Detection, Characterization and Control of Bacteria in UPW Systems

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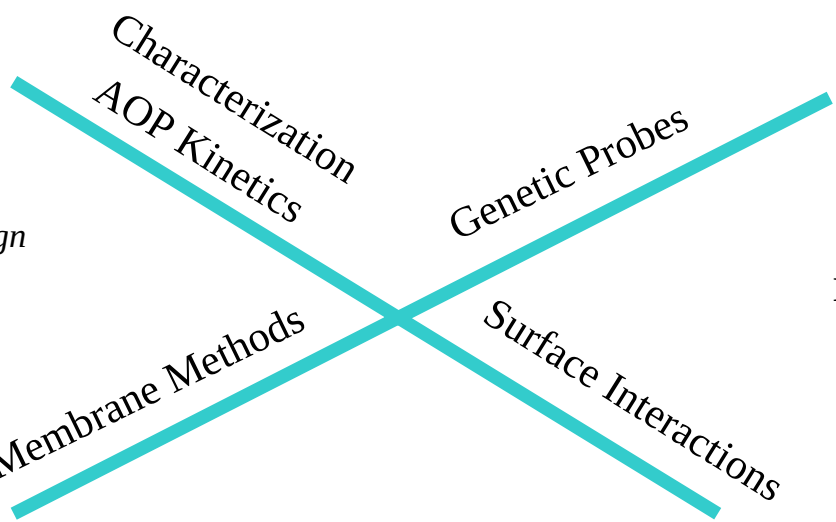
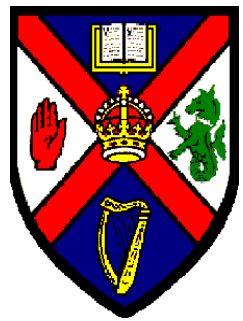
NSF/SRC *Engineering Research Center for Environmentally Benign Semiconductor Manufacturing*

NSF I/UCRC *Center for Microcontamination Control*





I/UCRC *Queen's University Environmental Science and Technology Research Centre*

NJIT
New Jersey Institute of Technology

NSF I/UCRC *Hazardous Substance Management Research Center*

University at Buffalo
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Center for Biosurfaces

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INNOVATION irtu INTO SUCCESS

Objectives

- Characterize Bacteria in UPW Systems
- Explore the interactions of biofilm bacteria, their metabolic products and growth substrates, and advanced oxidation reaction species
  - Develop a system for evaluating the adhesion characteristics of representative strains of bacteria within an UPW system
  - Examine the adhesion of bacteria when subjected to simple irradiation vs. AOP with and without the presence of potential energy sources
- Develop an efficient biofilm destruction approach
  - Formulate a biofilm control scheme with an emphasis on conditions for minimal adhesion

Biofilm Bacteria and Semiconductor Manufacturing

Oligotrophs

- Cells are often <0.2 μm in size
- May produce extracellular polysaccharides (EPS)
- Ability to scavenge a broad substrate range
- Capable of growth on <1mg carbon/liter
- Often the first bacteria to become established within biofilms

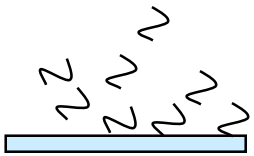
Advantages for Microorganisms in Biofilms

- Protection from shearing
- Constant flow of nutrients
- Genetic exchange between bacteria
- Increased resistance to bacteriocidal agents

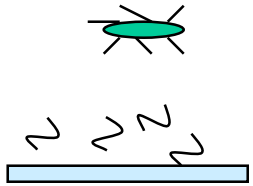
Current Industry Trends

- One bacterium is large enough to cause a short circuit given the current standard of 1 μm transistor linewidth.
- There is currently a general lack of knowledge regarding biofilm formation and behavior under ultrapure water and recycling conditions.

Bacterial Adhesion

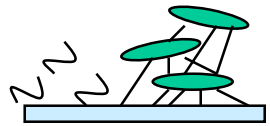


Conditioning

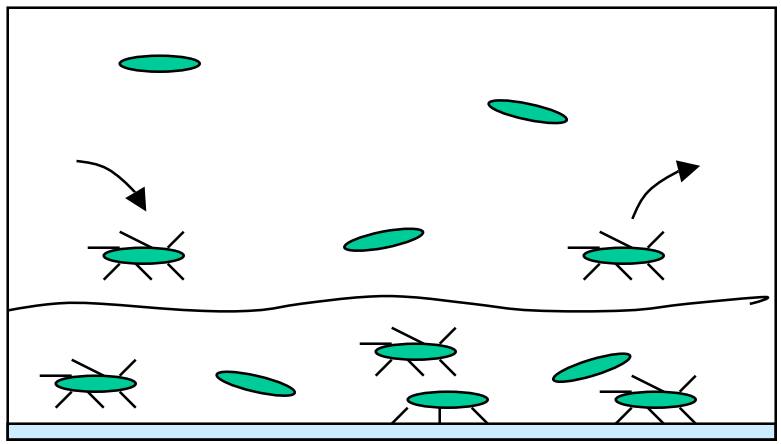


Transport
to Surface

Initial Adhesion



Irreversible
attachment



Mature Biofilm

Standard Biocidal Techniques

Ultrapure Water Production

Ultraviolet irradiation

- UV, 254 nm
- UV, 185 nm

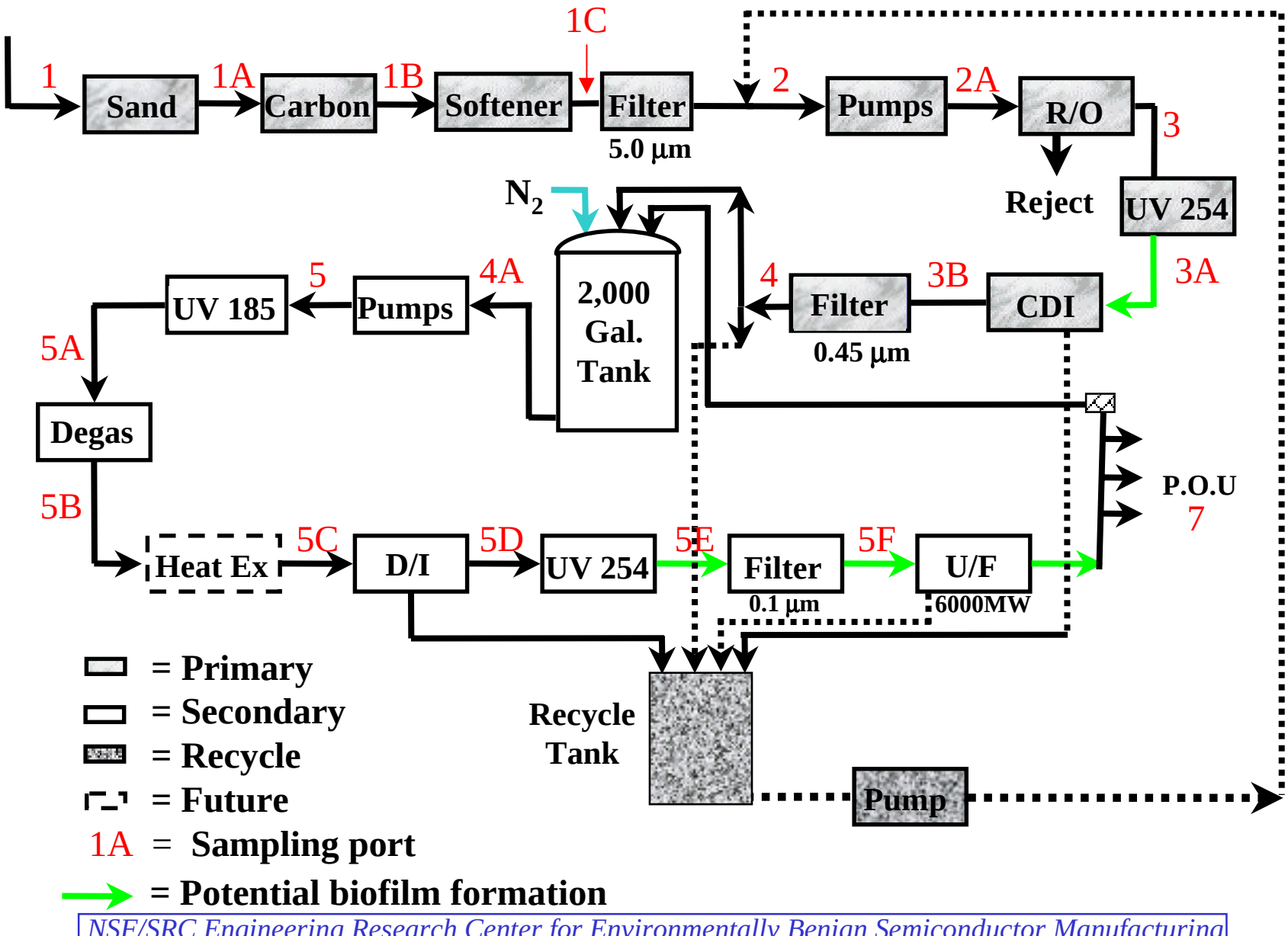
Chemical oxidation

- Chlorination
- Peroxide
- Ozone
- Proprietary chemical blends
- Advanced oxidation

Advanced Oxidation Processes

- Reactions that involve the generation of free radical intermediates, particularly •OH
- Capable of breaking down organic contaminants as far as CO_2 and H_2O
- Relatively non-invasive treatment: chemicals used decompose into “harmless” species
- Efficiency is often contaminant-specific and treatment environment-specific

Ultrapure Water Production



Isolation of Bacteria from UPWS

Incoming Water Strains

- 46 bacterial strains isolated
  (40 Gram –, 6 Gram +)
- Above strains generally not detected beyond port 3
- Growth on nutrient rich media
  (poor growth on OM media)

UPW Strains

- 58 bacterial strains isolated
  (54 Gram –, 4 Gram +)
- UPW bacteria may have adapted to a unique environmental niche
- Poor growth on nutrient rich media, positive growth on 1:10,000 R2A
- Potential heat-tolerant strains isolated (growth at ≤ 70°C)

Basic Characterization of Main Nuisance Bacteria

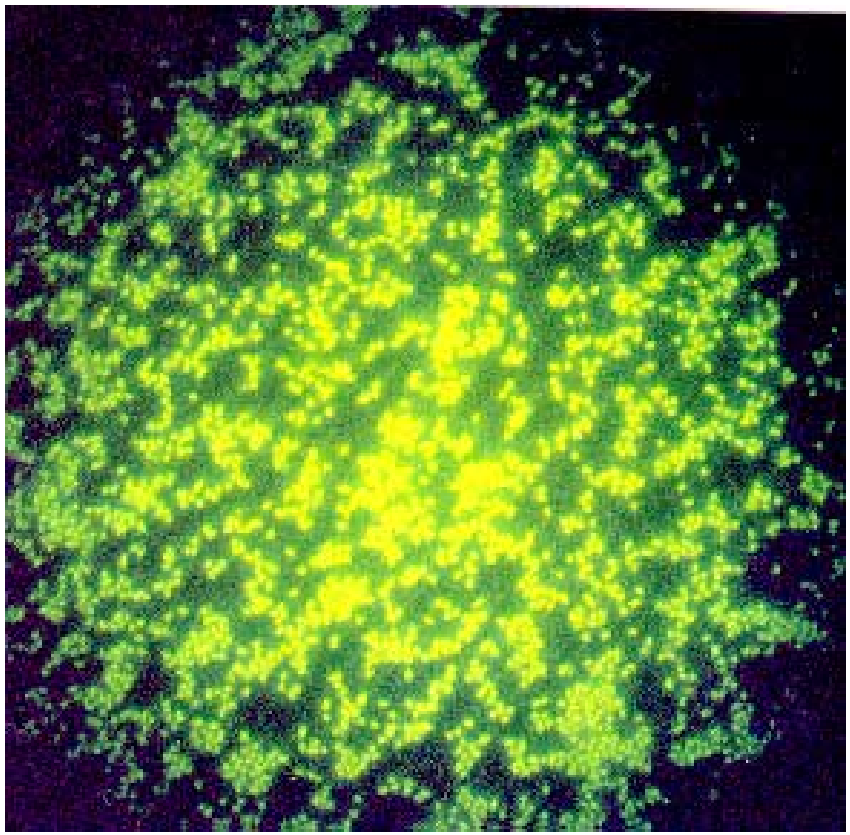
	MF254A	5E	5F3
Area of Isolation	After UV_{254}	After 2nd UV_{254}	After 0.1μm filter
Temperature range	25-42°C (37°C)	25-50°C (37°C)	25-37°C (30°C)
pH range	5.0-9.0 (6.5)	5.0-9.0 (6.0)	5.0-9.0 (7.0)
Growth under anaerobic conditions	+	+	+
Identification (16s rRNA Sequencing)	*Pseudomonas syzygii*	*Pseudomonas syzygii*	*Bradyrhizobium* sp.
% Homology	99%	98%	100%

Characteristics of Nuisance Bacteria

- Plate counts require incubation for one month at 25°C
  - 5x fold increase after one month compared to two weeks
- Many microorganisms are less than 0.2µm in size
- Viable bacteria represent approximately 10-28% of the total bacterial population

- MF254A / 5E - Growth on 33 out of 44 carbon sources tested (mainly amino acids and carbohydrates)
- 5F3 - Growth on 29 out of 44 carbon sources tested (mainly carbohydrates)
- Broad substrate range typical of oligotrophic bacteria
- Currently examining humic acid/ethanol utilization

FISH Probes



- Rapid Detection of Bacteria
- Generic Probes
- Species-specific Probes

Relevance to Industrial Systems

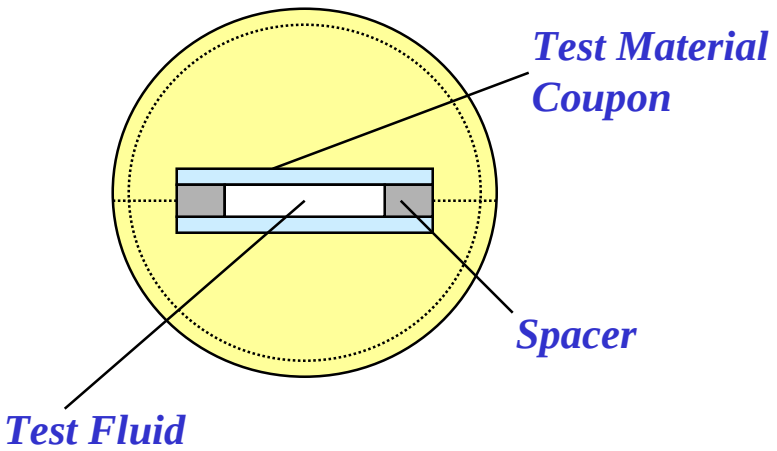
- *Psuedomonas syzygii*
  - Isolated after UV254
  - Found throughout our UPW system
  - Found in samples obtained from industrial partner system
    - No continuous ozonation
    - Confirmed via PCR
- *Bradyrhizobium* sp.
  - Isolate after 0.1 μm filter
  - Not yet detected in industrial partner systems

Materials and Methods

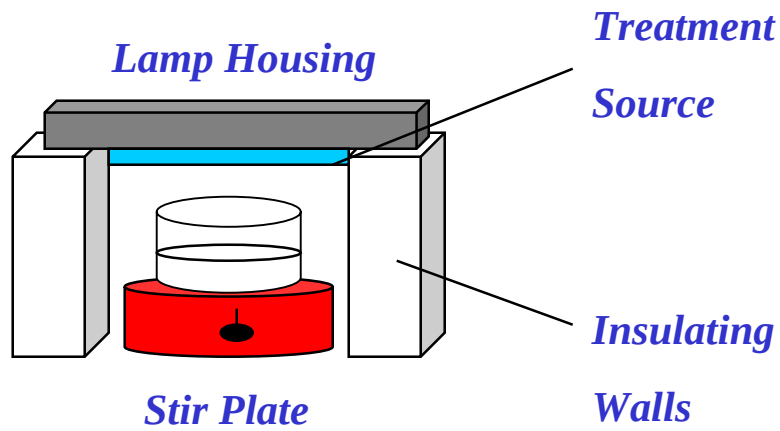
- **Isolation of representative biofilm-forming strains**
  - "MF254A": *Pseudomonas syzyggi* – rod-shaped, Gram (-) oligotroph
- **Foundation of approach - adhesion characterization**
  - Flow cell apparatus
    - Parallel plate design (Center for Biosurfaces, SUNY at Buffalo)
  - Water source
    - UPW Test-bed (UA)
  - Other environments
    - R2A medium
    - Humic acid
    - Buffer (phosphate, nitrite)
    - Treated humic acid
- **Treatment schemes**
  - Control: no treatment
  - AOP: UV_{185} lamp
  - Germicidal irradiation: UV_{254} lamp

Flow Cell and Apparatus Design

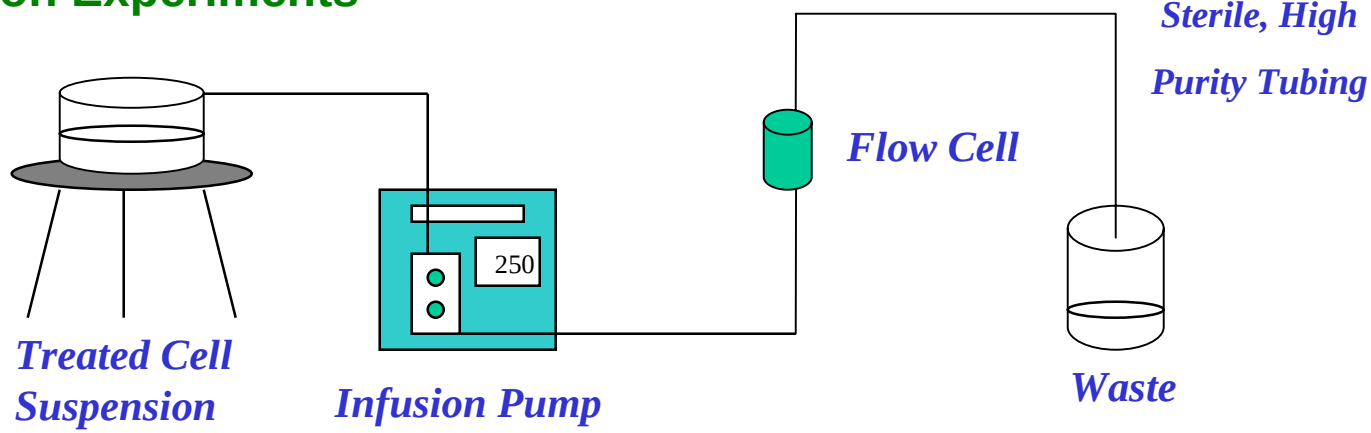
Flow Cell Cross-Section



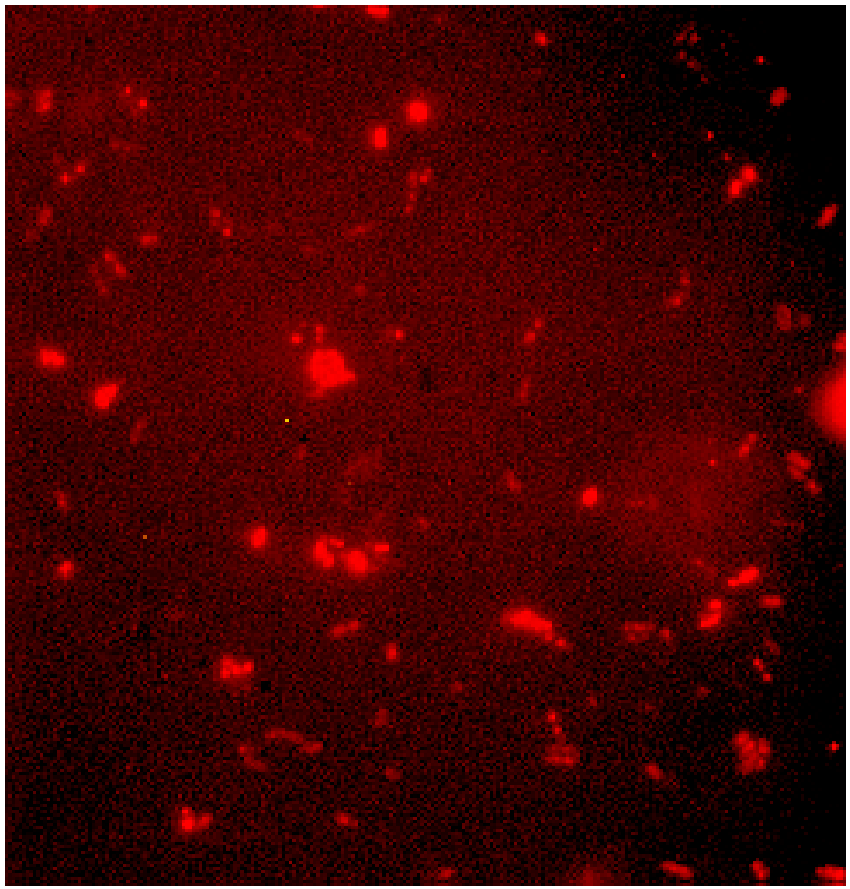
Cell Death Treatment



Adhesion Experiments

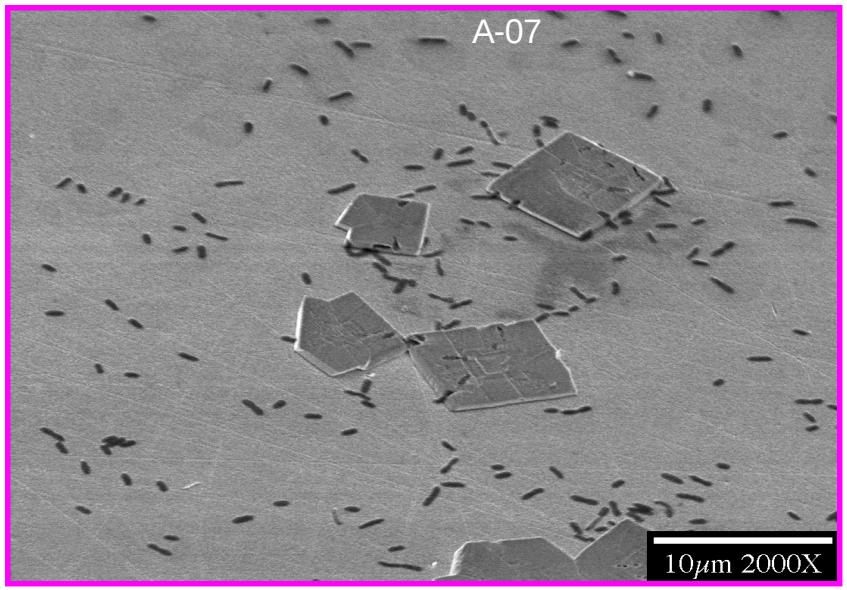


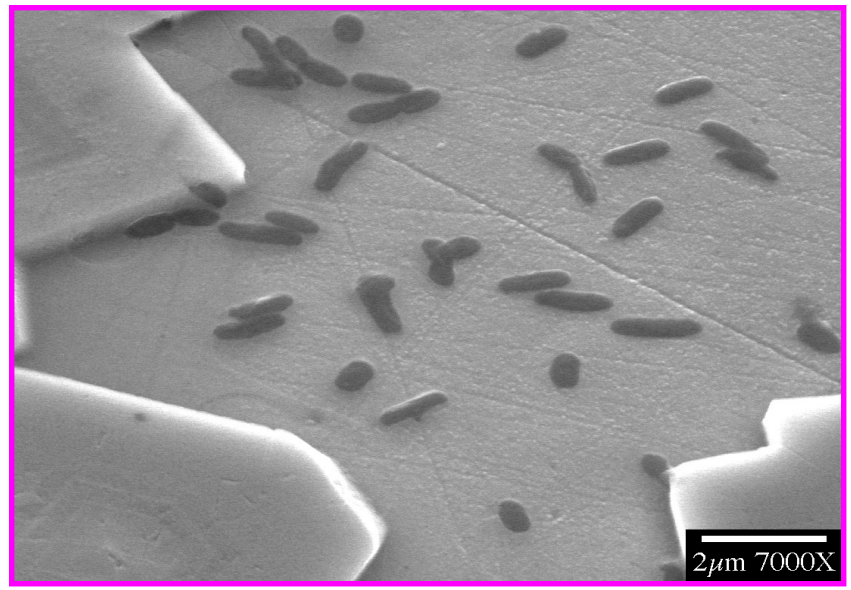
Bacterial Adhesion Analysis



- Epifluorescent Microscopy
- CTC – viable cells
- DAPI – total cells

MF254A Strain attached to Germanium IRE

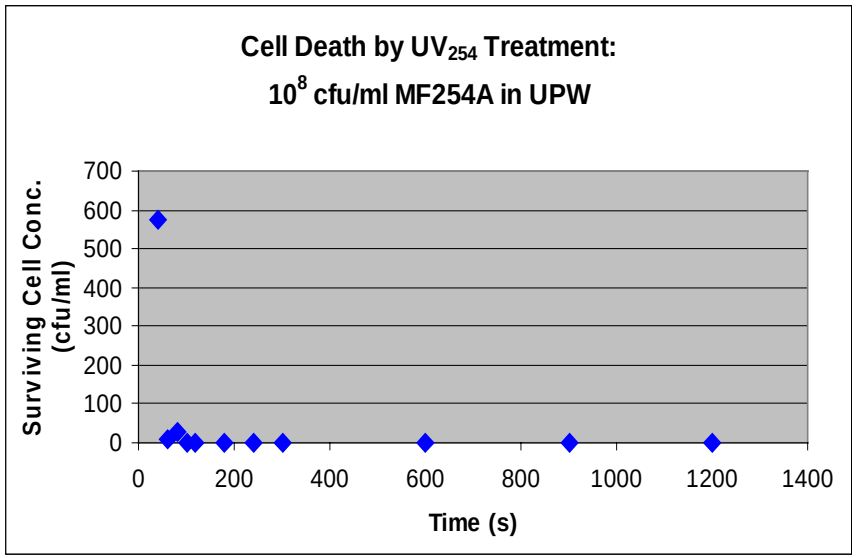


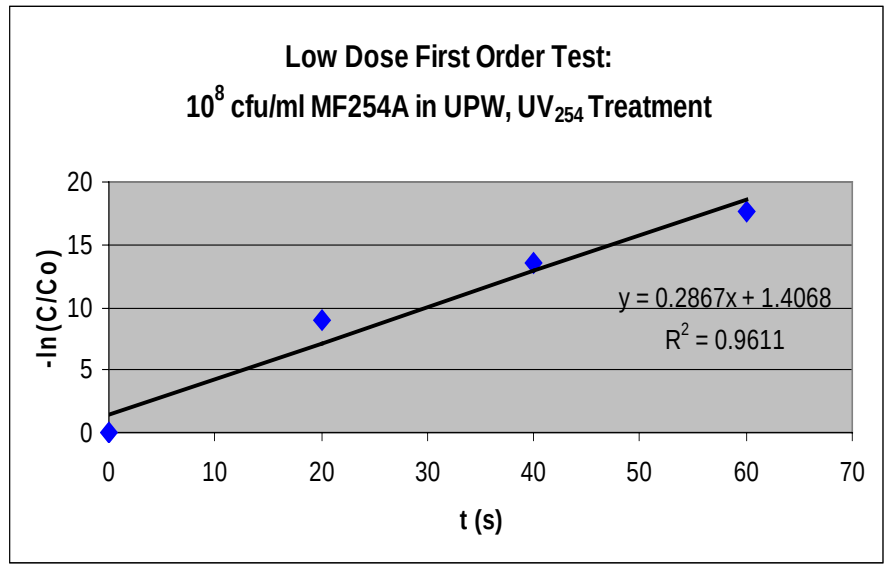


Analysis performed by Center for Biosurfaces, SUNY at Buffalo

- *Large ring-bound –OH fraction at bacteria surface (associated with strong bioadhesive characteristics)*
- *GeO_2/$Ge(OH)_x$ corrosion with attached microbes in a matrix*
- *Secondary attachment of additional microbes to the already-present oxide/hydroxide-microbe matrix*

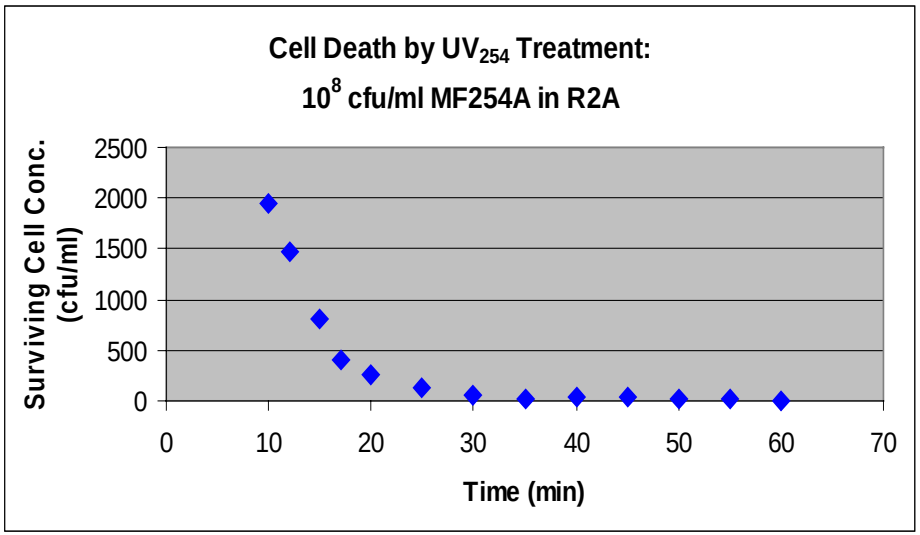
Cell Death Kinetics:
MF254A in UPW

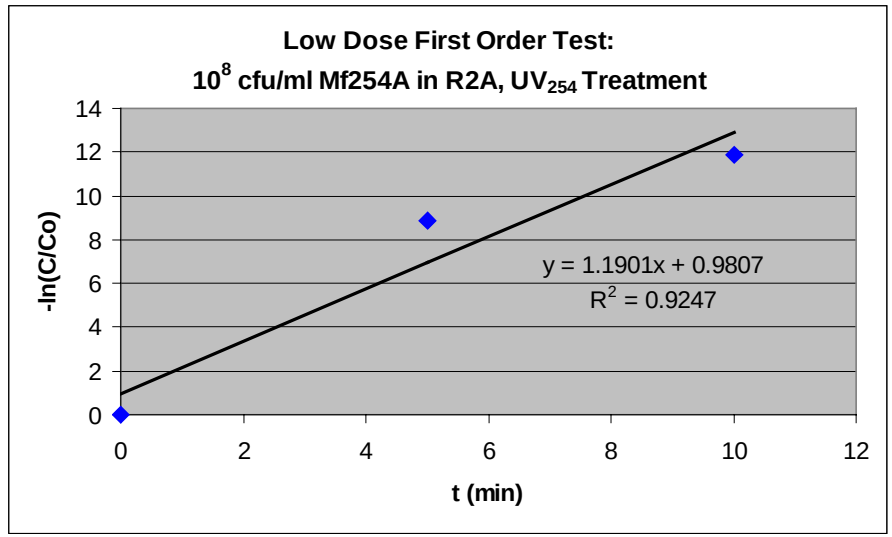




- UV_{254} Treatment: $C = C_0 e^{-k_d t}$, $k_d = 0.3\ s^{-1}$
- UV_{185} Treatment: $C = C_0 e^{-k_d t}$, $k_d = 0.2\ s^{-1}$
- 100 % cell death within 2 min of exposure

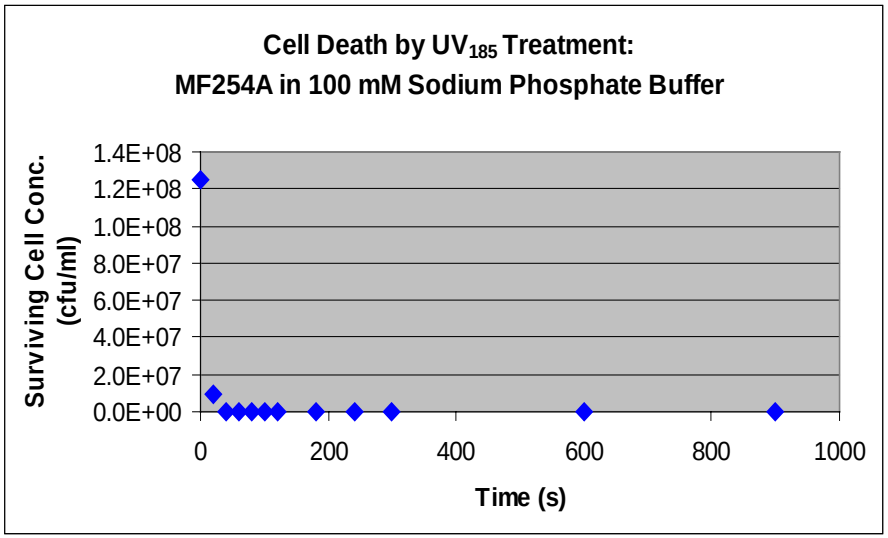
Cell Death Kinetics:
MF254A in R2A Medium

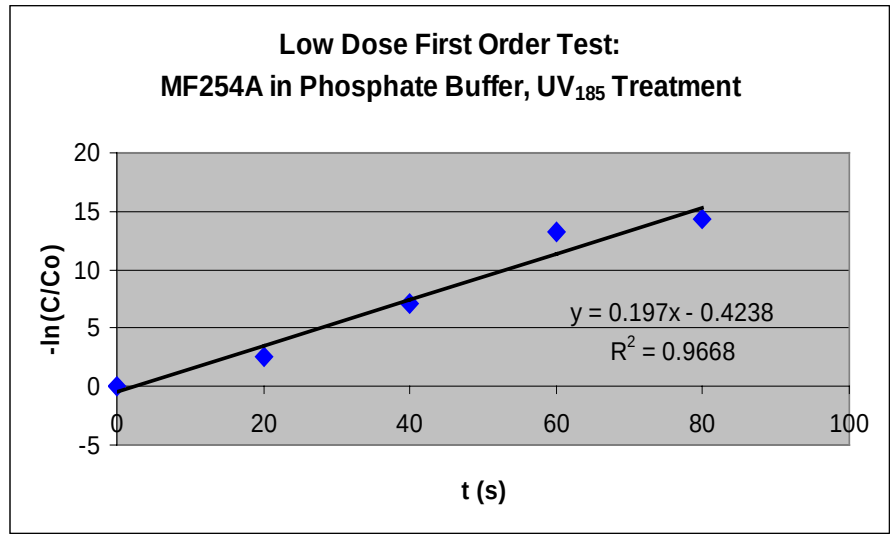




- UV_{254} Treatment: $C = C_0 e^{-k_d t}$, $k_d = 1.2\ s^{-1}$
- UV_{185} Treatment: $C = C_0 e^{-k_d t}$, $k_d = 0.6\ s^{-1}$
- Much longer doses needed for cell death compared to UPW

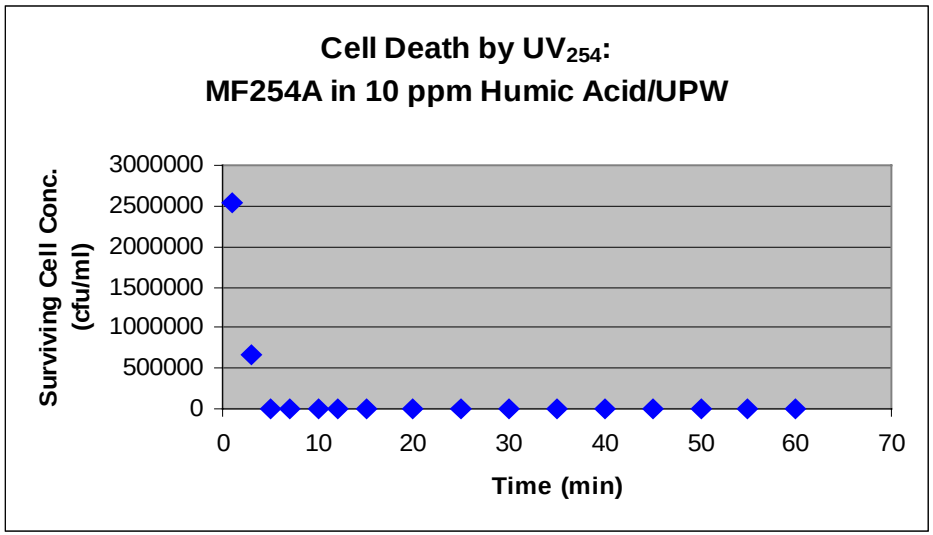
Cell Death Kinetics:
MF254A in 100 mM Sodium Phosphate Buffer

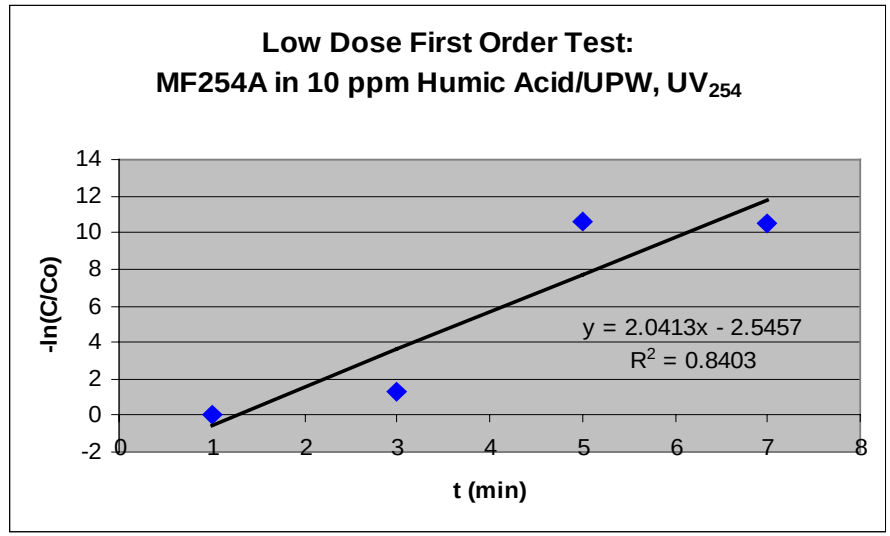




- UV_{254} Treatment: $C = C_0 e^{-k_d t}$, $k_d = 0.4\ s^{-1}$
- UV_{185} Treatment: $C = C_0 e^{-k_d t}$, $k_d = 0.2\ s^{-1}$
- Required dose for cell death similar to UPW

Cell Death Kinetics:
MF254A in 10 ppm Humic Acid/UPW

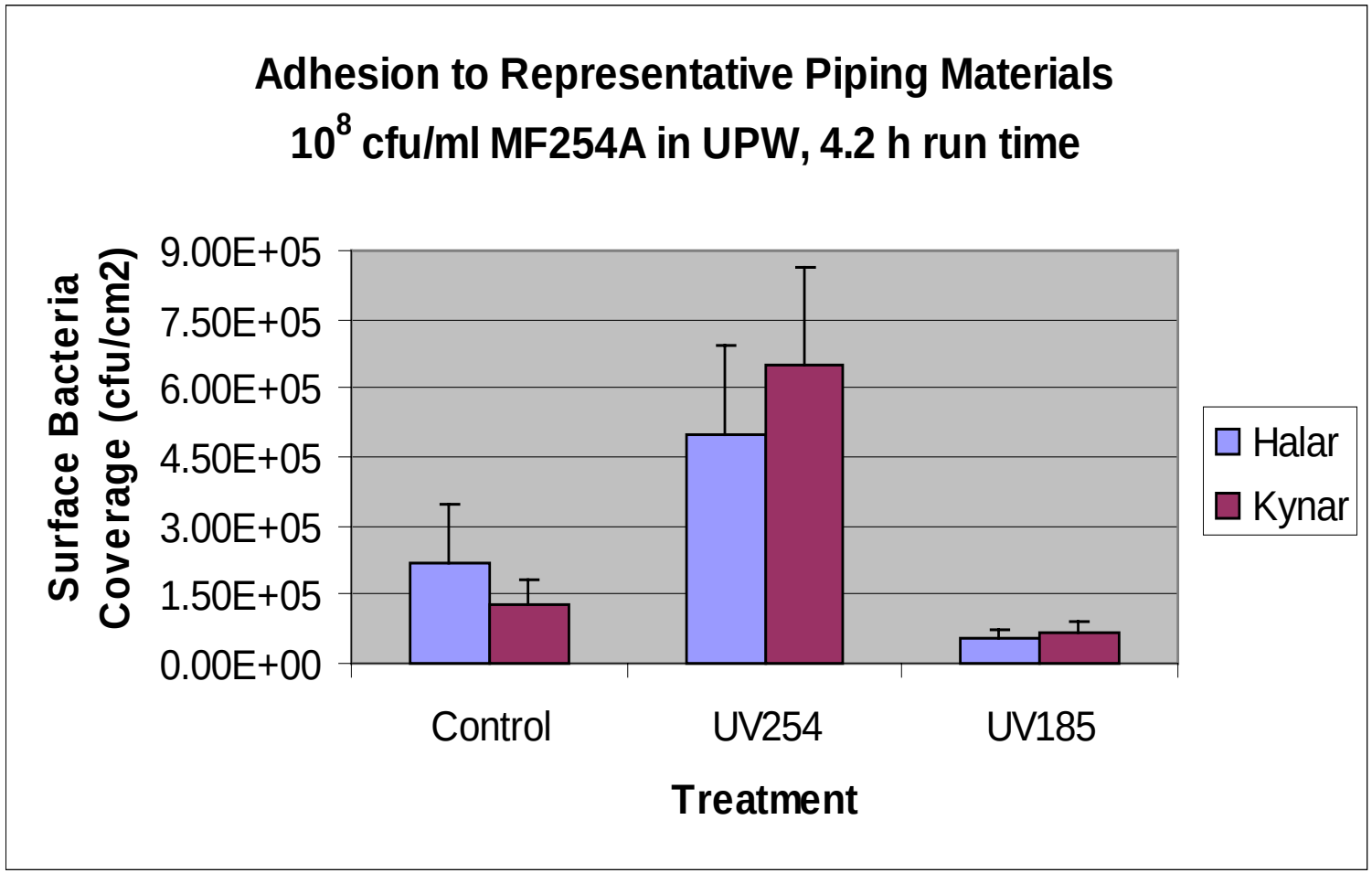




- UV_{254} Treatment: $C = C_0 e^{-k_d t}$, $k_d = 2.0\ s^{-1}$
- UV_{185} Treatment: $C = C_0 e^{-k_d t}$, $k_d = 4.6\ s^{-1}$
- Longest tailing section of any treatment environment tested

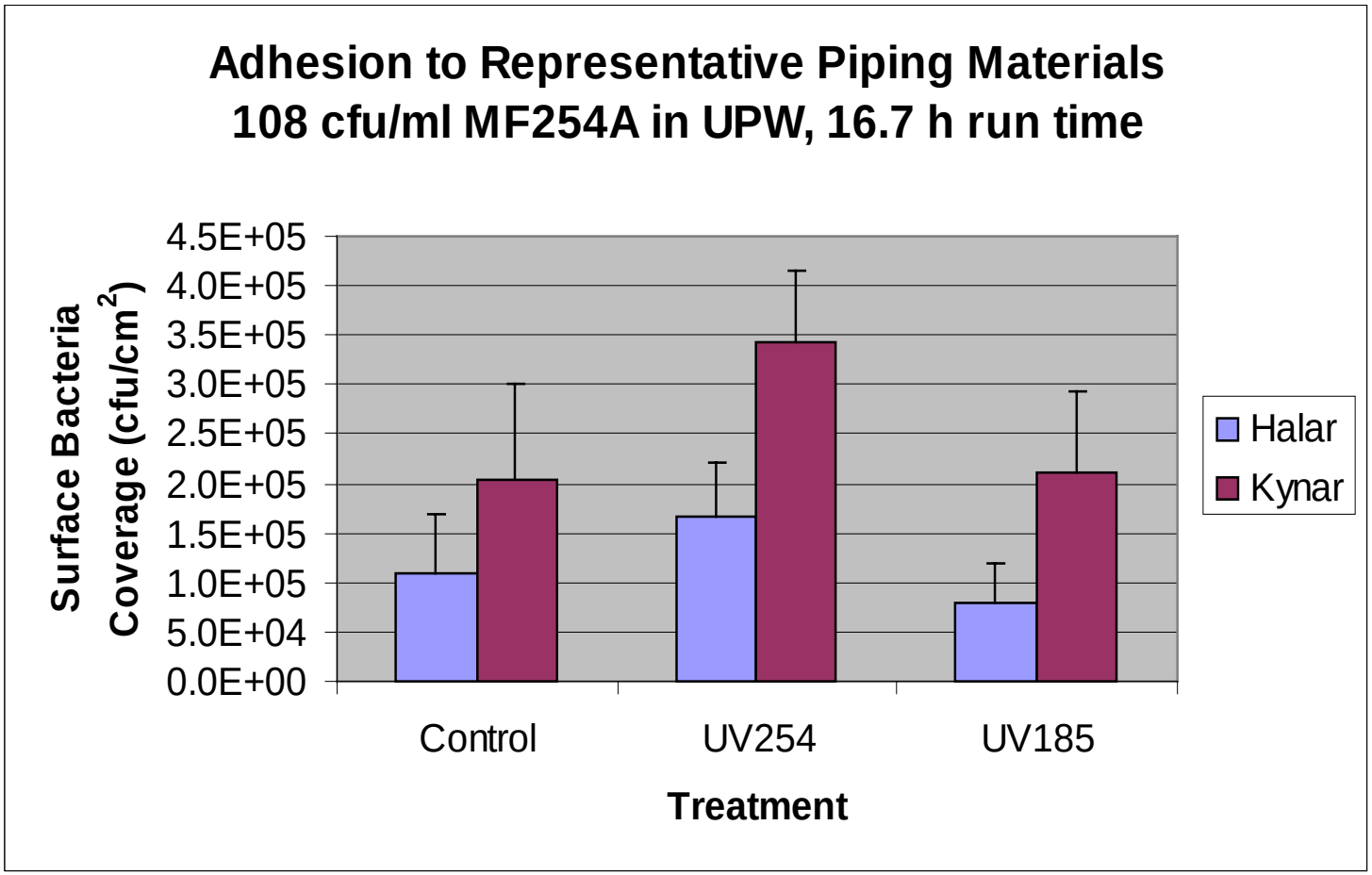
AOP vs. Germicidal Irradiation in UPW

Adhesion Comparisons, Short Course

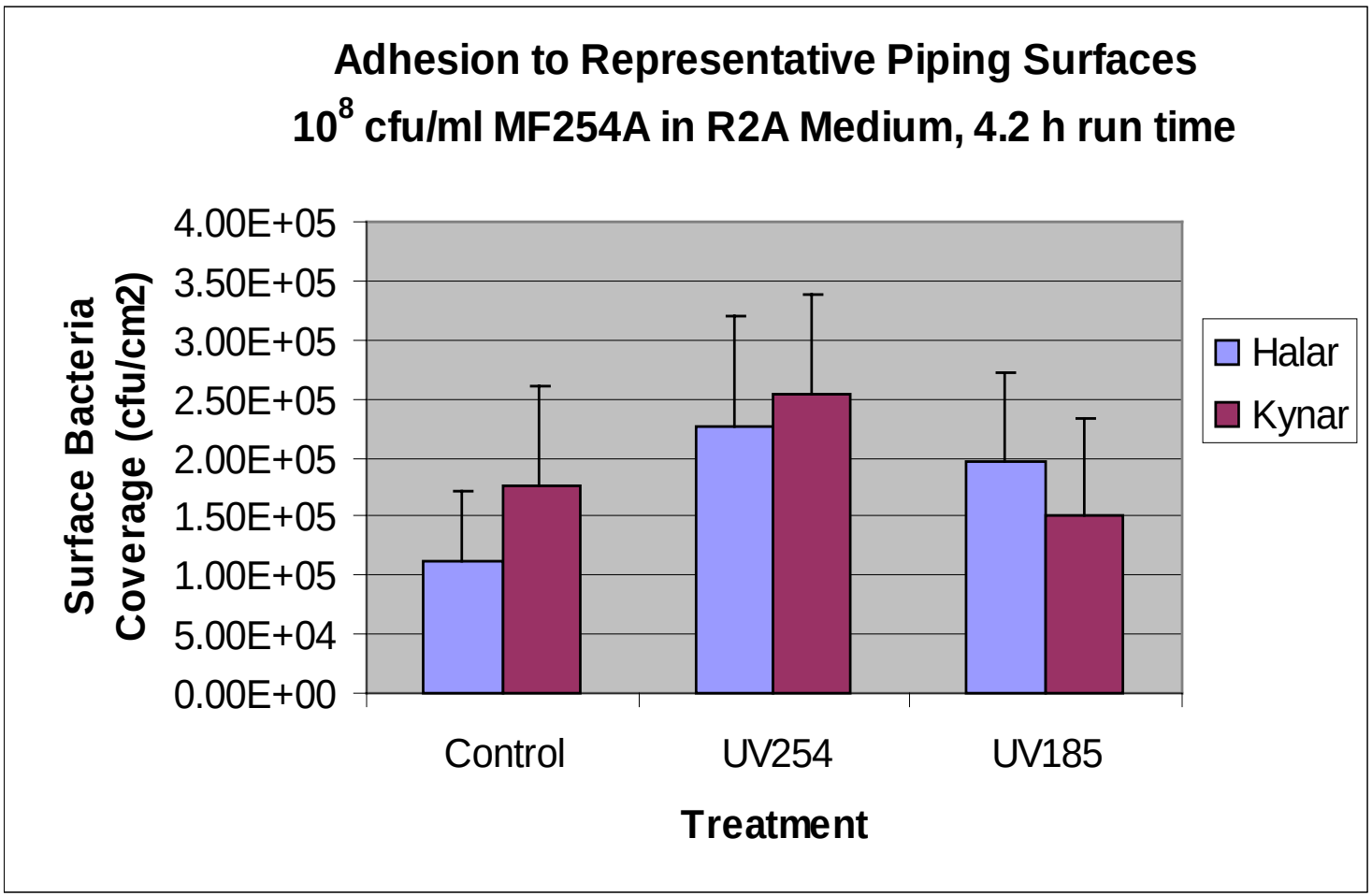


AOP vs. Germicidal Irradiation in UPW

Adhesion Comparisons, Long Course

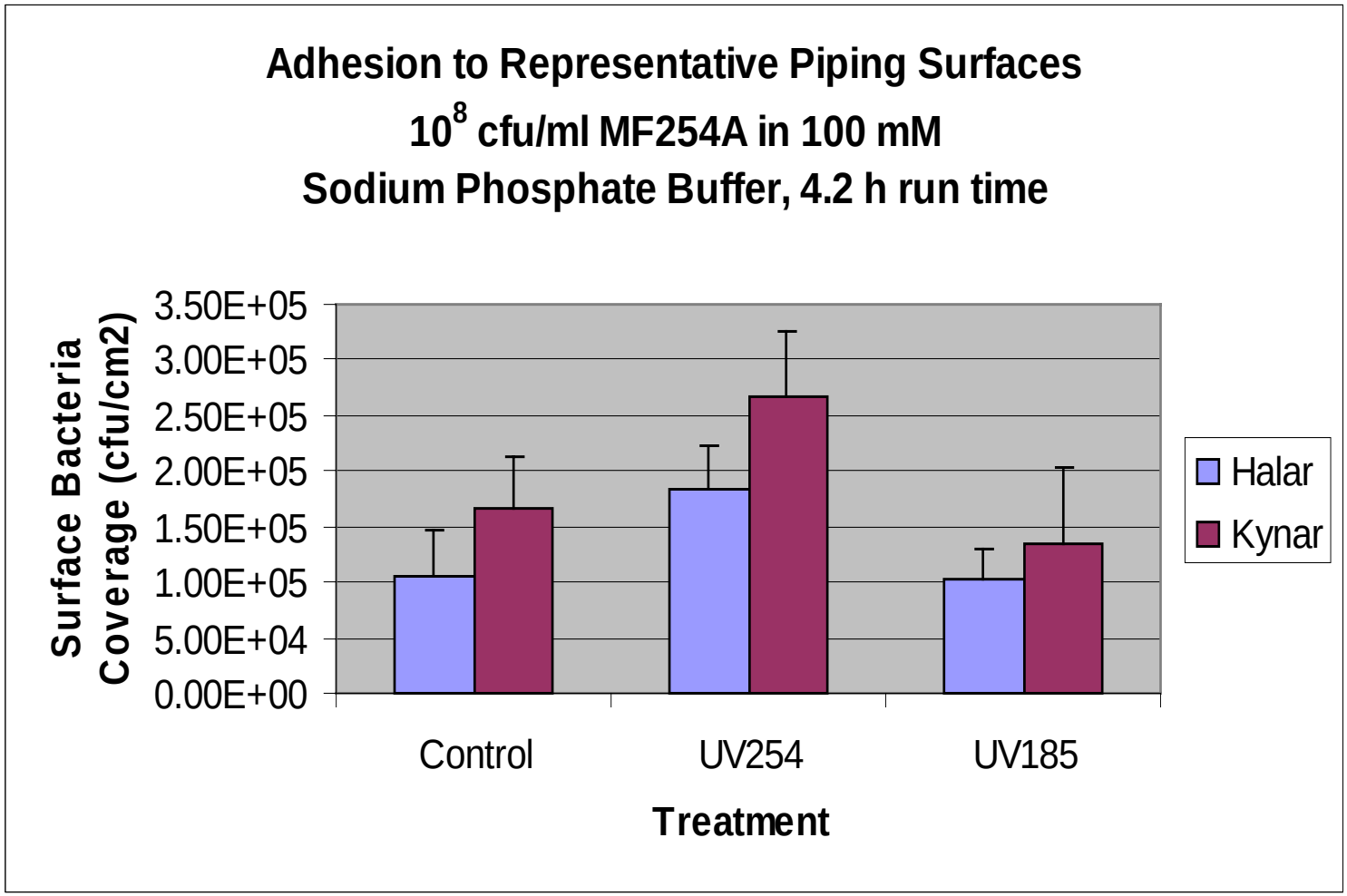


AOP vs. Germicidal Irradiation in R2A Medium



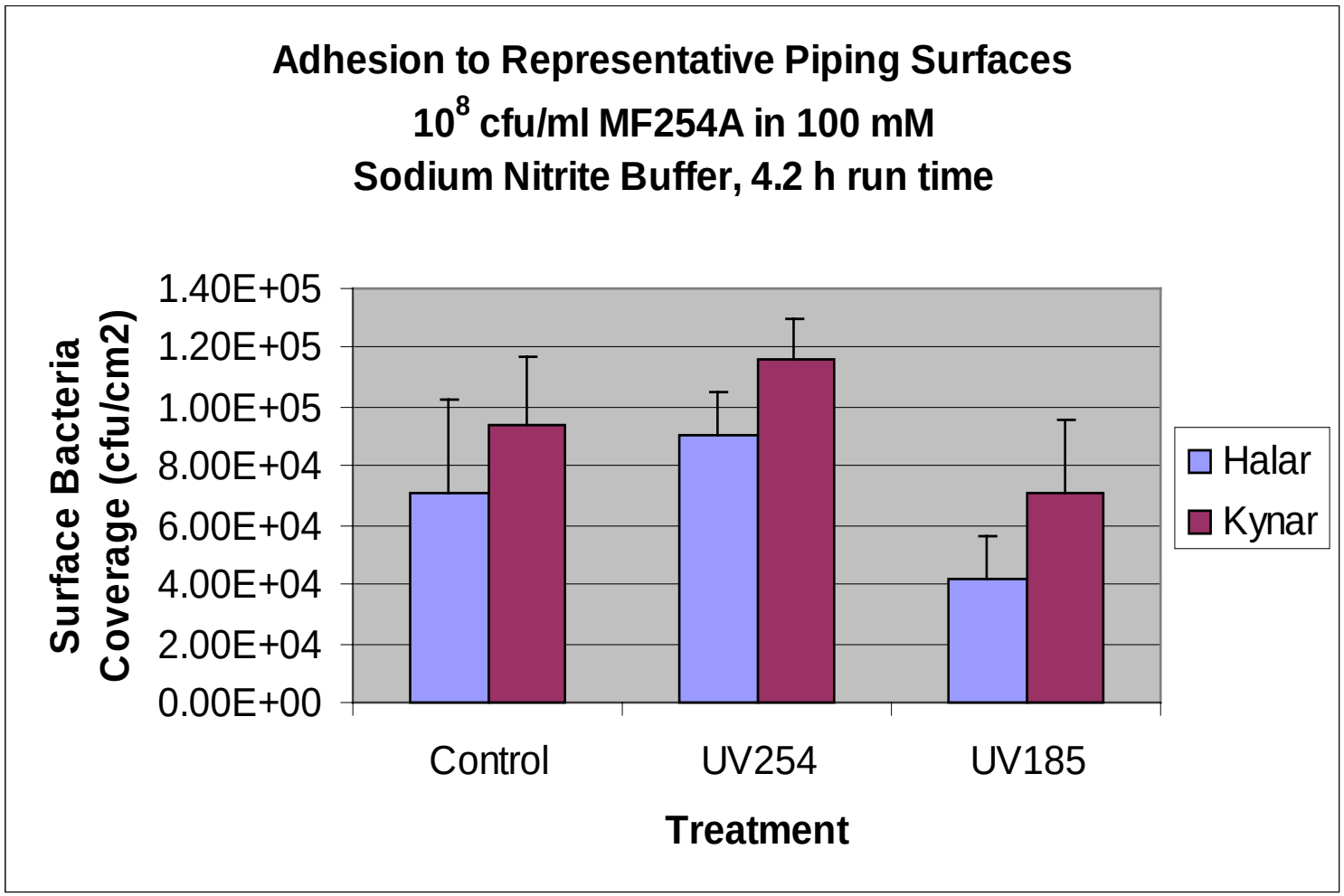
AOP vs. Germicidal Irradiation in Buffer

100 mM Sodium Phosphate



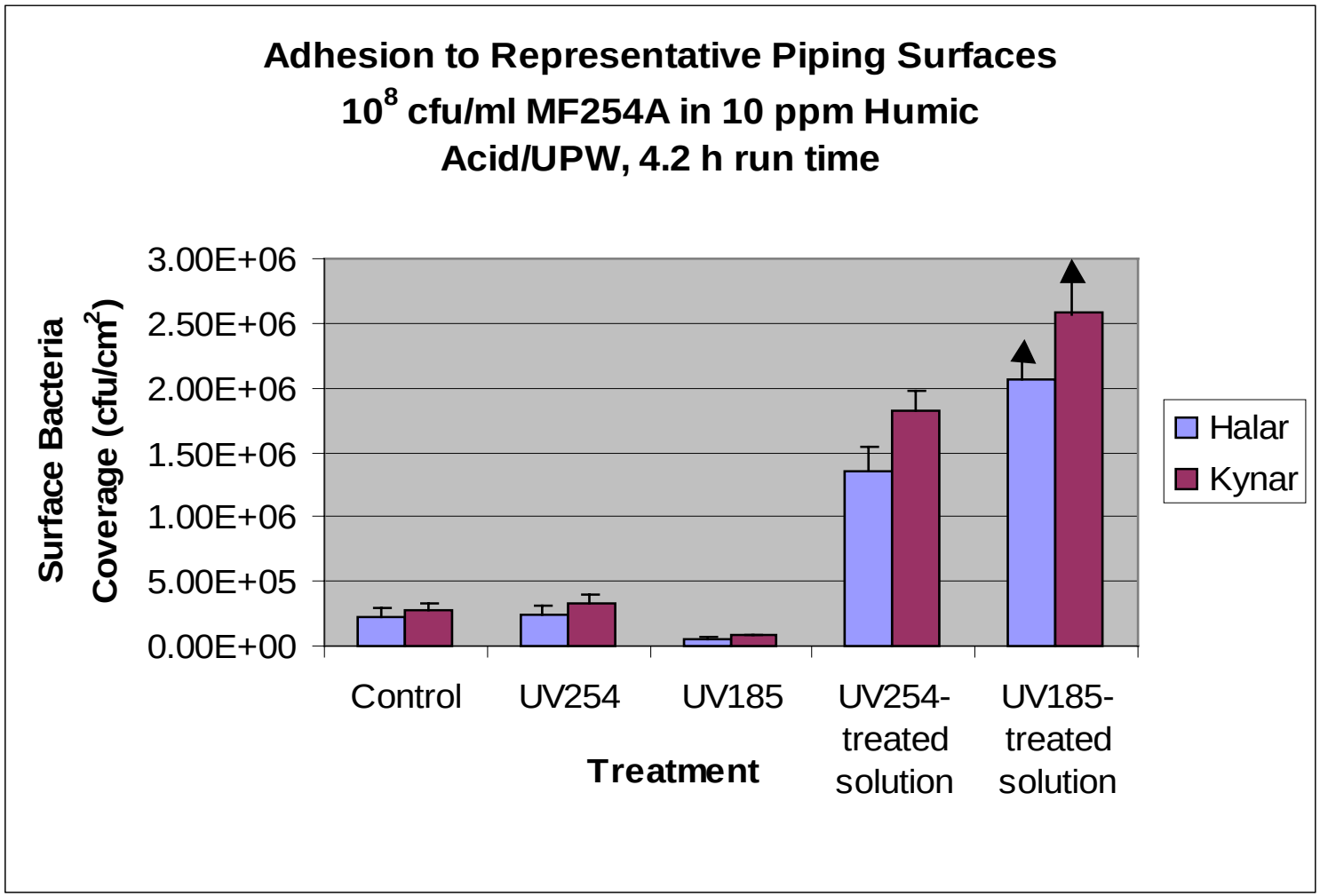
AOP vs. Germicidal Irradiation in Buffer

100 mM Sodium Nitrite



AOP vs. Germicidal Irradiation w/Nutrient Source

10 ppm Humic Acid and Treated Humic Acid



Adhesion Comparisons by Treatment and Environment

10^8 cfu/ml Adhesion on Halar®

	Control (x 10^5 cfu/cm^2)	UV_{254} (x 10^5 cfu/cm^2)	UV_{185} (x 10^5 cfu/cm^2)
UPW, short course	2.17 +/- 1.39	4.98 +/- 1.95	0.55 +/- 0.20
UPW, long course (avg)	1.18 +/- 0.44	1.53 +/- 0.58	0.77 +/- 0.28
R2A growth medium	1.12 +/- 0.61	2.26 +/- 0.93	1.97 +/- 0.76
100 mM phosphate buffer	1.06 +/- 0.41	1.84 +/- 0.39	1.03 +/- 0.27
100 mM nitrite buffer	0.71 +/- 0.32	0.90 +/- 0.15	0.42 +/- 0.14
10 ppm humic acid	2.26 +/- 0.71	2.44 +/- 0.60	0.57 +/- 0.13
10 ppm treated humic acid	2.26 +/- 0.71	13.6 +/- 1.75	> 20.6

- Differences in adhesion by treatment were more pronounced in UPW and humic acid/UPW than in media and buffers
- Adhesion comparisons on Kynar® follow same trends as Halar® with high adhesion in almost every case

Conclusions

- Cell death in UPW and similar environments using ultraviolet light follows conventional bacterial inactivation kinetics (first order exponential with tailing).
- Bacterial adhesion does not result in a uniform coverage of the solid surface, nor does it occur in a stepwise, uniform manner as time increases.
- In most cases, Halar® exhibits minimal adhesion over Kynar®.
- In both UPW and more nutrient-rich environments, germicidal irradiation treatment with UV_{254} results in greater adhesion than no treatment and advanced oxidation.
- Advanced oxidation treatment with UV_{185} varies in adhesion effects with the treatment environment.
- Treatment of potential biofilm nutrients prior to their contact with bacteria causes much greater adhesion than simultaneous treatment.

Further Work

- Perfect FISH Technique for specificity
- Continue detection of bacteria in industrial UPW systems
- Mimic tests on representative piping materials using higher water flow rates
- Further adhesion testing of alternative biofilm-forming bacteria species to piping surfaces
- Time-dependent series adhesion tests to determine rates of adhesion in the presence of various treatments
- Test different sources of advanced oxidation, such as UV/H_2O_2 or UV/TiO_2 catalyst

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