

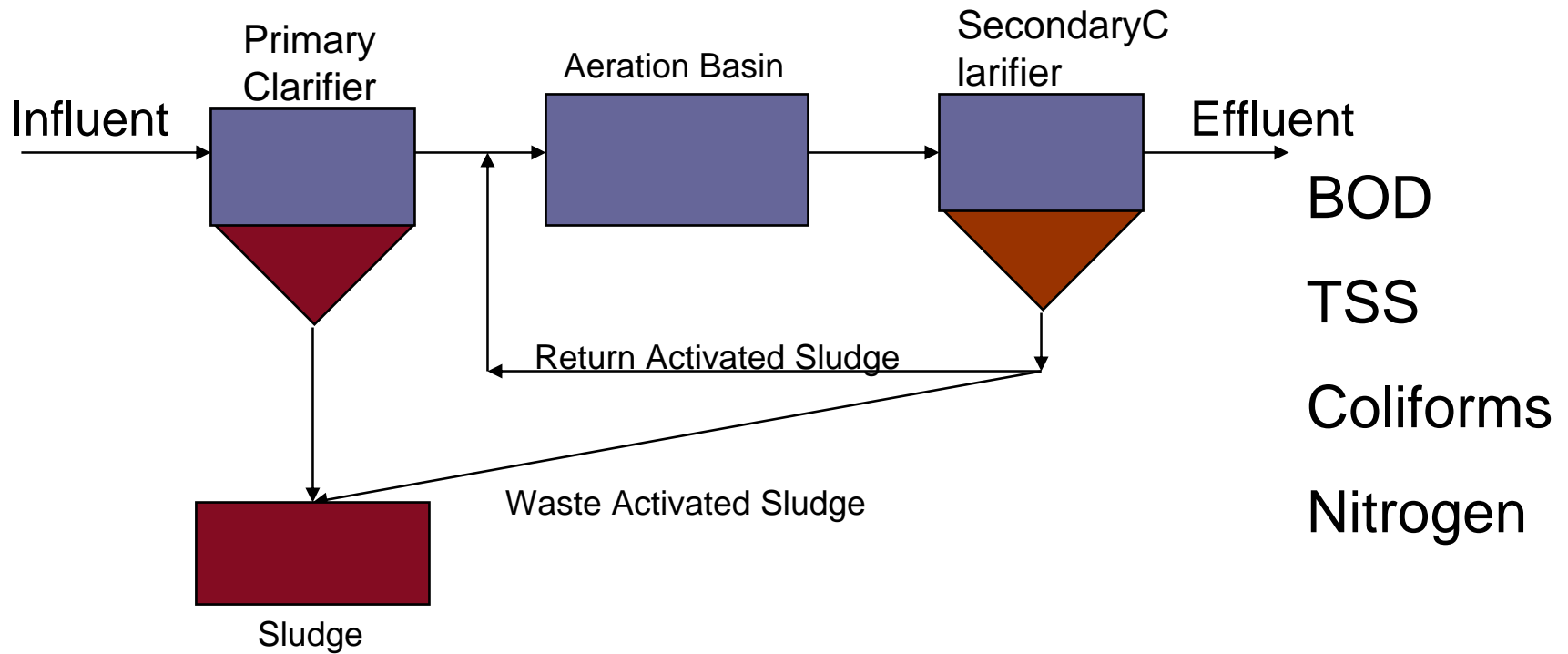
Characterization of Proteins in Domestic Wastewater Effluent Discharged to the Connecticut River

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Outline

- 1) Wastewater Treatment Plant
- 2) Regulatory Water Quality Parameters for Effluent
- 3) Water Quality Issues
- 4) Research
- 5) Future Work

Wastewater Treatment Plant Schematic



Regulatory Water Quality Effluent Parameters

- BOD = Biological Oxygen Demand
 - 5-day test, measure dissolved oxygen (DO) before & after
 - higher BOD means higher oxygen demand from effluent

- TSS = Total Suspended Solids
 - can carry micro-organisms and organics

- Coliform Bacteria
 - indicate pathogenic organisms

- Acute toxicity tests
 - twice per year
 - *Ceriodaphnia dubia*

Water Quality Parameters - NPDES Permit

2008 Reissuance, Page 2 of 15
NPDES No. MA0101818

Part I.A.

1. During the period beginning the effective date and lasting through expiration, the permittee is authorized to discharge treated effluent from outfalls 001 and 002* to the Connecticut River and Old Mill River respectively. Such discharge shall be limited and monitored by the permittee as specified below.

<u>EFFLUENT CHARACTERISTIC</u>	<u>EFFLUENT LIMITS</u>						<u>MONITORING REQUIREMENTS</u>	
	Mass Limits			Concentration Limits			MEASUREMENT FREQUENCY	SAMPLE TYPE ³
PARAMETER	AVERAGE MONTHLY	AVERAGE WEEKLY	MAXIMUM DAILY	AVERAGE MONTHLY	AVERAGE WEEKLY	MAXIMUM DAILY		
FLOW ¹	***	***	***	8.6 MGD	***	Report MGD	CONTINUOUS	RECORDER
FLOW ¹	***	***	***	Report MGD	***	Report MGD	CONTINUOUS	RECORDER
BOD ₅ ²	2152 lbs/Day 978 kgs/Day	3228 lbs/Day 1467 kgs/Day	Report	30 mg/l	45 mg/l	Report mg/l	2/WEEK	24-HOUR COMPOSITE ⁴
TSS ²	2152 lbs/Day 978 kgs/Day	3228 lbs/Day 1467 kgs/Day	Report	30 mg/l	45 mg/l	Report mg/l	2/WEEK	24-HOUR COMPOSITE ⁴
pH RANGE ⁵	6.0 - 8.3 SU SEE PERMIT PAGE 6 OF 15, PARAGRAPH I.A.2.b						1/DAY	GRAB
FECAL COLIFORM ^{6,6} (April 1- October 31)	***	***	***	200 cfu/100 ml	***	400 cfu/100 ml	2/WEEK	GRAB
<i>E. coli</i> ^{5,6} (April 1- October 31)	***	***	***	126 cfu/100 ml	***	409 cfu/100 ml	2/WEEK	GRAB
CHLORINE, TOTAL RESIDUAL ^{6,7} (April 1- October 31)	***	***	***	1.0 mg/l	***	1.0 mg/l	1/DAY (when in use)	GRAB

Water Quality Parameters - NPDES Permit

PARAMETER	AVERAGE MONTHLY	AVERAGE WEEKLY	MAXIMUM DAILY	AVERAGE MONTHLY	AVERAGE WEEKLY	MAXIMUM DAILY	MEASUREMENT FREQUENCY	SAMPLE TYPE ³
TOTAL NITROGEN ⁸	Report lbs/Day	***	Report lbs/Day	Report mg/l	***	Report mg/l	1/Week	24-HOUR COMPOSITE ⁴
TOTAL NITRITE + NITRATE	***	***	***	Report mg/l	***	Report mg/l	1/Week	24-HOUR COMPOSITE ⁴
TOTAL KJELDAHL NITROGEN	***	***	***	Report mg/l	***	Report mg/l	1/Week	24-HOUR COMPOSITE ⁴
TOTAL NITROGEN ⁸ (influent)	Report lbs/Day	***	Report lbs/Day	Report mg/l	***	Report mg/l	1/Week	24-HOUR COMPOSITE ⁴
TOTAL NITRITE + NITRATE (Influent)	***	***	***	Report mg/l	***	Report mg/l	1/Week	24-HOUR COMPOSITE ⁴
TOTAL KJELDAHL NITROGEN (Influent)	***	***	***	Report mg/l	***	Report mg/l	1/Week	24-HOUR COMPOSITE ⁴
TOTAL AMMONIA AS N	***	***	***	Report mg/l	***	Report mg/l	1/Week	24-HOUR COMPOSITE ⁴
WET ^{9, 10, 11}	ACUTE LC ₅₀ ≥ 50%						2/YEAR	24-HOUR COMPOSITE ⁴

Water Quality Issues

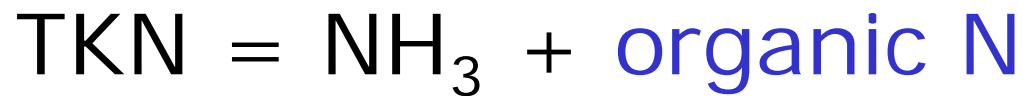
- Long Island Sound Study
 - eutrophication in summer huge problem
 - early 1990s – approx. 60% Nitrogen load from POTWs

- 12 POTWs discharge directly to CT River in Massachusetts

- 127 MGD permitted flow

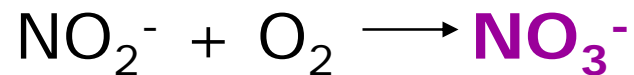
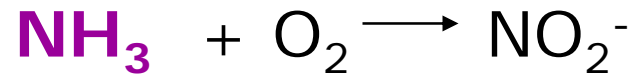
- 8,900 lbs Nitrogen released per day
assuming effluent concentration of 10 mg/L TN

Nitrogen

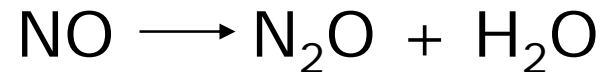


Nitrification / Denitrification

- Nitrification:



- Denitrification:



Research - Organic Nitrogen

- Dissolved organic nitrogen (DON) is Persistent
 - Free amino acids
 - Combined amino acids (Proteins)
 - Humic acids
 - amino acid fragments
 - other N-containing organic matter
 - EDTA
 - NDMA precursors

Research - Proteins

- What proteins are in the effluent from POTWs?
 - are they from influent?
 - are they from secondary treatment?

- Are they bio-available? When?

- Are there active enzymes in the effluent?
 - if so, how do they interact with the riverine environment?

- Proteins are difficult to measure
 - proteomics allows us to learn more about proteins

Research - Proteomics

Developed in Biology and Genetics:

- The study of the expression, location, functions, and interactions of proteins in a cell or organism.

Applied to an ecosystem (wastewater):

- The study of the presence, activity, and interactions- the fate and transport of proteins in an environment.

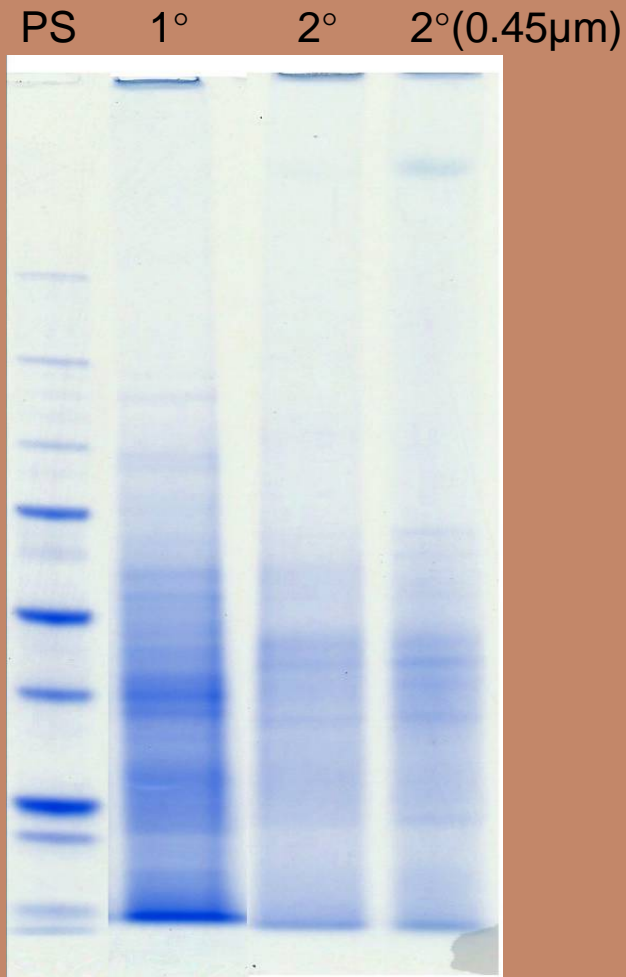
Research- Tools of Proteomics

- SDS-Page
 - sodium dodecyl sulfate polyacrylamide gel electrophoresis
 - separate protein fragments by size on gel
 - stain gel or protein fragments to visualize

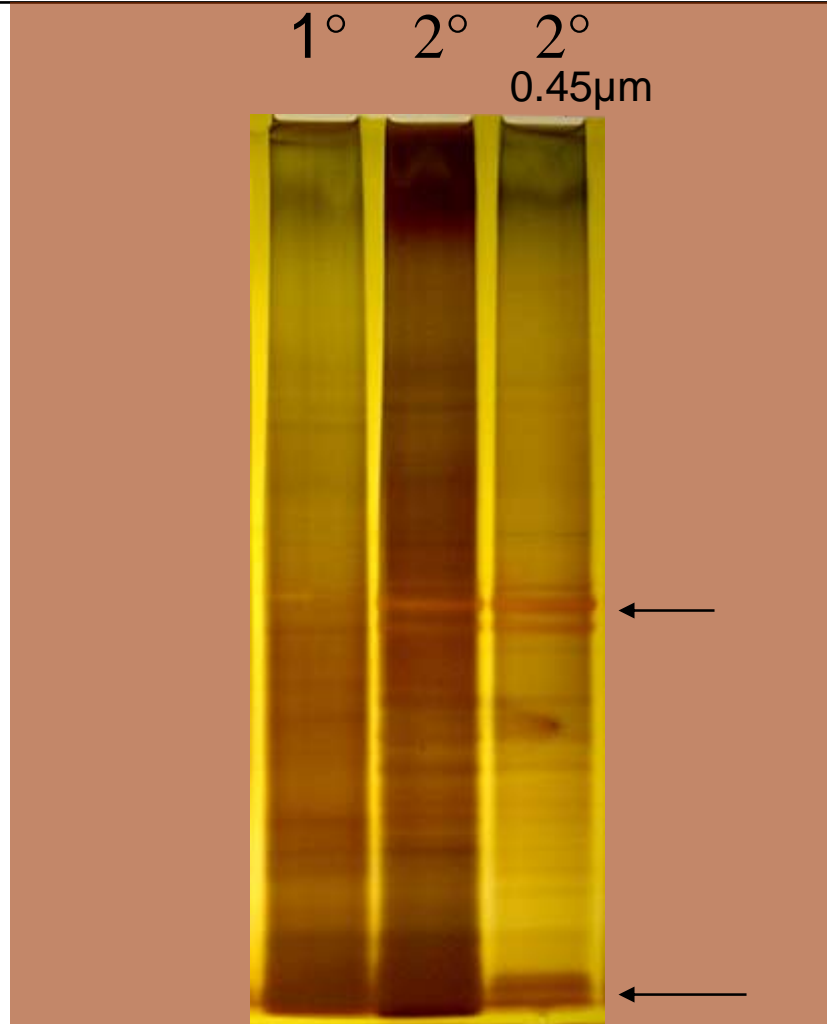
- Zymography
 - gel infused with proteins (casein, gelatin)
 - separate proteins on gel
 - incubate and stain
 - areas of gel with active enzymes won't stain

- Mass-spectrometry
 - Measures protein mass

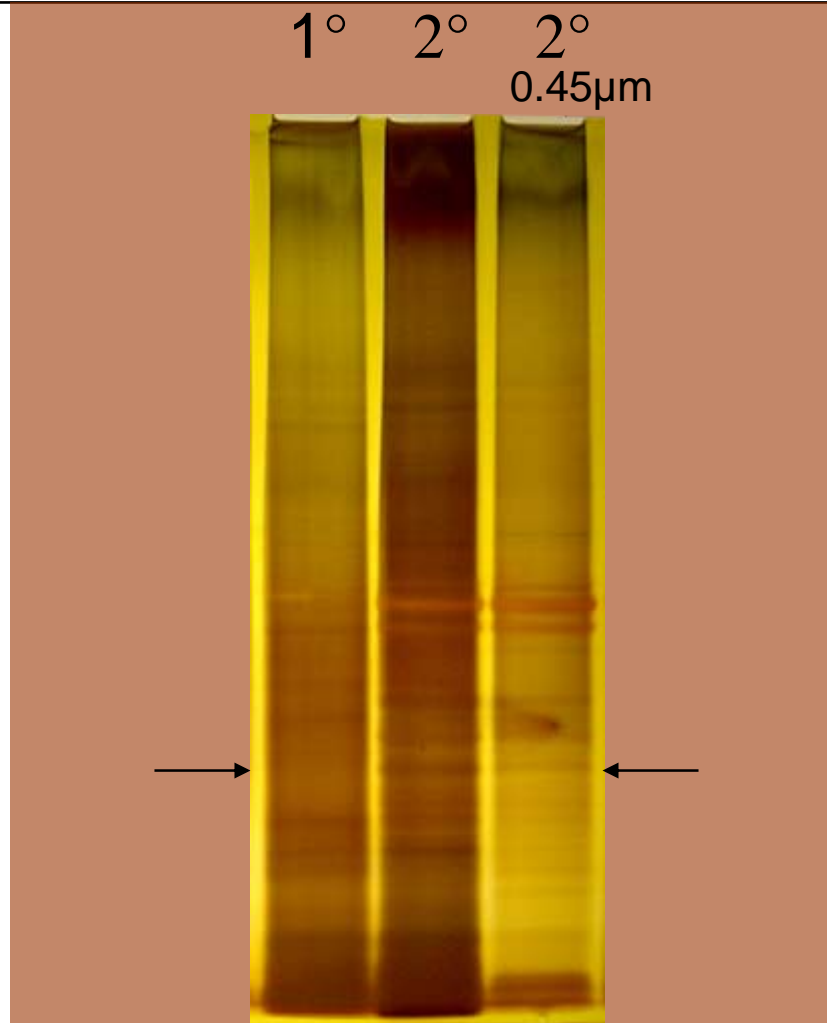
Research – Recalcitrant Proteins



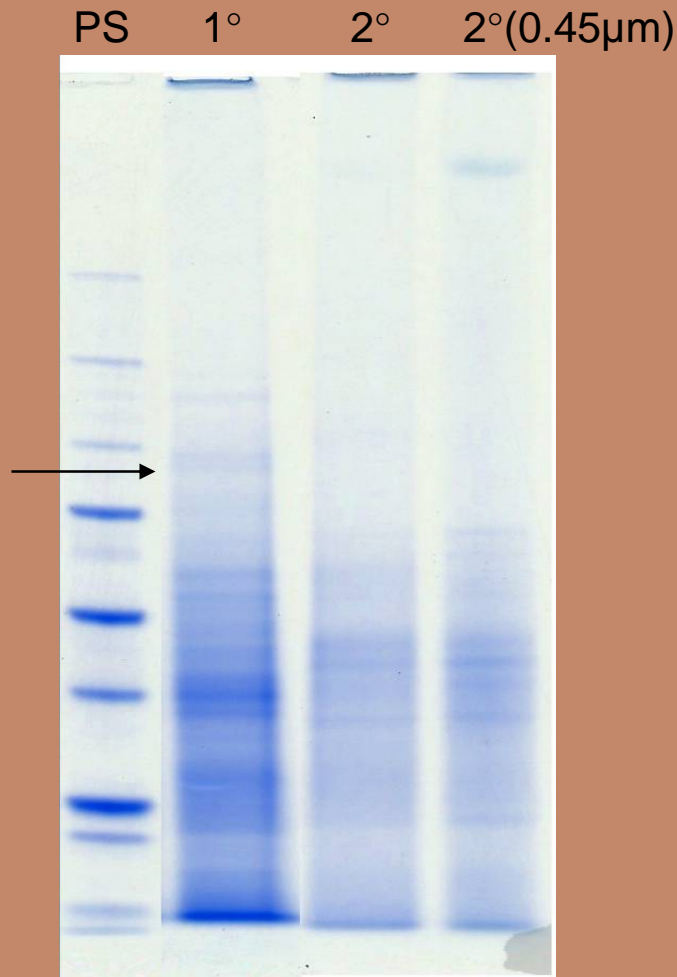
Research – Recalcitrant Proteins



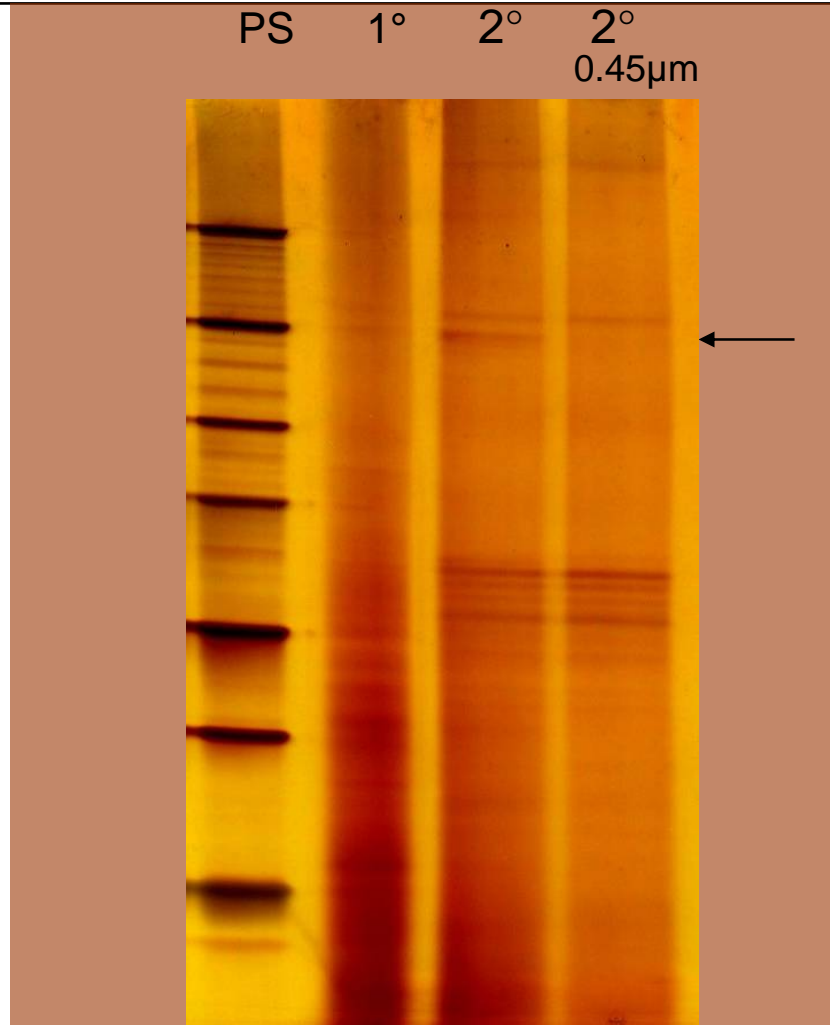
Research – Proteins Generated in Secondary Treatment



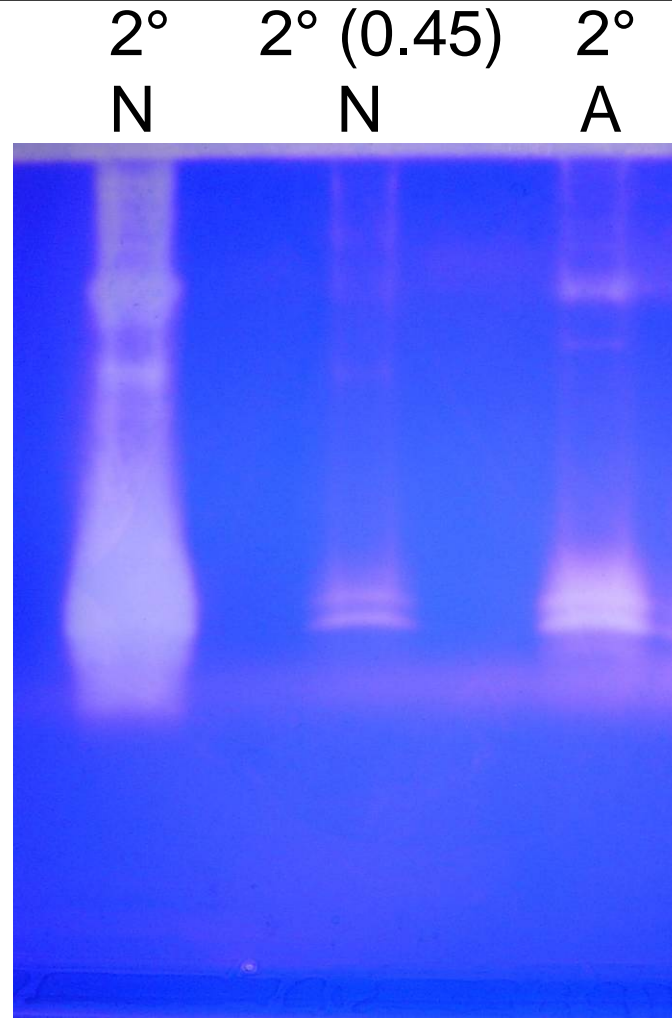
Research – Some Proteins Removed



Research – 0.45 μ m Filter Removes some Proteins



Research – Active Enzymes



Research – Parameters Measured

- Protein quantity and fragment patterns
- Enzyme activity
- TSS/VSS
- COD
- TN
- TP
- ammonia, nitrate, nitrite (IC)

Research - Conclusions

- Significant amount of proteins and organic N are transported to the CT River in POTW effluents.
- Some influent proteins *persist* and pass through secondary treatment processes.
- Some proteins *generated* during secondary treatment are released in the effluent.
- High level filtration (0.45 μ m) does not remove many of these proteins.
- Some effluent proteins are active enzymes.

Future Research

Short term:

- Continue to expand data set through collection & processing of samples
- Refine mass balance of proteins
- Identify protein bands of interest with mass spectrometry

Future Research

This summer:

- Expand mass spectrometry data set of effluent protein to create a mass 'fingerprint' from each plant.
- Laboratory bioassays – characterize proteins in effluent before and after incubation with receiving water to evaluate the bioavailability of effluent proteins.
- Field study - monitor the fate of proteins in the receiving water using proteomic datasets.

Acknowledgements

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