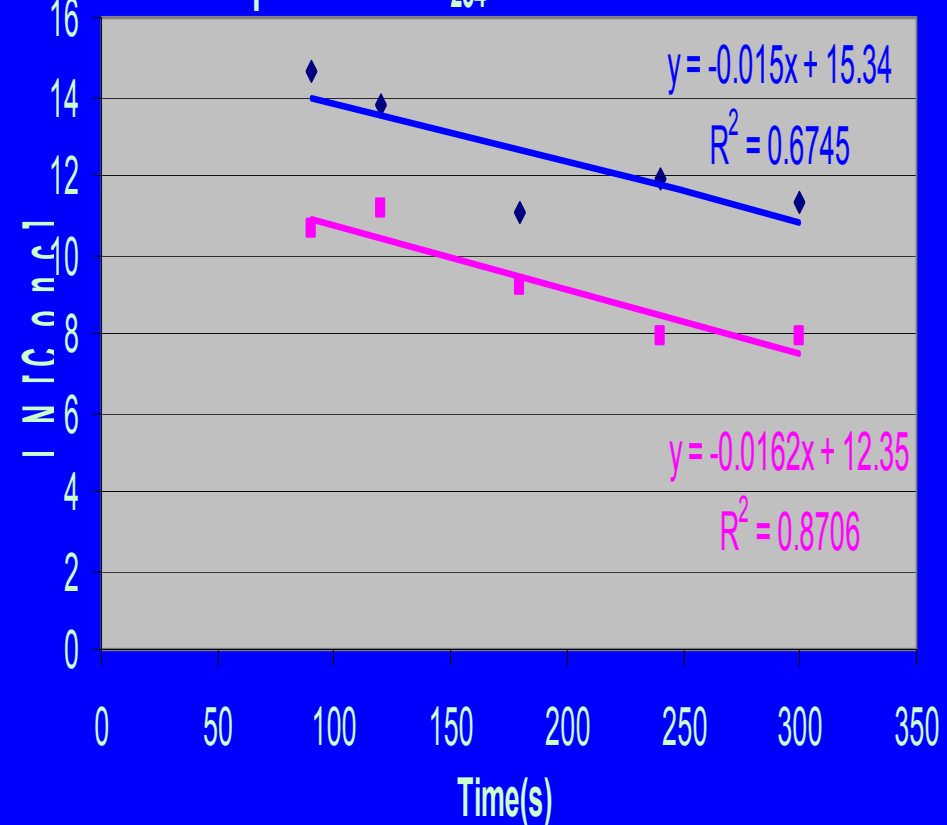
Concentration Dependence for first 90s: 10⁹ cfu (green), 10⁸ cfu(plum) , 10⁷ cfu(blue)

Previously Exposed Bacteria

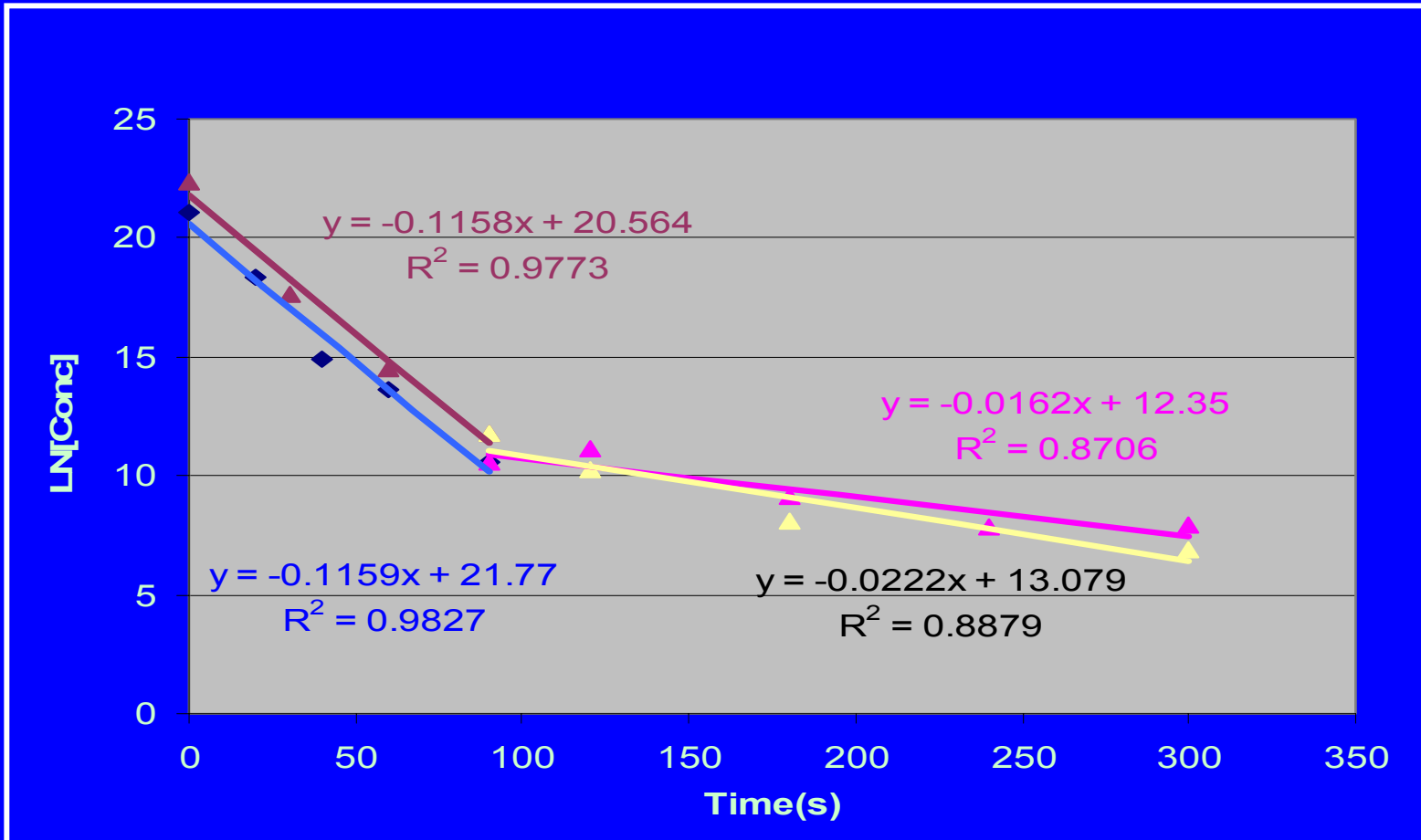
Ln[conc] vs Time for 3A1 Bacteria previously exposed to UV₂₅₄ for the first 90 seconds



Ln[conc] vs Time for 3A1 Bacteria previously exposed to UV₂₅₄ after 90 seconds



Relationship Between Previously and First Exposed Bacteria



First Exposure (Plum and yellow)

Second Exposure (Blue and Pink)

Results

Death Rate for First and Previously exposed 3A1 bacteria

	Time period(s)	Original Concentration	Death Rate	Standard Deviation
Exposed for the first time	0-90	10^9	0.098	0.03
		10^8	0.088	0.0002
		10^7	0.045	0.007
	90-300		0.020	0.004
Previously Exposed	0-90	10^9	0.10	0.01
	90-300		0.016	0.001

Future Approaches

- Mass spectrophotometry analysis on different spots in 2-D gels to determine type of protein
- Determine rate and mechanism of capsule formation
- Investigate the importance of the concentration as a parameter for death by UV light
- Continue to investigate cell adaptation to UV light