The Paul L. Busch Award

Recognizing Significant Advances in Water Quality Research

Wednesday, May 13, 2015
2:00 - 3:30 pm ET

THE WERF ENDOWMENT
FOR INNOVATION IN APPLIED WATER QUALITY RESEARCH
THE PAUL L. BUSCH AWARD

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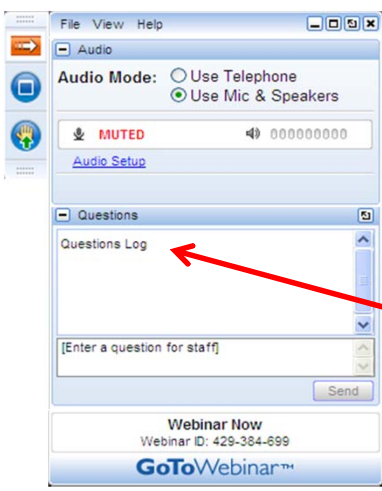
How to Participate Today



- **Audio Modes**
  - Listen using Mic & Speakers
  - Or, select “Use Telephone” and dial the conference (please remember long distance phone charges apply).
- **Submit your questions using the Questions pane.**
- **A recording will be available for replay shortly after this webcast.**

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Today's Moderator

Amit Pramanik, Ph.D., BCEEM
Director of Research
apramanik@werf.org

571-384-2101

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Today's Agenda

2:00 pm	Welcome and Introductions	Doug Owen / Amit Pramanik
2:10 pm	**Nexus of Water Sustainability and Public Health: Antibiotic Resistance in Recycled Water**	**Amy Pruden**
2:40 pm	**The Interplay Between Chemicals and Microorganisms in Urban Water Systems**	**Nancy Love**
2:55 pm	**Engineered Platforms and Pathways for Resource Recovery from "Waste"**	**Kartik Chandran**
3:10 pm	**Engineering Better Biofilms: Rational Design of Attachment Surfaces to Improve Their Performance**	**Andrew Schuler**
3:25 pm	Panel discussion / Q&A	All
3:40 pm	Adjourn	

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Douglas Owen, P.E., BCEE, ENV SP
ARCADIS
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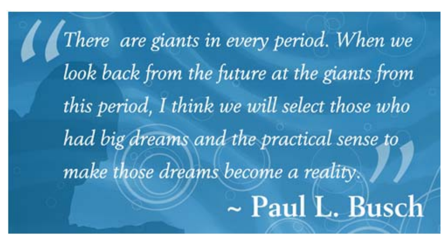

"WERF's goal of developing the scientific understanding and the technology which will improve the environment in a sustainable manner is a goal which everyone in our profession can share." – Paul Busch

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The Paul L. Busch Award

- 2001 - NANCY LOVE
- 2002 - LUTGARDE RASKIN
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- 2005 - DANIEL R. NOGUERA
- 2006 - PAUL WESTERHOFF
- 2007 - PAIGE NOVAK
- 2008 - ANDREW SCHULER
- 2009 - JAEHONG KIM
- 2010 - KARTIK CHANDRAN
- 2011 - VOLODYMYR TARABARA
- 2012 - ROBERT NERENBERG
- 2013 - CHUL PARK
- 2014 - AMY PRUDEN

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Amy J. Pruden, Ph.D.
Virginia Tech

Kartik Chandran, Ph.D.
Columbia University

Nancy G. Love, Ph.D., P.E., BCEE
University of Michigan

Andrew Schuler, Ph.D.
University of New Mexico

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Amy J. Pruden, Ph.D.
Virginia Tech

2014 Paul L. Busch Award Recipient

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Nexus of Water Sustainability and Public Health:

Antibiotic Resistance in Recycled Water

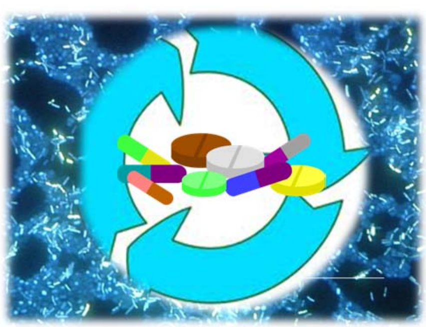

Image: Rodney M. Donlan, CDC

Paul L. Busch Award 2014

Amy Pruden
Professor

VirginiaTech Invent the Future®

Via Department of Civil & Environmental Engineering

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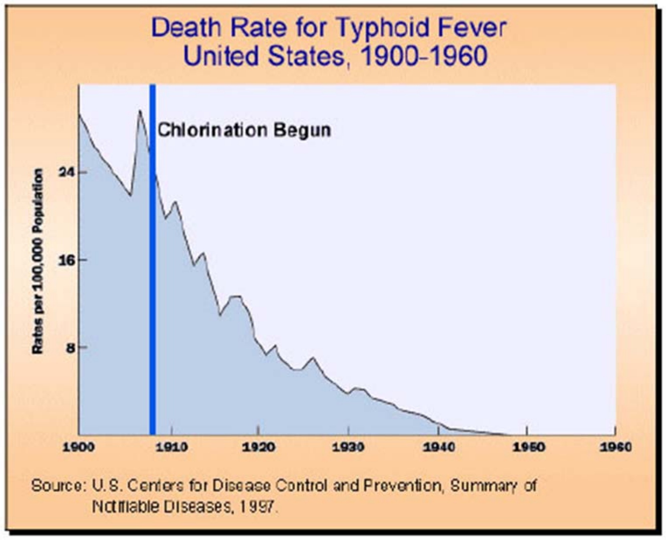
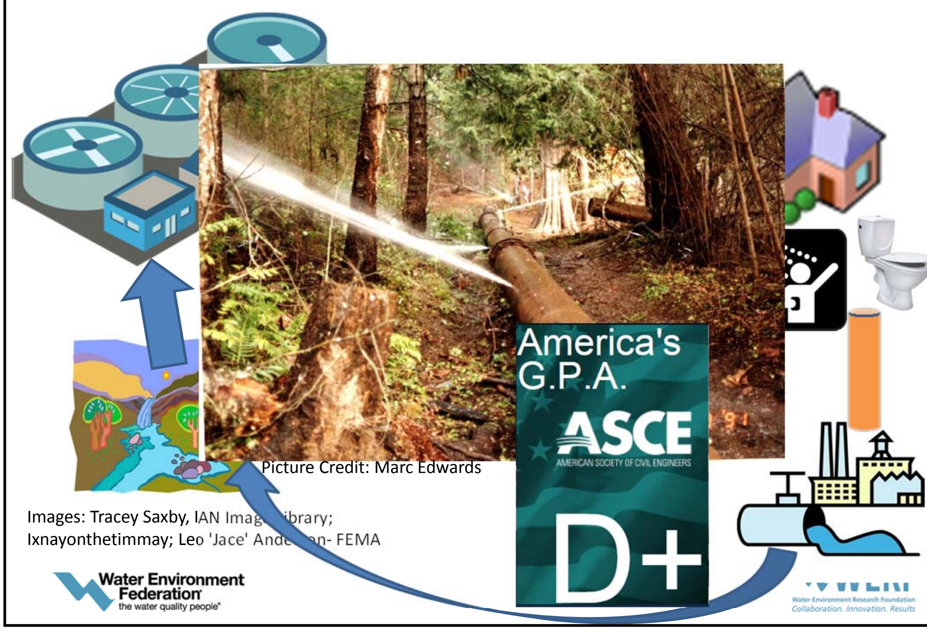
Human Progress: *Our Water Infrastructure*



Our Water Infrastructure

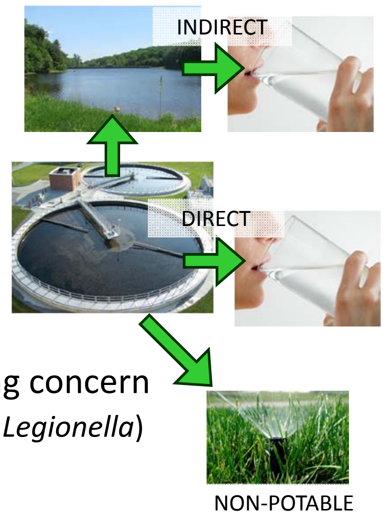


Picture Credit: Marc Edwards

Images: Tracey Saxby, IAN Imag[illegible]ibrary; Ixnayonthetimmay; Leo 'Jace' Ande[illegible]n- FEMA

Recycled Water

- Need for Water Sustainability
- Direct and Indirect Potable Reuse
- Nonpotable Reuse
- Role of bacterial regrowth for microbial constituents of emerging concern
  - Opportunistic Pathogens (OPs, e.g., *Legionella*)
  - Antibiotic Resistance Genes (ARGs)



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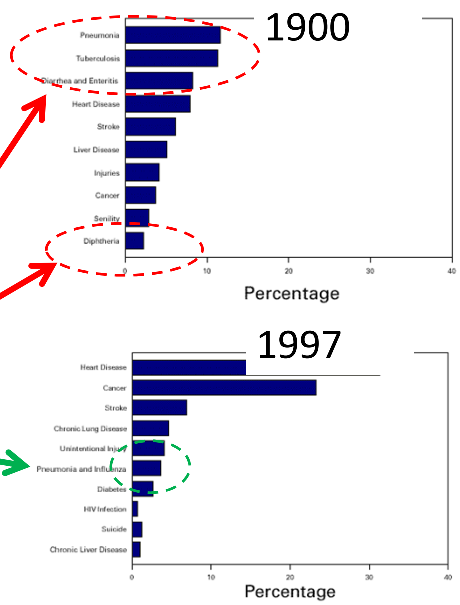
In addition to access to clean water, ***antibiotics are largely responsible for the high quality of life we enjoy today***

Bacterial infections

CDC, MMWR, July 30, 1999 / 48(29);621-629

FIGURE 2. The 10 leading causes of death as a percentage of all deaths — United States, 1900 and 1997



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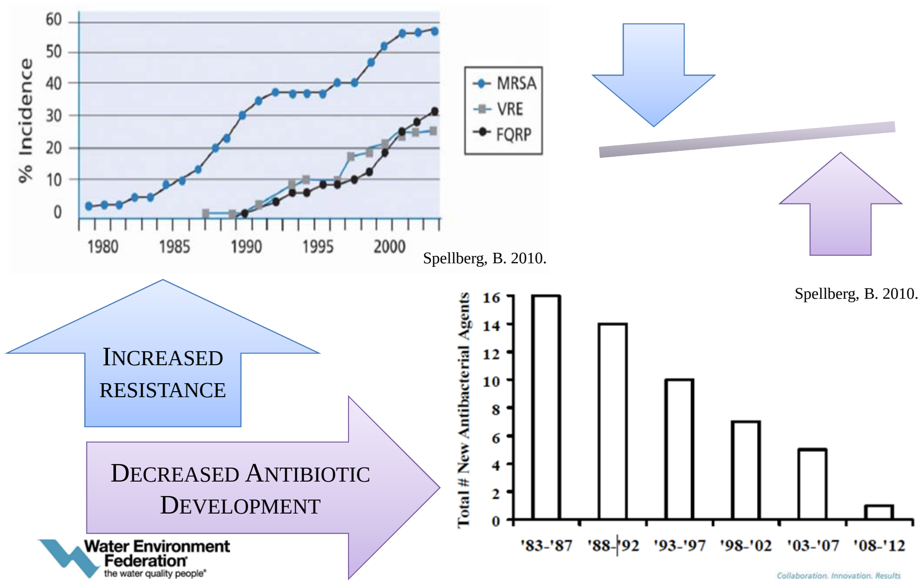
Sustainability of Antibiotic Use



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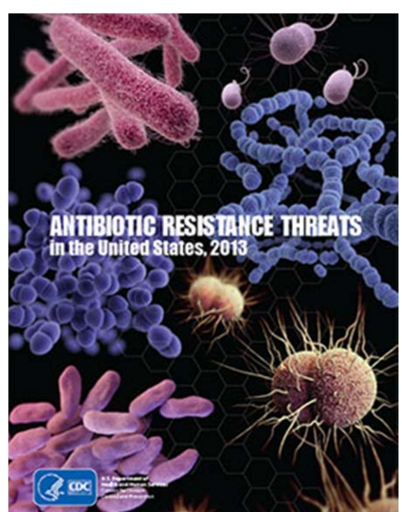
Antibiotic Resistance in the U.S.

- September 2013 CDC Report:
  - 2 million Americans fall ill from antibiotic-resistant bacteria
  - At least 23,000 die as a result (many more if count complications)
  - Community-acquired MRSA now surpasses hospital-acquired MRSA

"*Antibiotic-resistant infections can happen anywhere. Data show that most happen in the general community*"



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NATIONAL ACTION PLAN FOR COMBATING ANTIBIOTIC-RESISTANT BACTERIA

MARCH 2015

THE WHITE HOUSE
WASHINGTON

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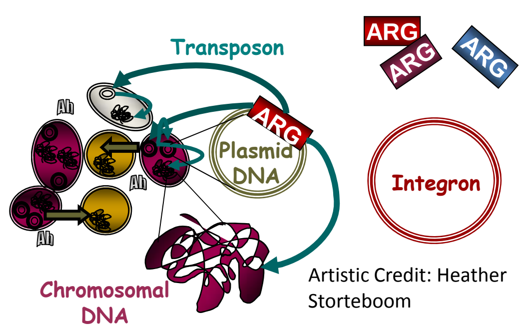
Antibiotic Resistance Genes (ARGs) as Environmental Contaminants

Antibiotic Resistance

- Mobile genetic elements (Antibiotic Resistance Genes or ARG)
- Mutations in chromosome



Artistic Credit: Heather Storteboom

Horizontal Gene Transfer (HGT): Traditional approach of killing bacteria may not be sufficient- ideally should think about destroying ARGs.

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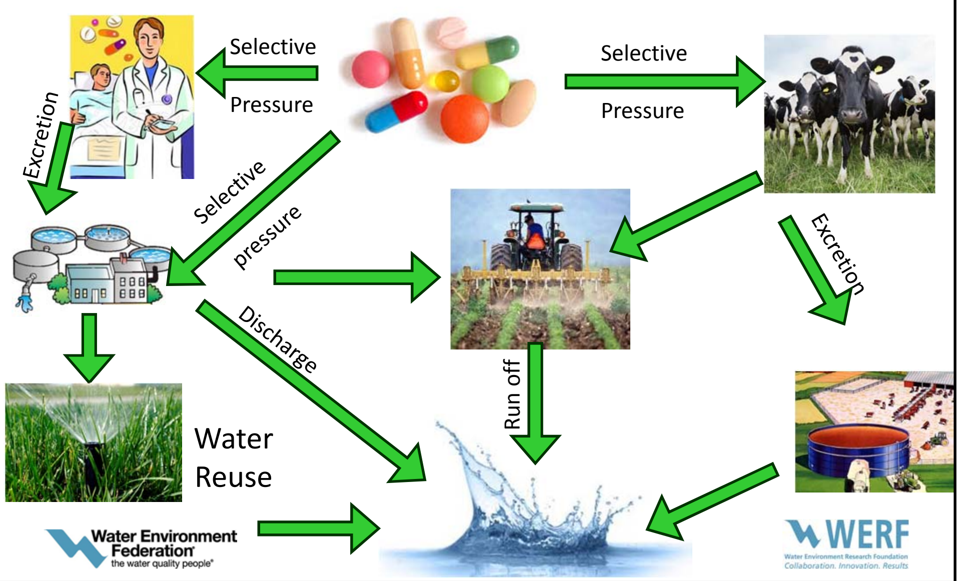
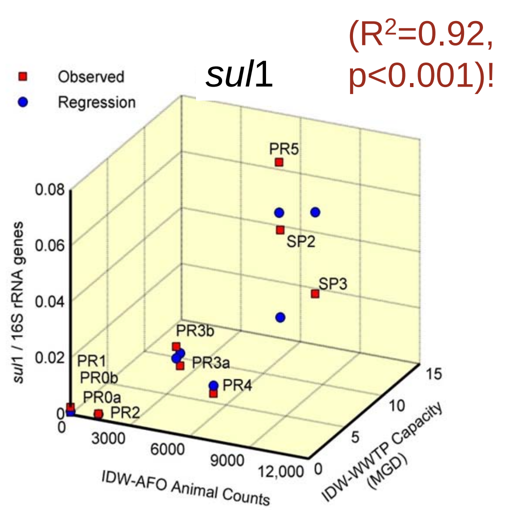
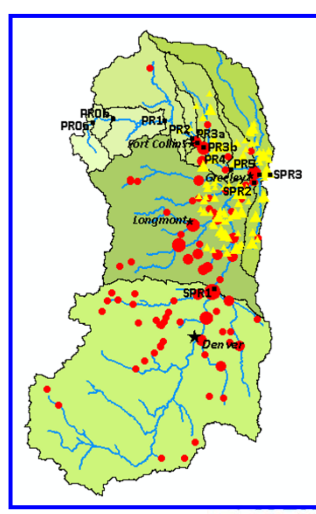
Antibiotic Resistance Genes (ARGs)



ARGs Correlate with Animal Feeding Operations and WWTPs in the Poudre River Watershed



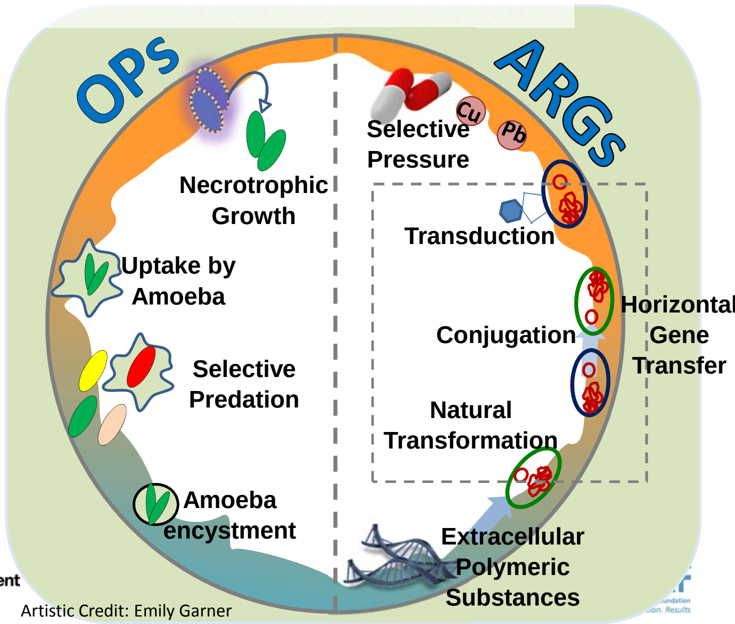


Pruden et al. ***ES&T*** **2012**

Microbial Ecology in Pipe Biofilms

Additional Parameters Contributing to Regrowth:

- **Decay of Disinfectant Residual**
- **Stagnation**
- **Elevated nutrient (C,N,P) concentration**
- **Temperature**



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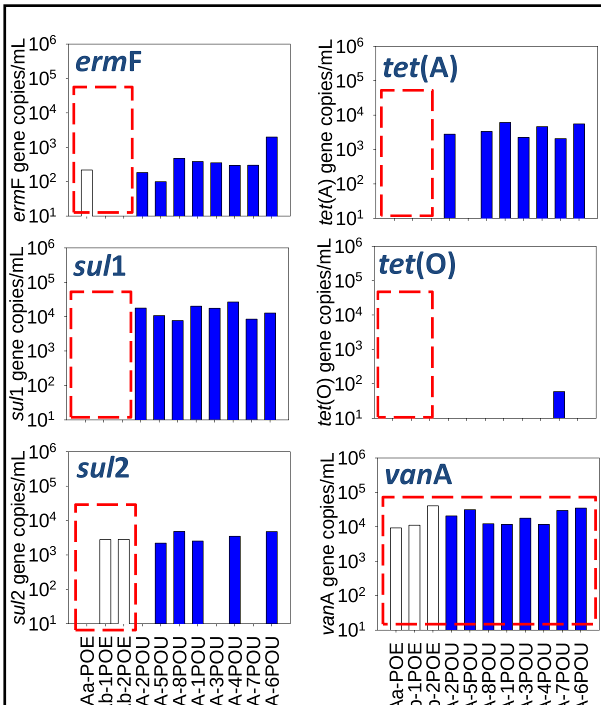
Artistic Credit: Emily Garner



Reclaimed Water Pipes

- Most ARGs detectable at the **point-of-use**, but not exiting treatment plant
- *van*A detectable throughout
- Highlights **importance** of considering the microbiology that happens **as water flows through pipes**

Fahrenfeld et al. *Frontiers in Microbiology* 2013

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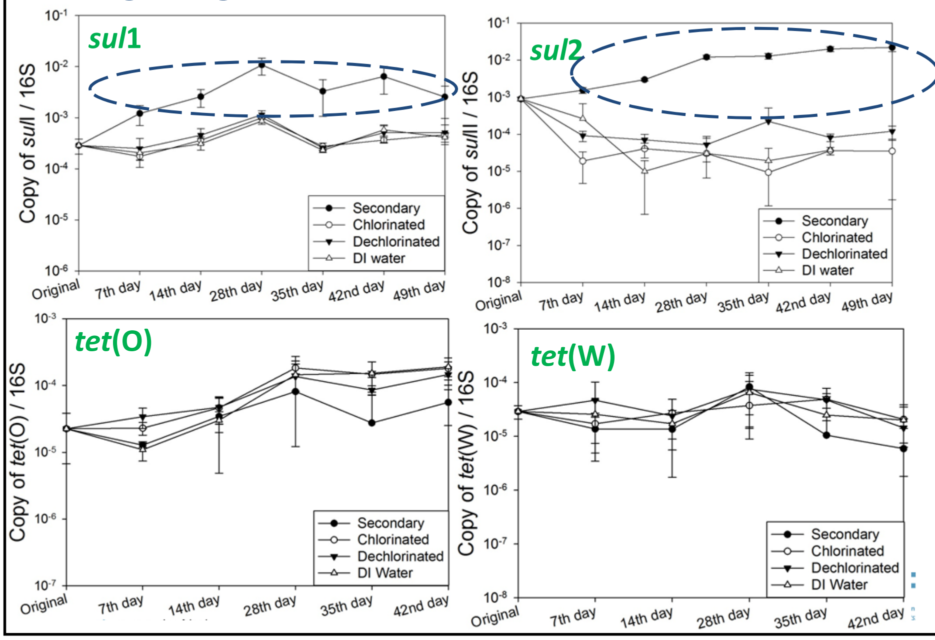
Fahrenfeld et al. *Frontiers in Microbiology* 2013

Irrigating Soil Slurries with Reclaimed Water



Potential Mitigation Endpoints

- Comparable to a defined control background
  - ARG diversity
  - ARG abundance
  - Absence of key clinical ARGs (e.g., NDM-1)
  - All of the above: HGT/multi-drug markers

The ISME Journal (2009) 3, 243–251
© 2009 International Society for Microbial Ecology All rights reserved 1751-7362/09 $32.00
www.nature.com/ismej

ORIGINAL ARTICLE

Functional metagenomics reveals diverse β-lactamases in a remote Alaskan soil

Heather K Allen[1,2], Luke A Moe[1], Jitsupang Rodbumrer[1,3], Andra Gaarder[1] and Jo Handelsman[1]
[1]Departments of Bacteriology and Plant Pathology, University of Wisconsin-Madison, Madison, WI, USA and [2]Microbiology Doctoral Training Program, University of Wisconsin-Madison, Madison, WI, USA

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Objective: Paul L. Busch Award

- Compare ARGs in reclaimed and potable water distribution systems
  - Potable water is an important “control”
  - Potable water distribution system management can inform distribution of recycled water
  - Examine role of microbial re-growth
  - Use next generation DNA sequencing for deep insight into microbial community and ARGs
  - Compare with culture-based methods

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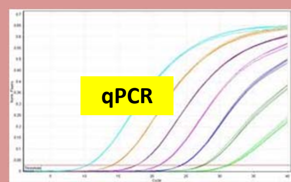
Distribution System Survey

Sample Collection

Microbiology Methods

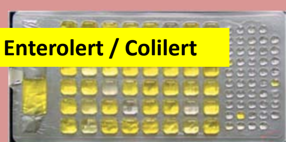
OPs

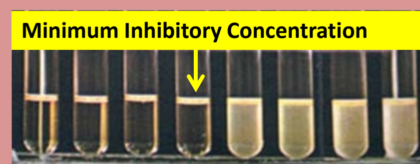


ARGs

MG-RAST
metagenomics analysis server

E. coli and *Enterococcus* resistance profiles





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Overview of Systems

	POTABLE WATER	RECLAIMED WATER	
System	Disinfectant	Summary of Treatment	Disinfectant
A	Cl_2 ($ClNH_2$ Residual)	Plant #1 – Advanced wastewater treatment-Bardenpho Process Plant #2 – Activated sludge, secondary clarification, denitrification	Cl_2
B	Cl_2; occasional ClO_2	Plant #1 – Advanced wastewater treatment – Bardenpho Process; Plant #2 – Biofiltration, secondary sedimentation	Cl_2 UV ($ClNH_2$ Residual)
C	Cl_2	Dual media filters or membrane bioreactors	Cl_2 ($ClNH_2$ Residual)

Note: All potable water sources are a combination of surface and groundwater

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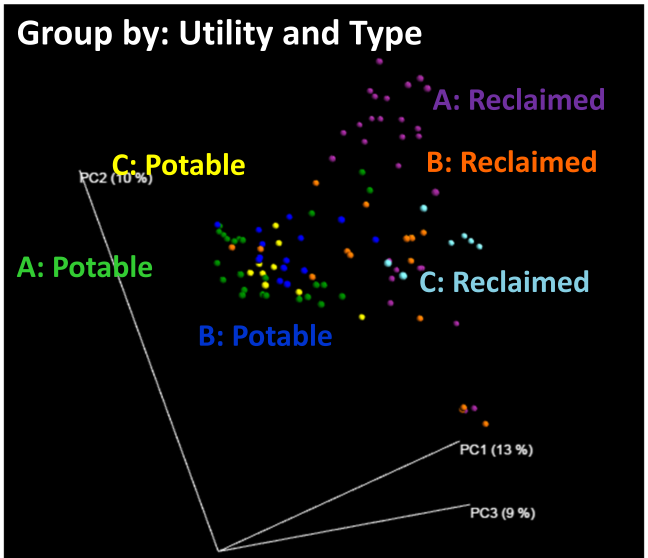
Microbial Community: Illumina Amplicon Sequencing



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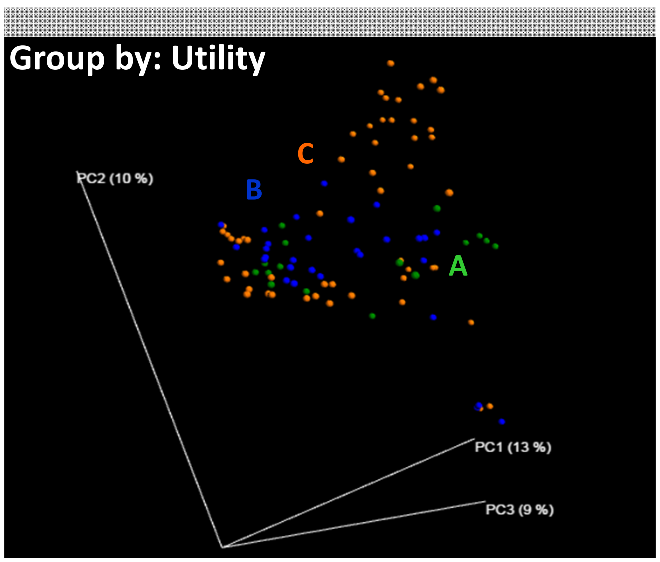
Microbial Community: Illumina Amplicon Sequencing



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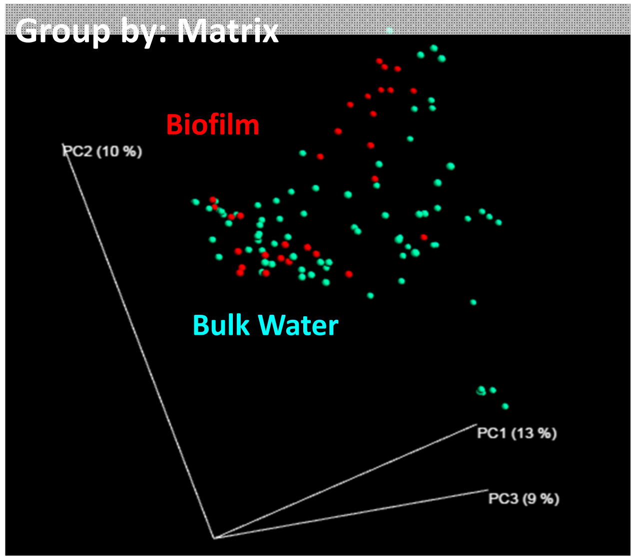
Microbial Community: Illumina Amplicon Sequencing



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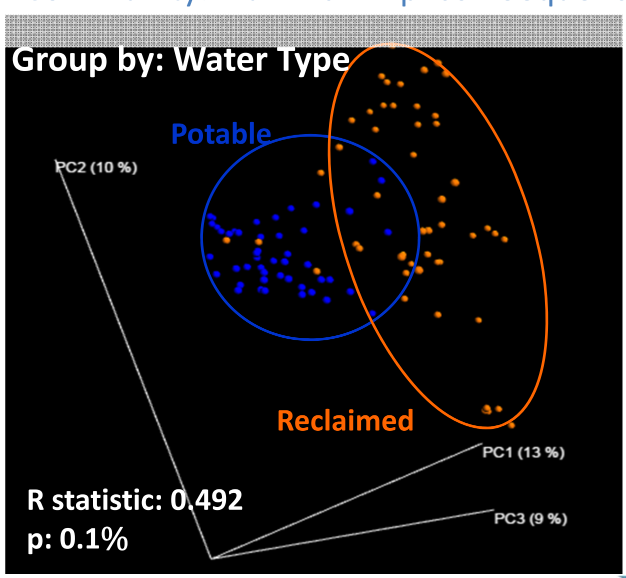
Microbial Community: Illumina Amplicon Sequencing



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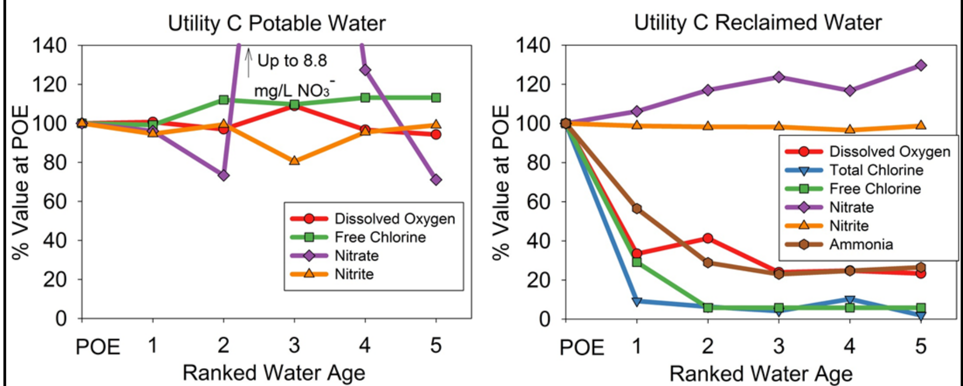
Water Chemistry as a function of Water Age



*Disinfectant Residual: Cl_2

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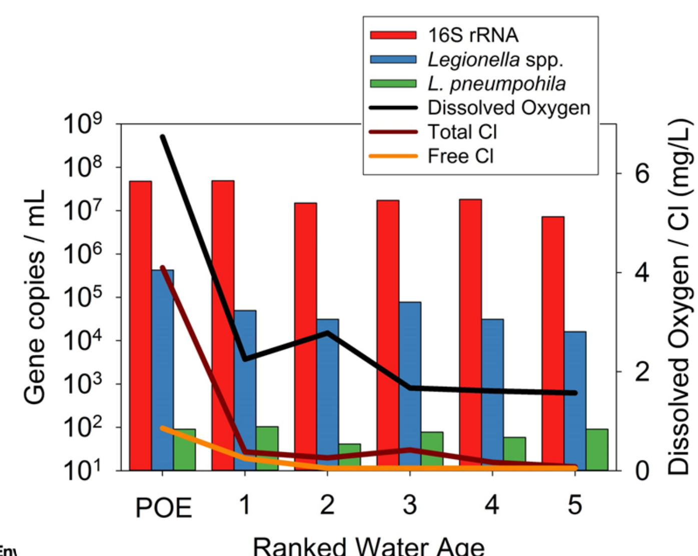
Impact of Water Chemistry on OPs



Regrowth of OPs

Utility A: Potable (Residual: $ClNH_2$)

Bulk Water [log (copies / mL)]

	POE	1	2	3	4	5
16S rRNA	4.0	3.3	3.6	4.1	4.8	6.2
Legionella spp.	2.1					3.2
L. pneumophila						3.0
Mycobacterium spp.					2.6	3.4
M. avium	2.0				1.8	1.8
N. fowleri						
Acanthamoeba spp.					3.0	
V. vermiformis			1.0			1.8

Biofilm [log (copies / cm²)]

	1	2	3	4	5
16S rRNA	4.1	3.9	4.0	3.9	4.4
Legionella spp.		3.3			2.7
L. pneumophila		2.8			2.8
Mycobacterium spp.	3.1	2.7	2.7		3.0
M. avium					
N. fowleri					3.0
Acanthamoeba spp.					
V. vermiformis					2.4

Utility A: Reclaimed (Residual: Cl_2)

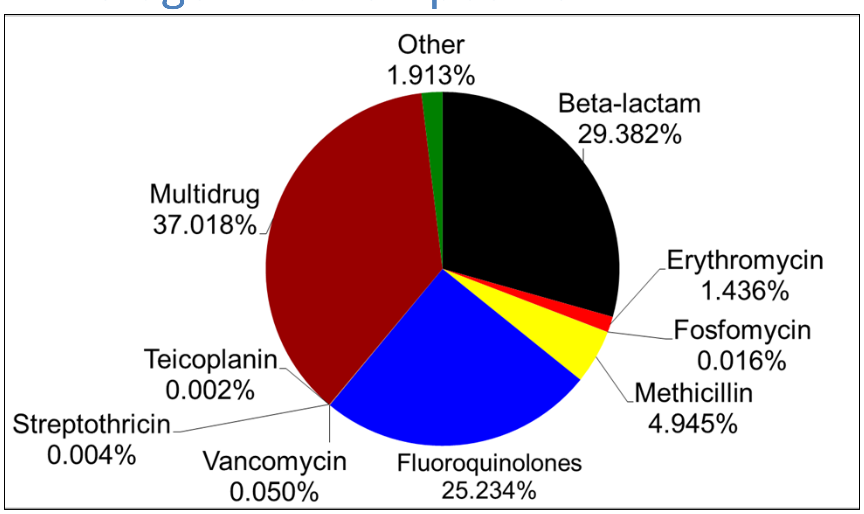
Bulk Water [log (copies / mL)]

	POE	1	2	3	4	5
16S rRNA	5.4	6.6	6.4	6.8	6.6	7.3
Legionella spp.		4.0	3.8	3.2	4.0	4.4
L. pneumophila	2.6	2.6	2.7		2.6	3.0
Mycobacterium spp.		2.5	2.6	2.7	2.8	2.5
M. avium						
N. fowleri		2.5				
Acanthamoeba spp.			2.5			
V. vermiformis						

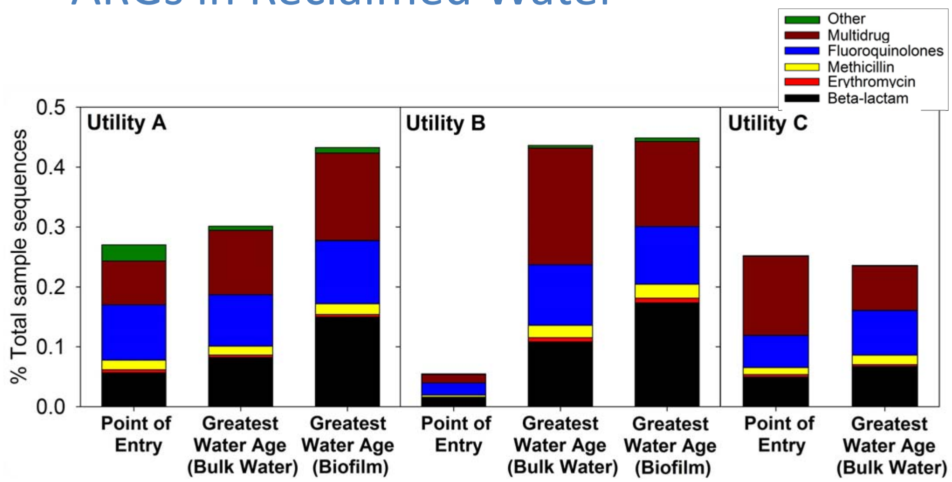
Biofilm [log (copies / cm²)]

	1	2	3	4	5
16S rRNA	5.0	4.9	5.0	4.4	5.0
Legionella spp.	3.4	3.4	3.4	3.3	3.1
L. pneumophila	2.7	3.4	3.7	2.5	2.8
Mycobacterium spp.					3.1
M. avium					
N. fowleri					
Acanthamoeba spp.					
V. vermiformis					

Metagenomics: Average ARG Composition



Metagenomics: ARGs in Reclaimed Water



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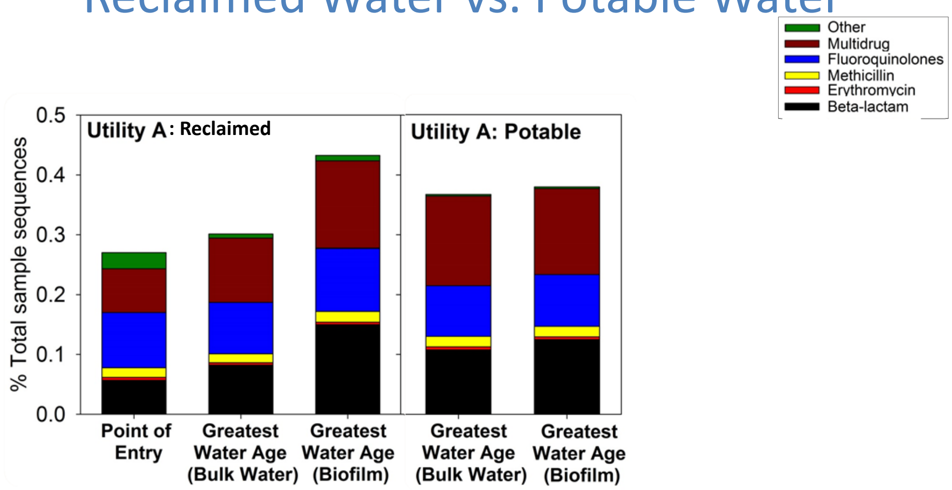
Metagenomics: ARGs in Reclaimed Water vs. Potable Water



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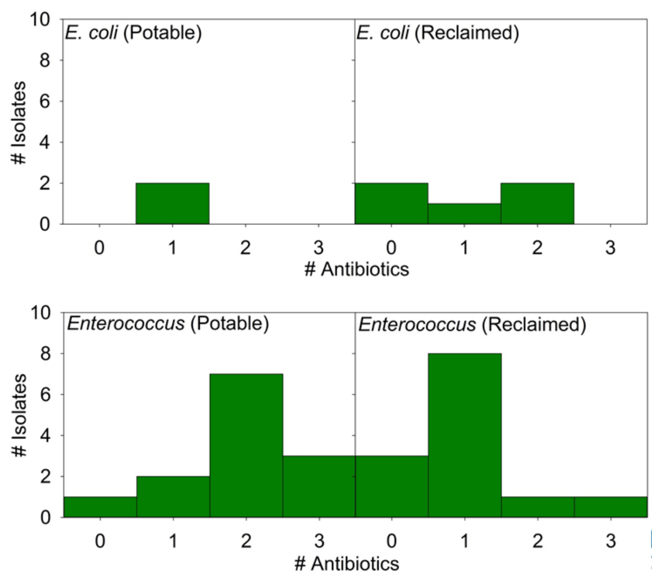
Antibiotic Resistant Indicator Bacteria

E. coli	Utility	Cephalexin	Erythromycin	Sulfamethoxazole
POTABLE	A	ND	ND	ND
	B	ND	ND	ND
	C	0/2	2/2	0/2
RECLAIMED	A	0/5	5/5	3/5
	B	ND	ND	ND
	C	ND	ND	ND

Enterococcus	Utility	Cephalexin	Erythromycin	Vancomycin
POTABLE	A	ND	ND	ND
	B	ND	ND	ND
	C	10/13	8/13	8/13
RECLAIMED	A	5/5	4/5	3/5
	B	ND	ND	ND
	C	6/8	2/8	3/8

Presented as [# resistant / # isolates tested]
ND indicates no isolates present

Multiple Antibiotic Resistance



Conclusions

- OPs
  - *Legionella* spp. and *L. pneumophila* gene markers were detected throughout but did not increase at higher water ages
- ARGs
  - Increase of ARGs from POE to POU (2/3 cases)
  - Relative Abundance of ARGs in reclaimed water comparable to potable water (1/1 case)
  - Multiple antibiotic resistance observed in potable and reclaimed water isolates

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Environmental Science & Technology

Feature

pubs.acs.org/est

Balancing Water Sustainability and Public Health Goals in the Face of Growing Concerns about Antibiotic Resistance

Amy Pruden[1,*]

[1]Via Department of Civil and Environmental Engineering, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, United States

the United States and other developed countries, our water infrastructure has reached its design lifespan, as evidenced by the American Society of Civil Engineers combined grade of "D" for the U.S. drinking water and wastewater infrastructure.[5] Thus, we as a society face a key moment in history where we will either proactively take on the challenge of sustainable water infrastructure, or generally continue a much costlier reactionary approach. Clearly there is need for innovation, both technological and institutional, as recently reviewed by Kiparsky and colleagues.[6]

Initiatives, such as ReNUWit, a National Science Foundation Engineering Research Center lead by Stanford University and partners,[7] challenge us to envision the city of the future in

Pruden, *ES&T* 2014 Editor's Choice: Best Feature Article

Next Steps

- Continue field survey- four total locations and four events
- Extend principles of examining OP and ARG regrowth into direct potable reuse (DPR) systems:
  - Water Research Foundation Project 4536 "*Blending Requirements for Water from Direct Potable Reuse Treatment Facilities*" (PI Andrew Salveson, Carollo Engineers, Inc.)

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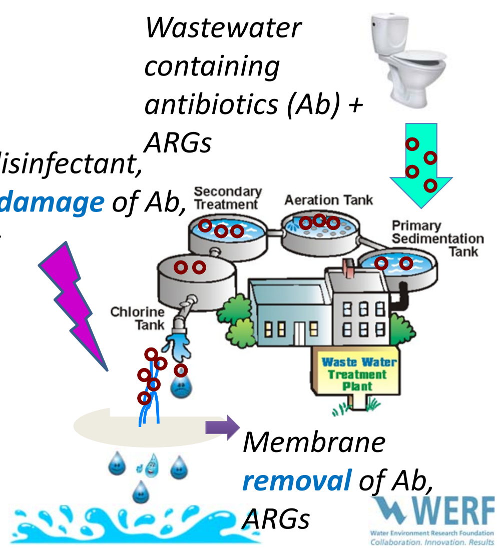
Management of Antibiotic Resistance Risk in Sustainable Water Systems

- DNA/Ab **Removal**
- DNA/Ab **Damage**
- Management of **Distribution System** and Other Infrastructure

Wastewater containing antibiotics (Ab) + ARGs

*UV, disinfectant, AOP **damage** of Ab, ARGs*



*Membrane **removal** of Ab, ARGs*

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Acknowledgements- People

- Emily Garner, NSF Graduate Research Fellow
- Jeannie McLain, University of Arizona
- Marc Edwards, Virginia Tech
- Andrew Salveson, Carollo Engineers
- Our many supportive and helpful utility partners

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Acknowledgements- Funding

WERF

- Water Environment Research Foundation Paul L. Busch Award 2014
- NSF Graduate Research Fellowship
- Alfred P. Sloan Foundation Microbiology of the Built Environment
- Water Research Foundation Project 4536

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Nancy G. Love, Ph.D., P.E., BCEE
University of Michigan

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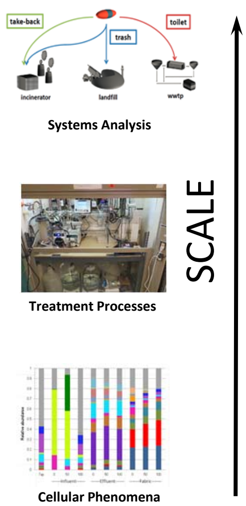
2001 - 2010
PAUL L. BUSCH AWARD
Celebrating 10 Years of Innovative Research

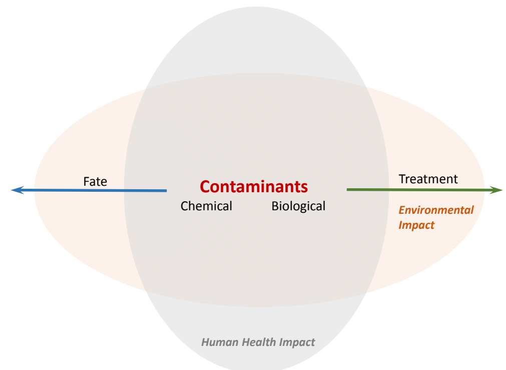
Nancy Love used the award in her effort to create a protein-based warning system that will help plant operators quickly detect changes in the influent, prevent upsets, and optimize the treatment process.

Nancy Love
University of Michigan

2001 Paul L. Busch Award Recipient

The Interplay Between Chemicals and Microorganisms in Urban Water Systems





Nancy G. Love, Ph.D., P.E., BCEE
University of Michigan
May 13, 2015

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Busch Award (2001): Working Hypothesis

Stress responses, which are controlled at the molecular/cellular level, play a significant role in defining how biological treatment processes perform at the macroscopic level.

chemical → microorganism → (*Stress response*) → process performance impact

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Busch Award (2001): Working Hypothesis

Stress responses, which are controlled at the molecular/cellular level, play a significant role in defining how biological treatment processes perform at the macroscopic level.

Biological basis for sensor that indicates chemical stress

chemical → microorganism → *Stress response* → process performance impact

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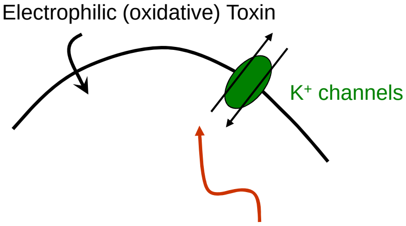
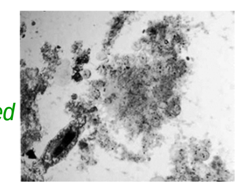
Electrophilic (oxidative) Toxin

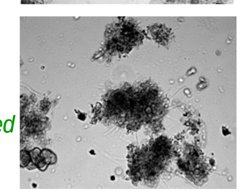


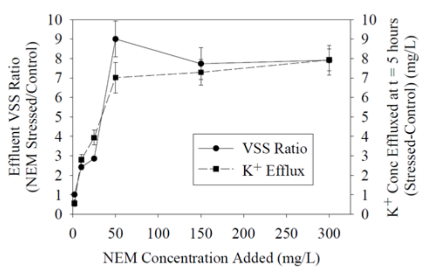
Glutathione + Toxin → Conjugate OR oxidized glutathione

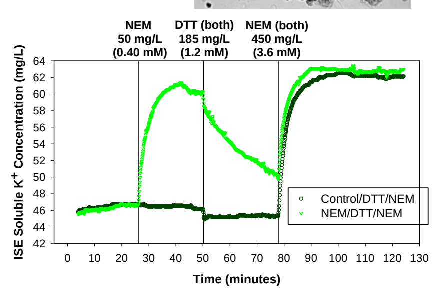
Well flocculated



Poorly flocculated







Bott and Love, *Water Environ Res*, 2002; Bott and Love, *Appl Environ Micro*, 2004

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Busch Award (2001): Working Hypothesis

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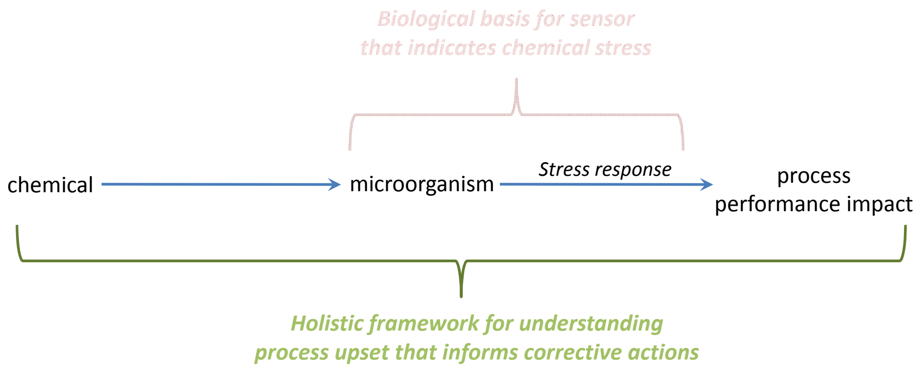

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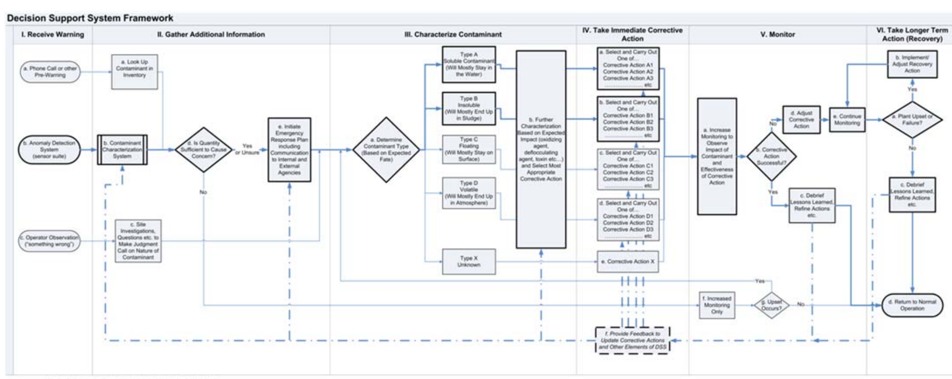
A decision support system framework was developed to guide responses to influent anomalies.



Love, N. G., A. J. Pinto, J. S. Guest, S. Hardin and A. Shaw. 2009. Determining and Assessing Corrective Action Strategies for Treatment Plants Exposed to Chemical Toxins. Water Environment Research Foundation, Report No. 04-CTS-11S, Alexandria, VA, 191 pages.

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Busch Award (2001): Working Hypothesis

Stress responses, which are controlled at the molecular/cellular level, play a significant role in defining how biological treatment processes perform at the macroscopic level.

Biological basis for sensor that indicates chemical stress

chemical → microorganism → *Stress response* → process performance impact

Holistic framework for understanding process upset that informs corrective actions

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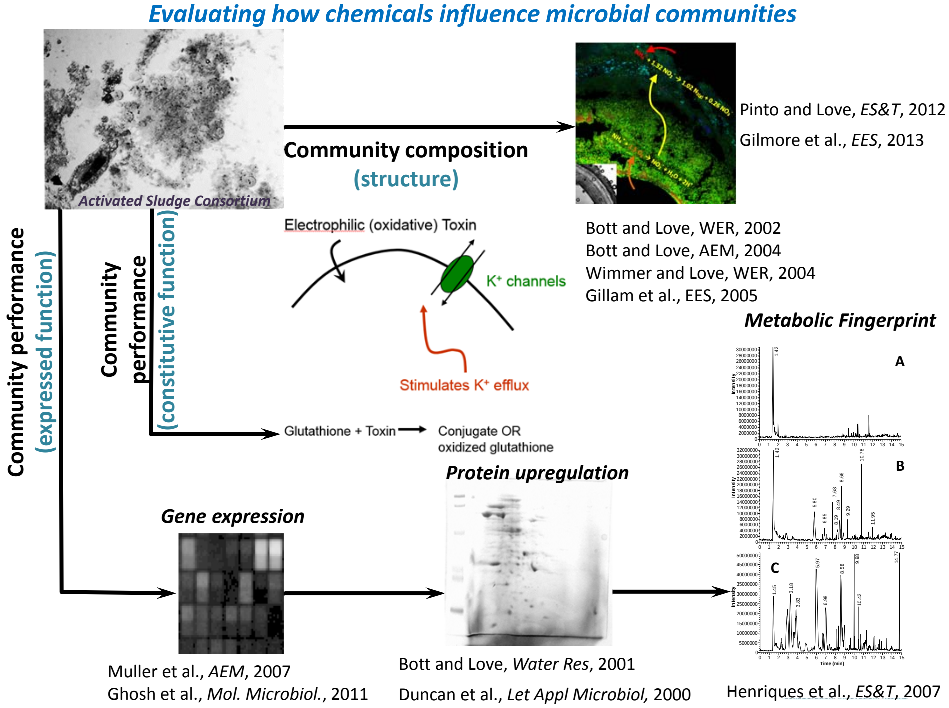
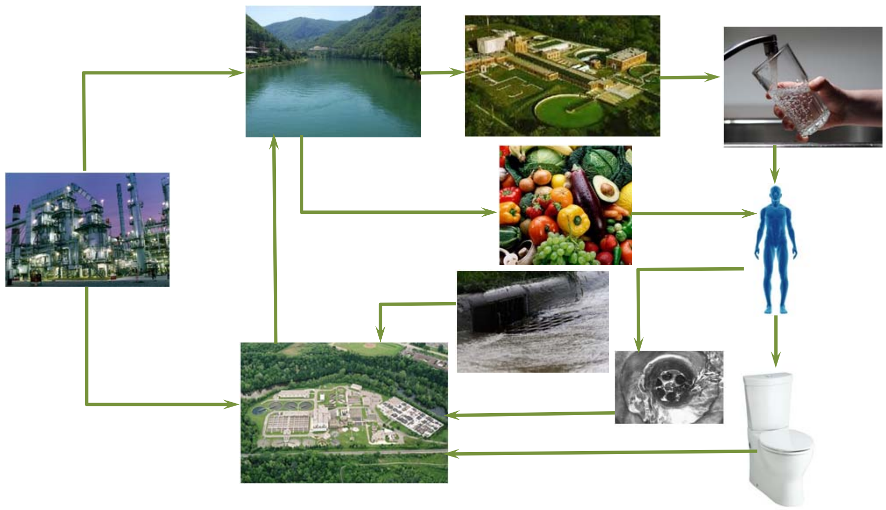
Evaluating how chemicals influence microbial communities



The interplay between chemicals and microorganisms in urban water systems.



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Growth in the industrial- and technology-based economy brings increased chemical production.

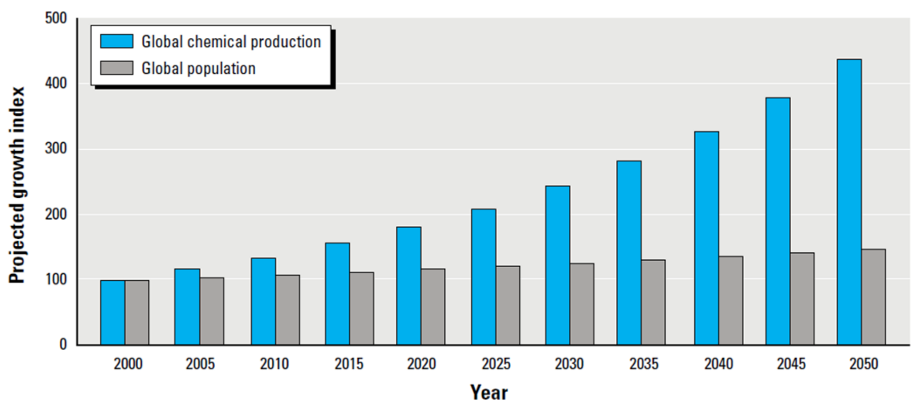

Figure 2. Global chemical production is projected to grow at a rate of 3% per year, rapidly outpacing the rate of global population growth, estimated at 0.77% per year. On this trajectory, chemical production will double by 2024, indexed to 2000 (American Chemistry Council 2003; OECD 2001; United Nations 2004).

Wilson and Schwarzman (2009) Toward a New U.S. Chemicals Policy: Rebuilding the Foundation to Advance New Science, Green Chemistry, and Environmental Health, *Environ. Health Perspect.* **117**:1202-1209.

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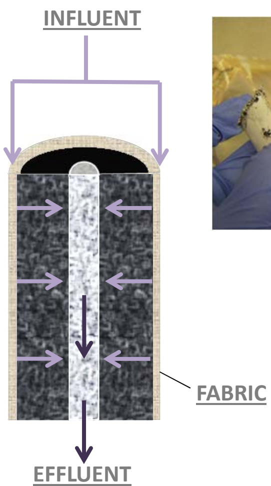
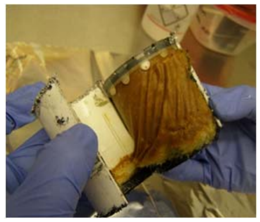
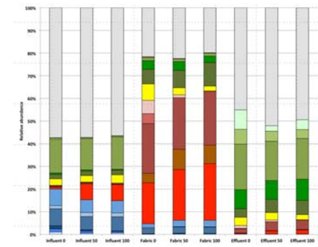
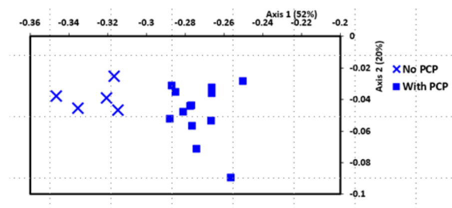
Point-of-use filters and low levels of disinfection byproducts change the drinking water microbiome.









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Low levels of chemicals influence microbial structure and function which, in turn, changes microbial communities and our exposure risk.

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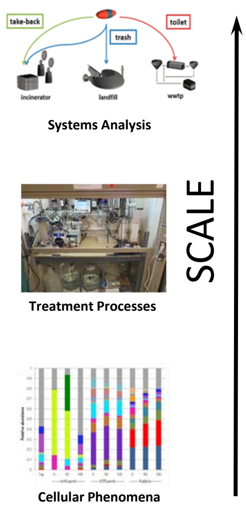
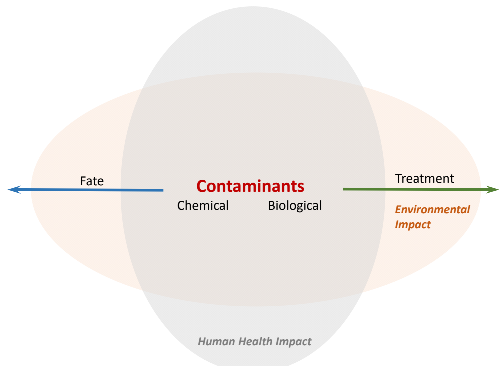
The Interplay Between Chemicals and Microorganisms in Urban Water Systems





Nancy G. Love, Ph.D., P.E., BCEE
University of Michigan
May 13, 2015

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Kartik Chandran, Ph.D.
Columbia University

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2001 - 2010
PAUL L. BUSCH AWARD
Celebrating 10 Years of Innovative Research

Kartik Chandran is pursuing a promising new biological treatment process that transforms methane, a potent greenhouse gas, into a green fuel – methanol. This offers wastewater treatment plants a more affordable, environmentally friendly process for producing this alternative fuel and help reduce nitrogen in effluents.

Kartik Chandran

Columbia University

2010 Paul L. Busch Award Recipient

Andrew Schuler, Ph.D.
University of New Mexico

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Engineered Platforms and Pathways for Resource Recovery from "Waste"

Kartik Chandran
Columbia University

WEF WERF Webcast Paul Busch Award
May 13th, 2015

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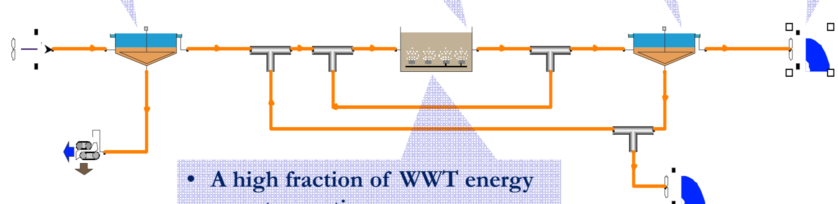
Overview of biological sewage treatment

Solids, inerts separation

Aerobic C &N removal (oxidation)

Recycle of bacteria

Disinfection and discharge



- A high fraction of WWT energy goes to aeration
- $MM in organic chemical purchase
- Bacteria could produce unwanted products (N_2O)

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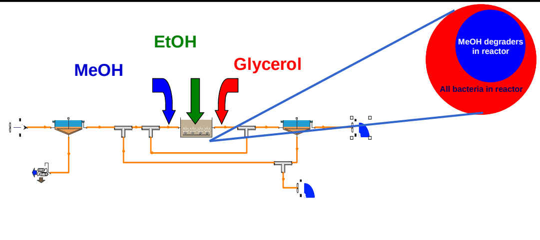
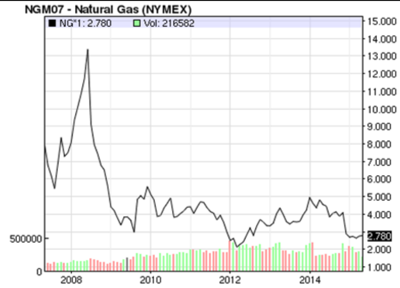
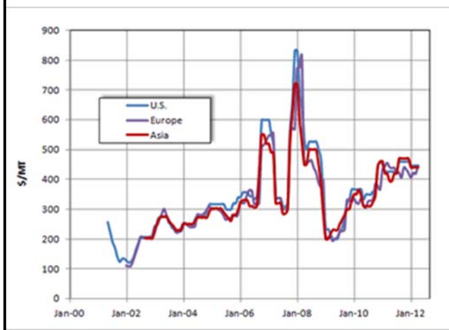
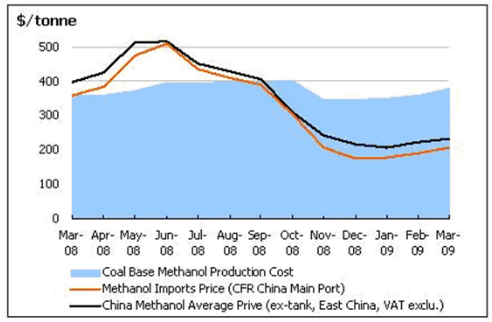


Baytshtok et al., 2008, 2009, Lu et al., 2010, 2011, 2012, 2014







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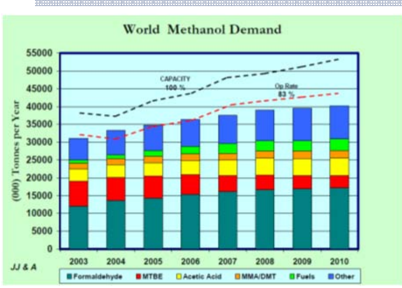
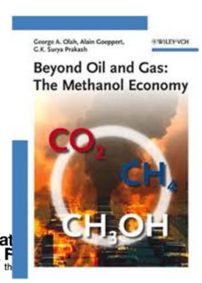
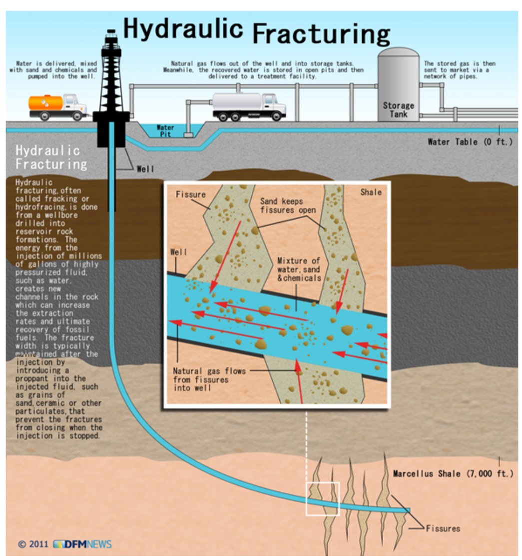
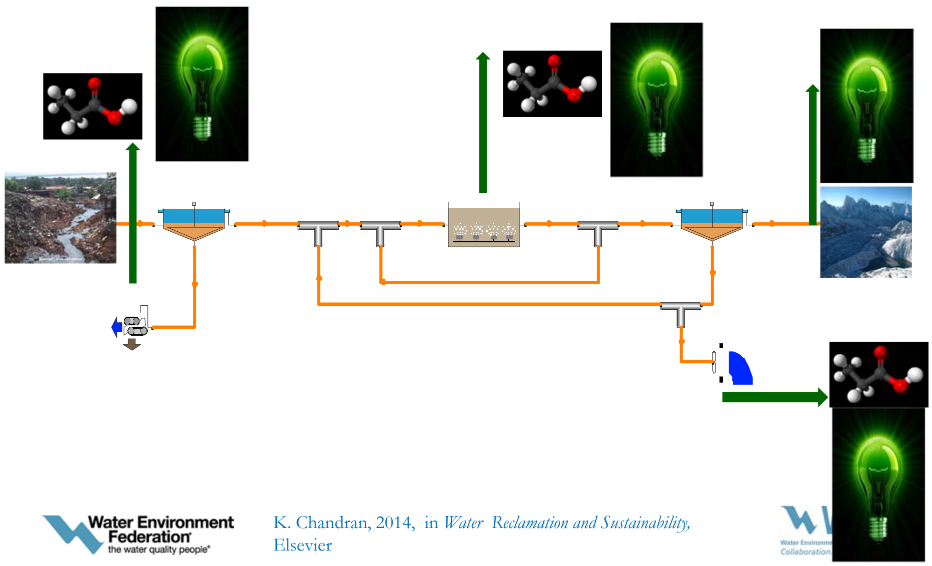
Additional drivers







Shifting to Engineered Resource Recovery from 'Waste' Streams



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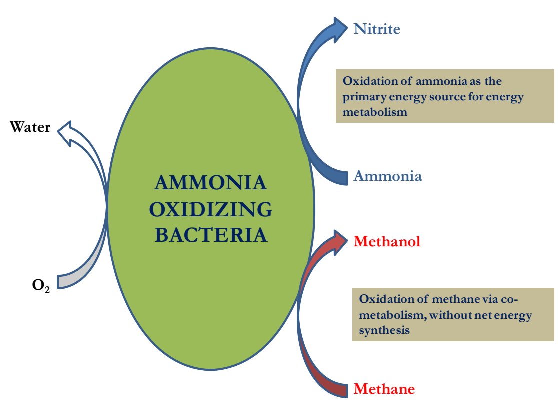
K. Chandran, 2014, in *Water Reclamation and Sustainability*, Elsevier

From Greenhouse Gas to Green Fuel



- Concomitant oxidation of CH_4 and CO_2 fixation
- Prospect of combining C &N cycles

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WERF Paul Busch Award, 2010
Taher and Chandran, ES&T, 2013

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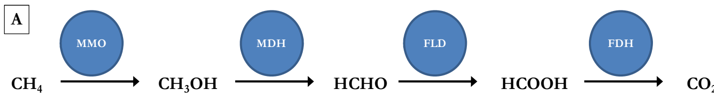
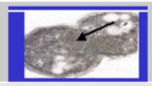
Biological production of methanol



	Type I methanotroph	Type II methanotroph
		
Phylogeny	Gamma proteobacteria	Alpha-proteobacteria
CH_4 oxidation and carbon assimilation	Ribulose mono-phosphate	Serine
Monooxygenase	pMMO	sMMO

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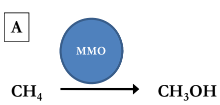
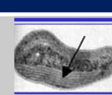


	Type I methanotroph	Type II methanotroph
		
Phylogeny	Gamma proteobacteria	Alpha-proteobacteria
CH_4 oxidation and carbon assimilation	Ribulose mono-phosphate	Serine
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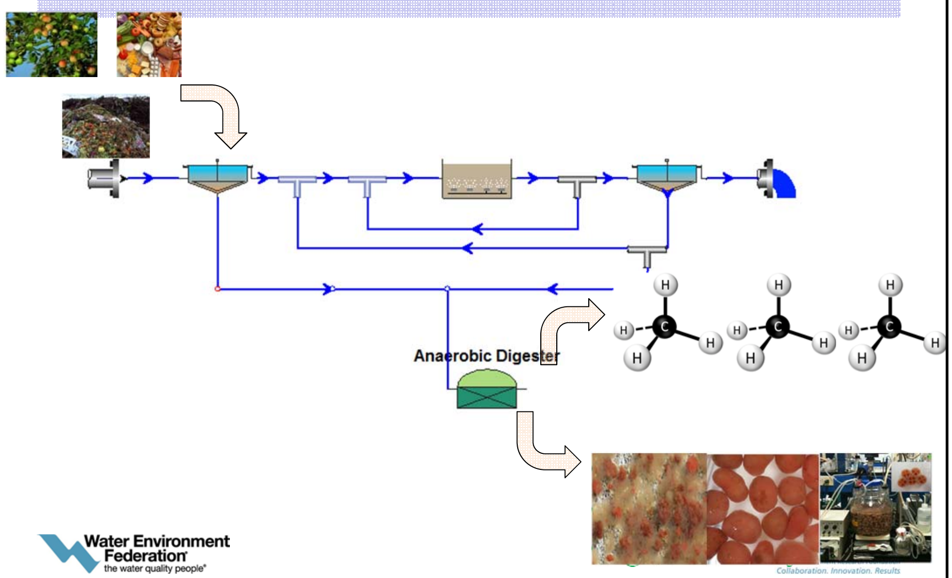
Water Quality-Energy



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Maximum CH_3OH production rate $\frac{\text{mg } CH_3OH \text{ COD}}{\text{mg biomass COD-d}}$	Peak CH_3OH concentration (mg COD/L)	Microbial system used	Reference
0.21	23.47 ± 0.50	Mixed nitrifying cultures NH_3 only feed (FS1)	Taher and Chandran (2013) Paul Busch Award study
0.30	27.50 ± 0.78	Mixed nitrifying cultures NH_2OH only feed (FS2)	
0.22	31.52 ± 1.19	Mixed nitrifying cultures NH_3 and NH_2OH co-feed (FS3)	
0.20	40.71 ± 0.16	Mixed nitrifying cultures NH_3 and NH_2OH alternating feed (FS4)	
0.82	59.89 ± 1.12	Mixed nitrifying cultures NH_2OH only feed with biomass replenishment (high rate)	
0.37	28.8	Pure suspended cultures of Nitrosomonas europaea	Hyman and Wood, 1983
0.31-0.54	NA	Pure suspended cultures of N. europaea	Hyman et al.,, 1988
0.02-0.1	6.2 ± 4.9	Pure immobilized cultures of N. europaea	Thorn, 2007

Phase II. Production in continuous reactors

Electron Source	Max S_{MeOH} $mgCODL^{-1}$	Biomass Normalized Methanol Production Rate $mg\text{-}CH_3OH\text{-}COD^{-1}(mg\text{-}X_{TOT}\text{-}CODd^{-1})$ Maximum	Steady state	HRT (h)
NH_2OH	41 ± 3.4	1.488 ± 0.120	0.084 ± 0.024	7.5
NH_2OH	21 ± 4.6	1.272 ± 0.240	0.144 ± 0.096	2
NH_3	7 ± 2.8	0.192 ± 0.048	0.048 ± 0.024	2

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Sathyamoorthy et al., *unpublished*

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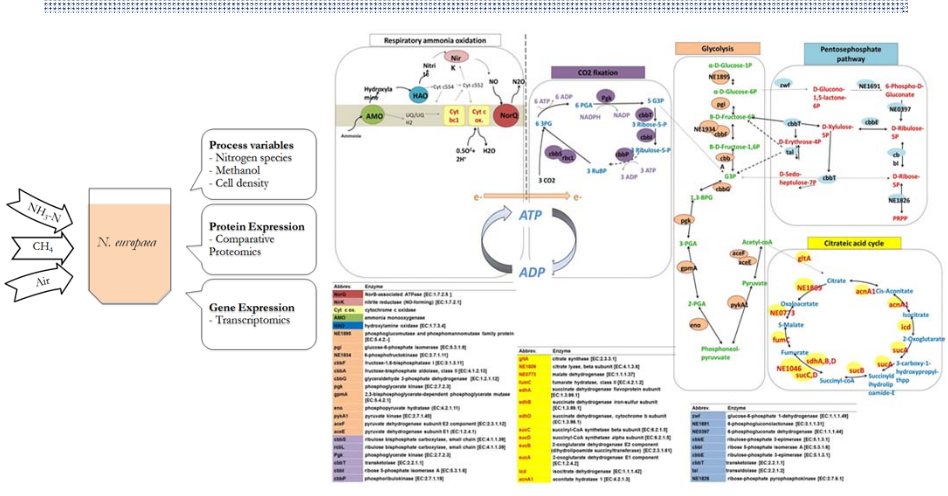
What are the metabolic pathways in AOB?

N-oxidation, CO_2 fixation, MeOH production… and more



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Su *et al., unpublished;* Jiang *et al.,* 2015

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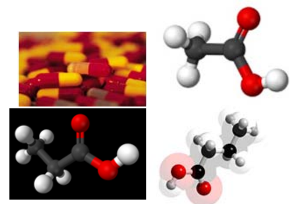
Think beyond CH_4

All based on anaerobic (+) technologies

Biofuels

Biodiesel from food waste at $0.71/L



Commercial chemicals

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Acknowledgements

Kartik Chandran
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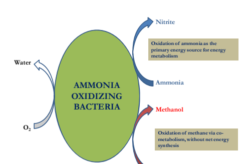

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2001 - 2010
PAUL L. BUSCH AWARD
Celebrating 10 Years of Innovative Research

Andrew Schuler is adapting advances in materials science in order to engineer surfaces of biofilm-based wastewater treatment systems . These systems could one day remove trace organic compounds at rates greater than currently possible.

Andrew Schuler

University of New Mexico

2008 Paul L. Busch Award Recipient

Engineering Better Biofilms: Rational Design of Attachment Surfaces to Improve Their Performance

Andrew Schuler
University of New Mexico

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UNM

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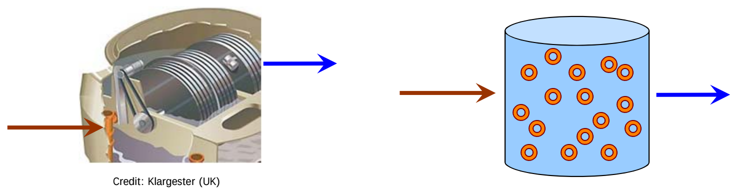
Outline

- Biofilms!
- Can we build a better mousetrap?
  - Surface Chemistry
  - Geometry
- Conclusions, future work

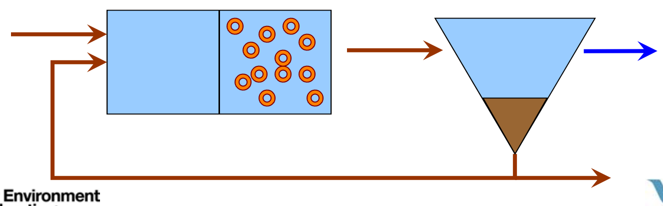
Biofilms are used in many wastewater treatment technologies

Trickling filters and rotating biological contactors

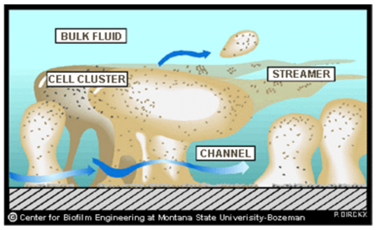
Packed/Moving Bed Bioreactors (MBBRs)



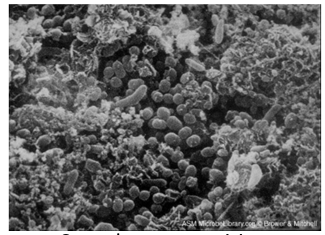
Integrated fixed film activated sludge (IFAS)



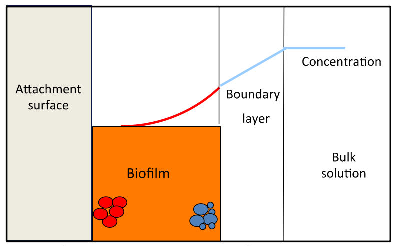
Biofilms are complex!



Complex structures



Complex composition



Complex interactions with environment

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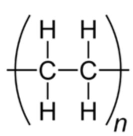
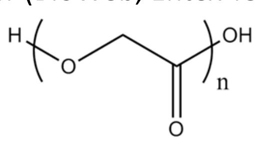
http://microwavesscience.blogspot.com/2011/05/biofilms-at-11.html

Biofilm attachment surface media

- Many shapes and sizes
- Commonly hydrophobic plastic, e.g. high density polyethylene (HDPE)
  - Durable, extrudable, inexpensive



- Polyester (BioWeb, Entex Technol.)



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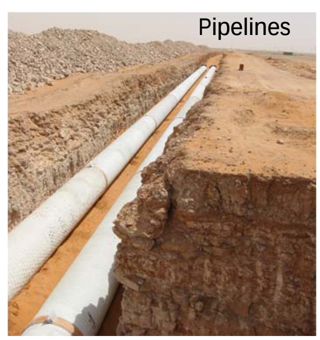
Can we do better?

Objective: strategically design surfaces to improve performance, and for specific functions

Much research previously devoted to reducing bacterial attachment

- Focus on control of biofouling – modified surface chemistries



We have the reverse goal: designing surfaces to enrich for beneficial biofilms

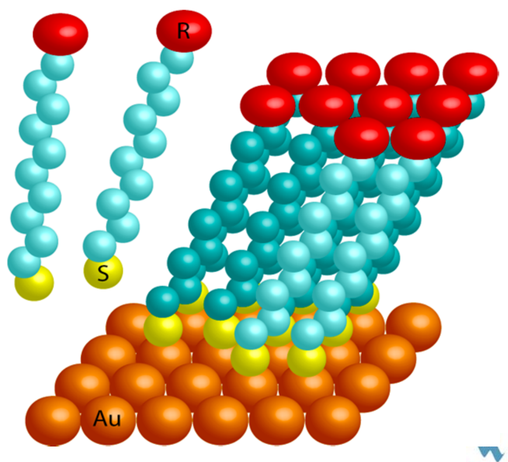
A tool to create chemically well-defined surfaces: Self-assembled monolayers (SAMs)

Terminal functional group (designable)

Alkane chain

Sulfur attachment group

Gold substrate



Examples:
NH_2^+,
CH_3,
OH^-,
COO^-

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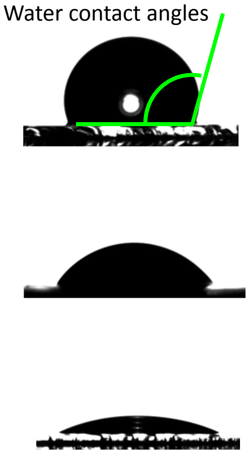
Changing functional groups changes surface properties

Water contact angles



	Contact Angle Θ_w	Surface Energy (mJ/m^2)
CH_3 SAM	108°	16.1
Nitrosomonas multiformis	56°	45.2
OH^- SAM	23°	50.2
	Decreasing hydrophobicity	Increasing surface energy

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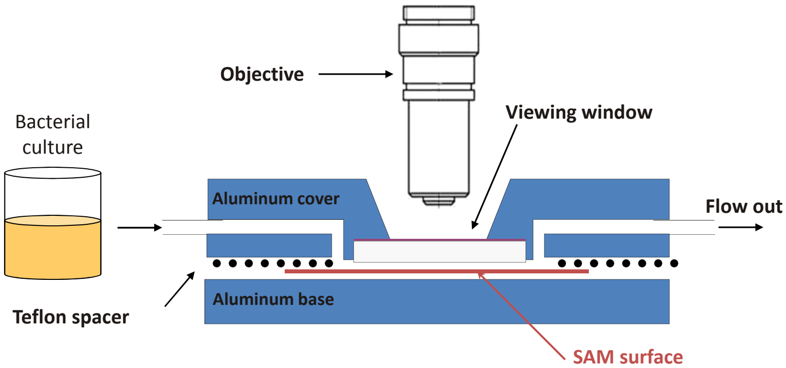
What we’re really interested in:
Can we relate surface energy to attachment?



Microscope-mounted flow cell for monitoring of bacterial attachment to SAMs

Focus on nitrifiers - Ammonia oxidizing bacteria (AOB)

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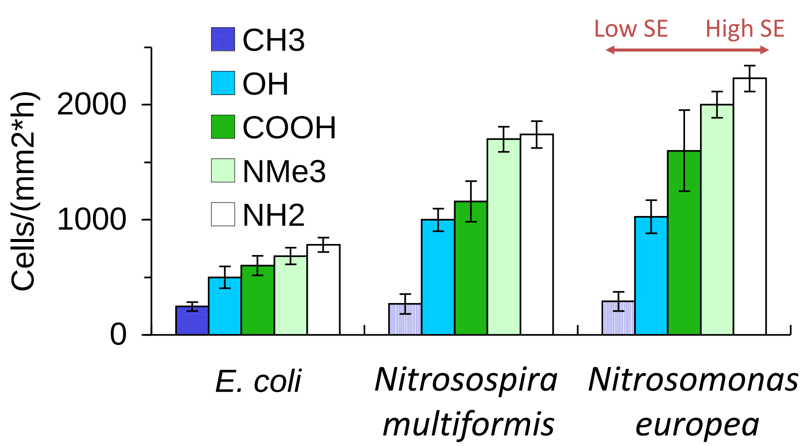
All strains attached preferentially to higher surface energy (SE) surfaces



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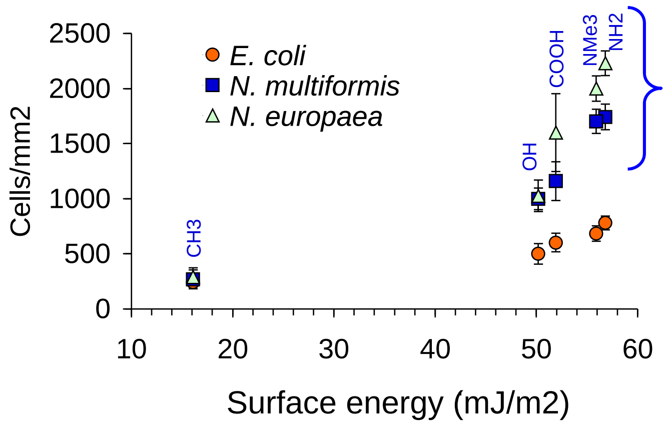
Surface energy was a good predictor of attachment



Nitrifiers attached particularly well to high SE surfaces

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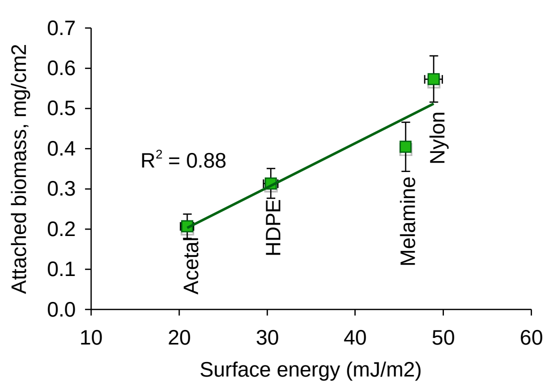
Apply results to growth on real plastics: More biofilm on higher surface energy surfaces



Plastic sheets incubated in activated sludge for 2 weeks

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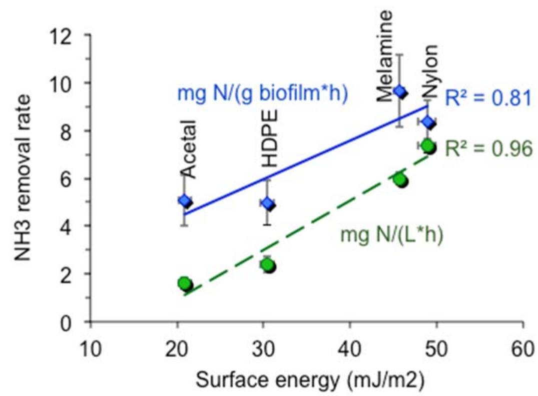
More nitrification on higher surface energy surfaces



High SE gave more biomass, <u>and</u> greater activity/ biomass

Biomass was enriched with nitrifiers.

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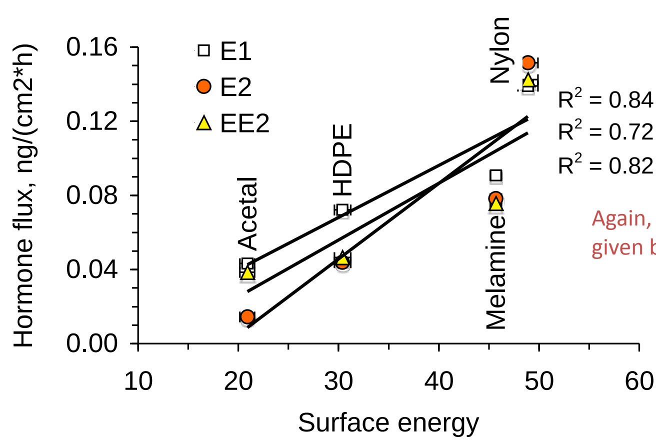
More hormone removal on higher surface energy surfaces



Again, not surprising, given biomass result

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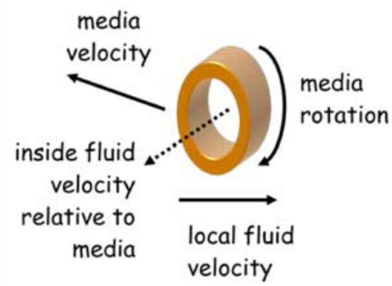
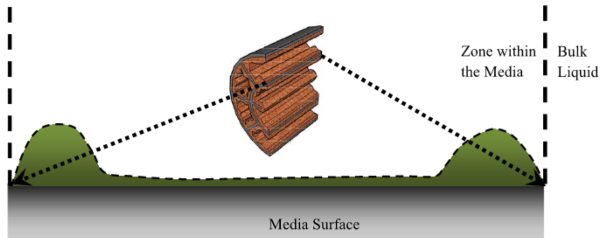
Media Geometry

Attachment media are available in many shapes and sizes.
How does this affect populations and their activity?



Melcer and Schuler (2014), WERF Report U4R11

Insight: most biofilm located near ends of media.
So should design maximize edges, minimize interior?



Bjornberg et al. 2009. Effect of temperature on biofilm growth dynamics and nitrification kinetics in a full-scale MBBR system, WEFTEC proceedings

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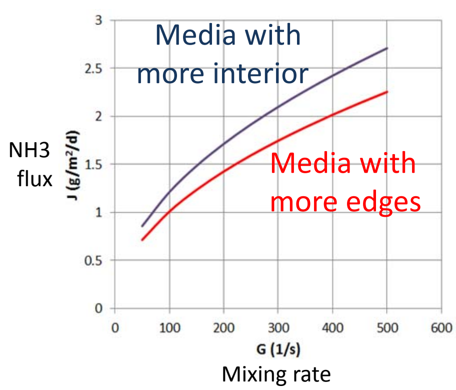
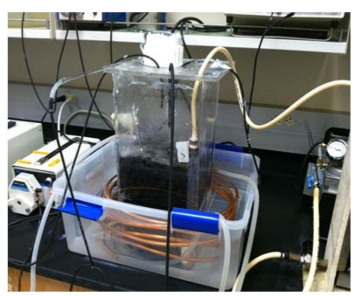
WERF study on mixing effects





Worse performance by "high edge" media seems to contradict goal of minimizing media "interior"

Melcer and Schuler (2014), WERF Report U4R11

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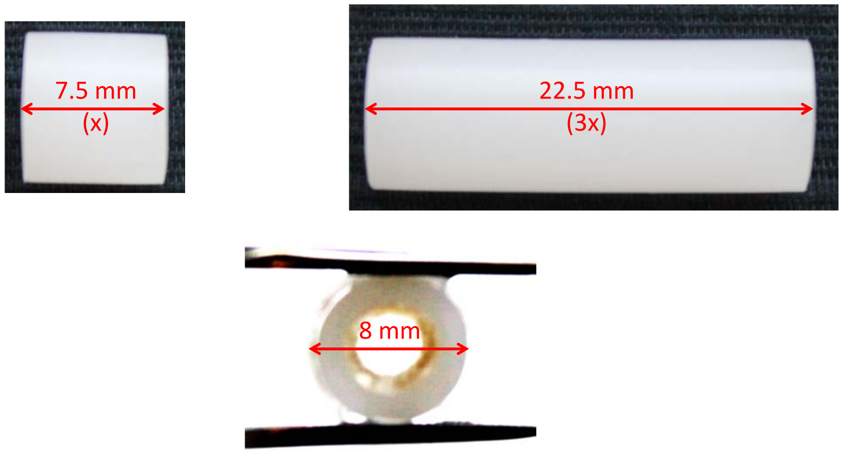
Test end effects in systematic manner using custom media



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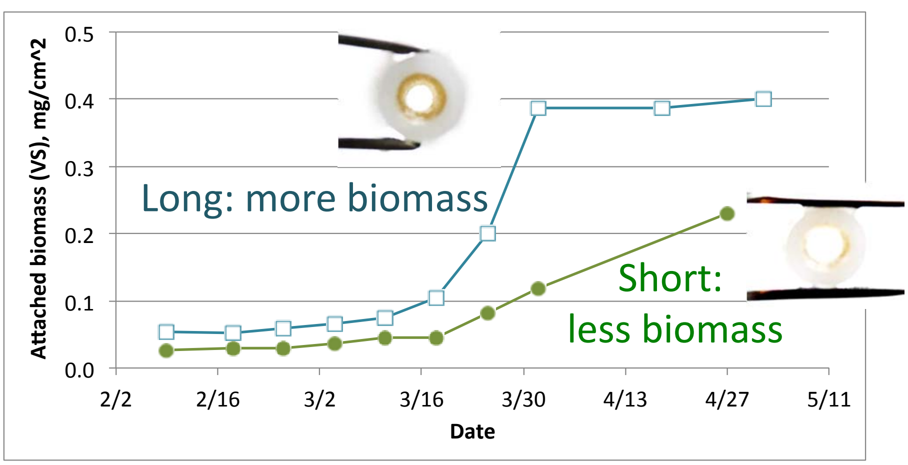
Long media produces more biofilm/area (surprise?)



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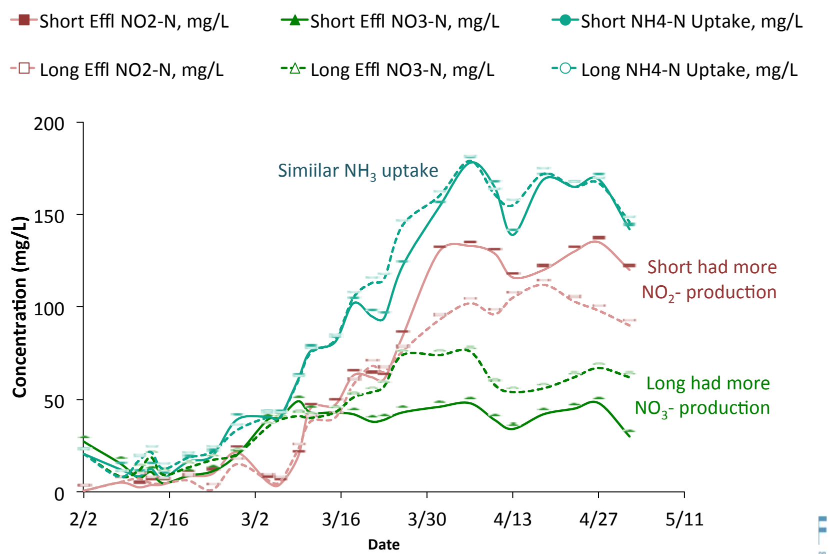
Longer media gave more "complete" nitrification (to NO_3^-)



3D printing of media

channel depth

1mm 2mm 3mm 5mm

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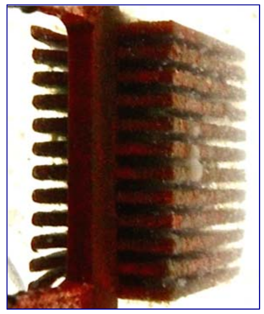
Growth on 5mm Depth channels over time

Growth on domestic primary effluent

10 days



15 days

50 days

Next steps

- Apply 3D printing to study of biofilm depth, geometry effects
- Combine chemical modifications with geometric modifications
- Analyze effects on community spatial heterogeneity

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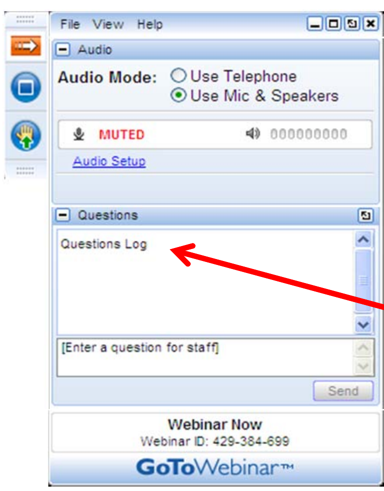
How to Ask Your Questions



- **Audio Modes**
  - Listen using Mic & Speakers
  - Or, select "Use Telephone" and dial the conference (please remember long distance phone charges apply).
- **Submit your questions using the Questions pane.**
- **A recording will be available for replay shortly after this webcast.**

Thank You

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