

SCOPE AND APPLICATION OF ANAEROBIC MEMBRANE BIOREACTORS (AnMBR) IN WASTEWATER TREATMENT

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**EU-CHINA WORKSHOP ON RESOURCE RECOVERY FROM BIOMASS AND
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1) Background

Energy, C footprint, solids, resource recovery, SAMBRs, effect of SMPs/colloids on membrane fouling

2) Results and analysis:

- A1) Measuring “critical flux” over time, and membrane fouling layer composition
- A2) Characterisation of proteinaceous materials and carbohydrates in wastewater systems
- A3) Effect of critical gassing rate and HRT on reactor supernatant composition/viscosity/floc size, and membrane fouling layer
- B) Rapid toxicity measurement using a fluorescent assay, and toxicity amelioration
- C) Control of organic and metal toxicity in AD using powdered activated carbon (PAC) and EDTA (chelating agent)

3) Conclusions

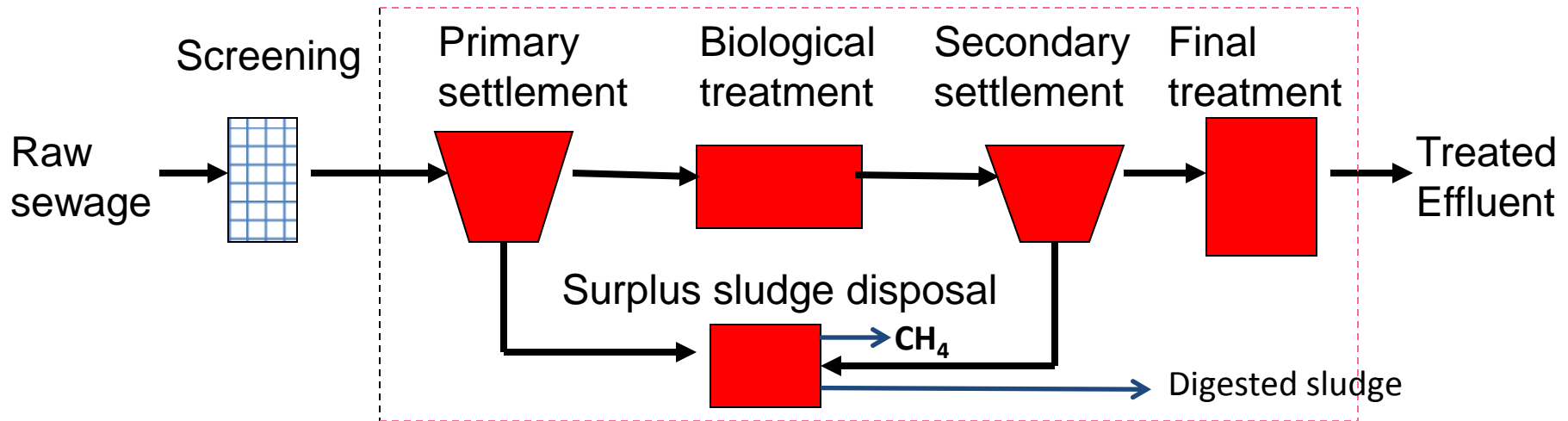
1) GENERAL BACKGROUND

- Issues today- energy use, solids disposal, carbon footprint, water scarcity, and nutrient recycling(?).
- Conventionally aerobic treatment chosen- more historical accident than rational choice.
- Few “drivers” to change choice until recently.
- **Anaerobic processes** most “rational” choice to address issues above. However, must compete with aerobic on removal efficiency, short HRTs, stability to toxins.
- With slow anaerobic growth rates must separate hydraulic retention time (HRT) from solids retention time (SRT) through reactor design, and make them more “robust” to toxins and fluctuating loads.


1) SAMBR BACKGROUND

- A1) When we measure “critical flux” over time does it change, and how does the membrane fouling layer build up over time?
- A2) Are the colorimetric methods we use today to measure protein and carbohydrates in WW accurate-what do they measure?
- A3) Is there a “critical gassing rate” for fixed fluxes, and how does this affect the fluid dynamics in a SAMBR, and the membrane fouling layer?
- B) AD treating industrial has issues with toxicity-can we develop a rapid (<10 min) toxicity sensor to warn us?
- C) If we do know about toxicity events, how can we ameliorate them?

“CONVENTIONAL TREATMENT TRAIN”



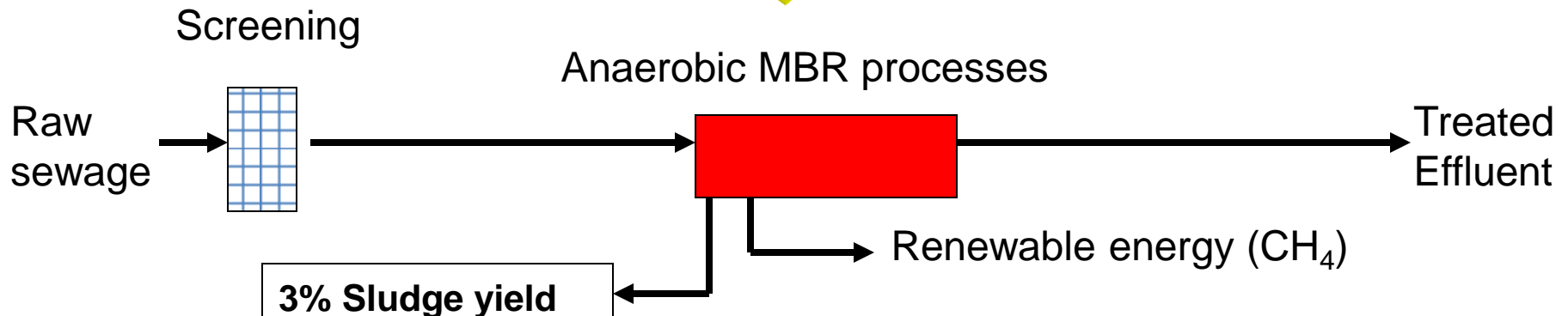
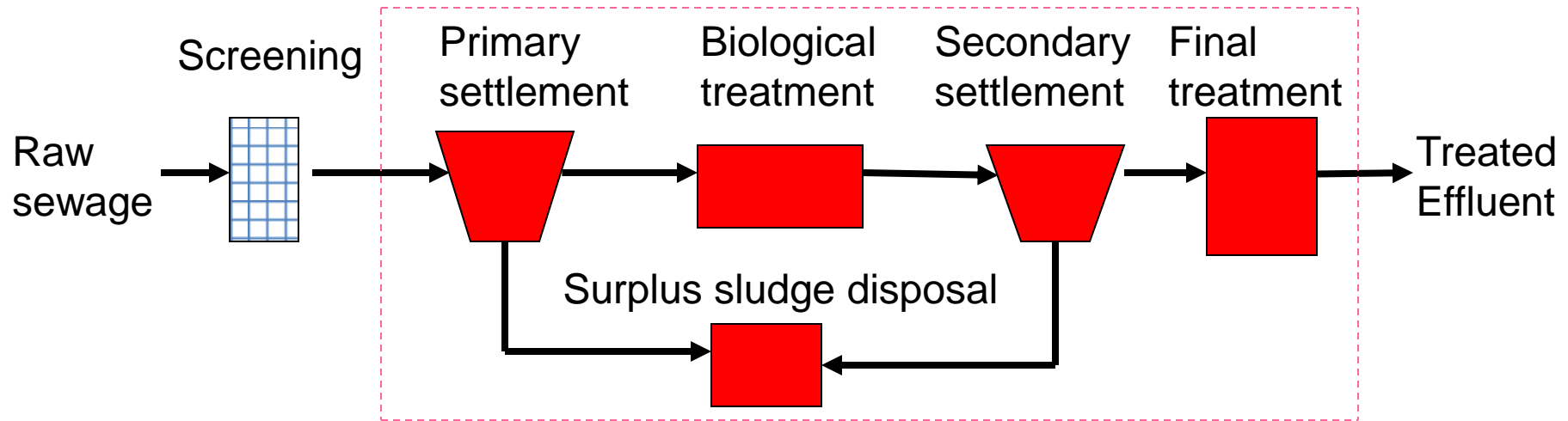
Why was this process flowsheet chosen? Historical!

- High energy demanding due to aeration-no product
- High solids generating- $Y \sim 0.4$
- Low organic loading rates $\sim 0.5 \text{ kgCOD/m}^3.\text{d}$
- Volatiles to atmosphere, AND CO_2 , CH_4 , N_2O -GHG
- Not “rational” today! 

**??? Submerged Anaerobic
Membrane Bioreactor (SAMBR or AnMBR)**

WHY USE A SAMBR?

CAPABLE OF “TURN UP/TURN DOWN” DURING PEAK FLOWS?



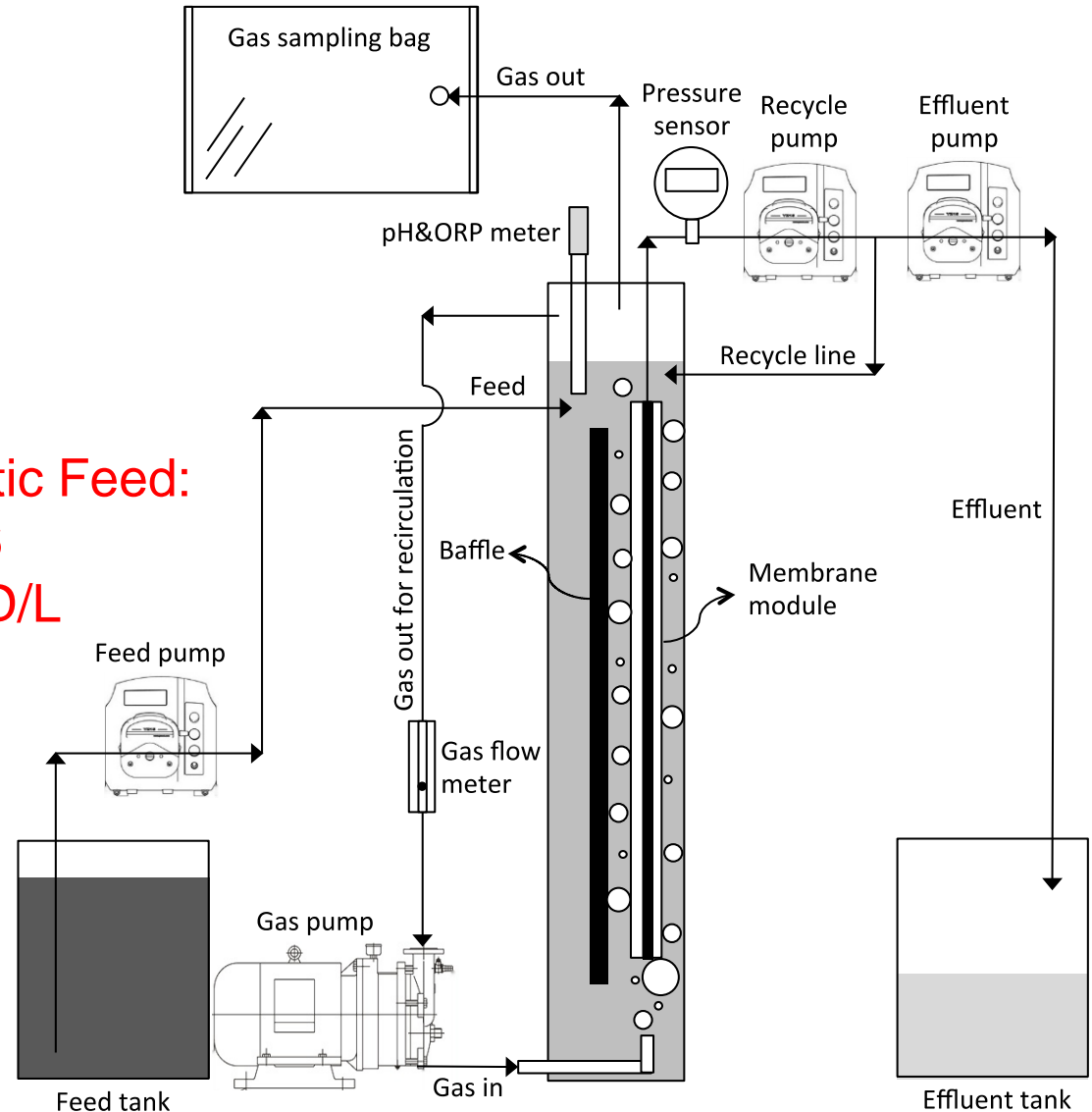
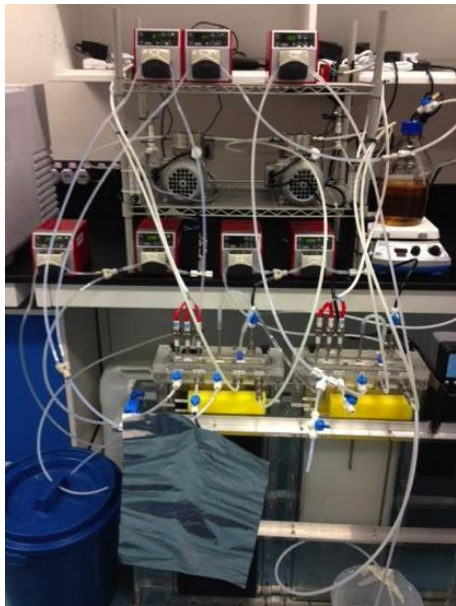
What is a SAMBR?

Operating parameters & SAMBR set up

HRT: 12, 8, 6, 4, 2, 1 hr
(SRT 200 days)

SRT: 50, 100, 200 days
(HRT 6 hrs)

Synthetic Feed:
 507 ± 56
mgCOD/L

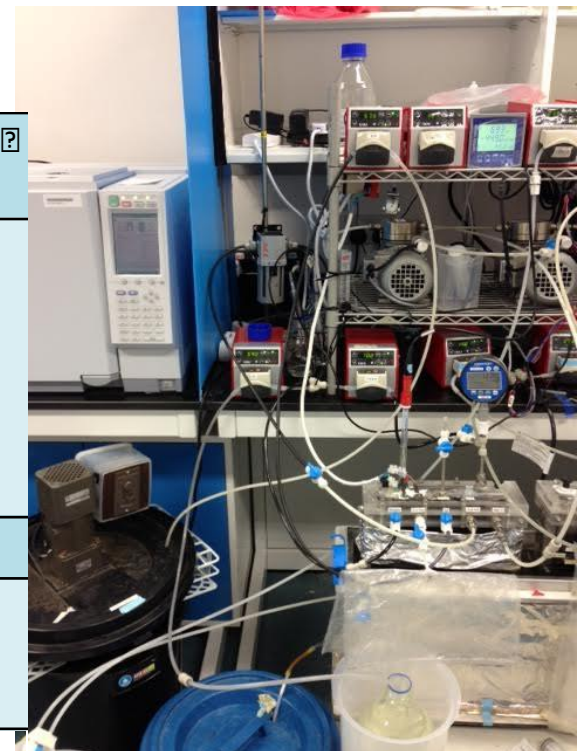


SAMBR PERFORMANCE AT DIFFERENT HRTs AND HYDRAULIC SHOCK

SAMBR (Flat Sheet)

HRT (hr)	Period (day)	COD _{inf} (mg/L)	COD _{eff} (mg/L)	Eff. (%)	Mass COD removed (g COD/d)	Methane (%)
12	69	533±68	14±8	97±2	3.1	69±2
8	23	502±34	12±4	98±1	4.4	72±2
6	46	520±35	16±4	97±0	6.0	74±1
4	23	484±62	12±4	97±2	8.5	75±3
2	6	458±73	26±6	94±2	15.6	80±2
1	12 hrs	432±29	85±3	80±1	25.0	78±3
SRT (day)						
50	44	474±15	17±2	96±1	5.5	74±3
100	57	544±22	15±1	97±1	6.3	74±1
200	46	520±35	16±4	97±0	6.0	74±1

Note: Eff. = Efficiency



HRT: 12hr → 8 hr → 4 hr → 2hr → 1hr

Membrane- flux: 12-15-28 LMH, gas rate 3-4 LPM, pH: 6.8 – 7.2

A1) MEASURING “CRITICAL FLUX” OVER TIME, AND MEMBRANE FOULING LAYER COMPOSITION

- Does the time one holds the constant flux to measure “critical flux” affect the value, and do flat sheet and hollow fibre have different CFs?
- What is the composition and MW of the fouling layer? How accurate are the current analytical methods?

FACTORS INFLUENCING MEMBRANE FOULING

1) Membrane fouling → lower fluxes → greater area/cost.
BUT, fouling leads to ↑ COD removal due to gel layer.

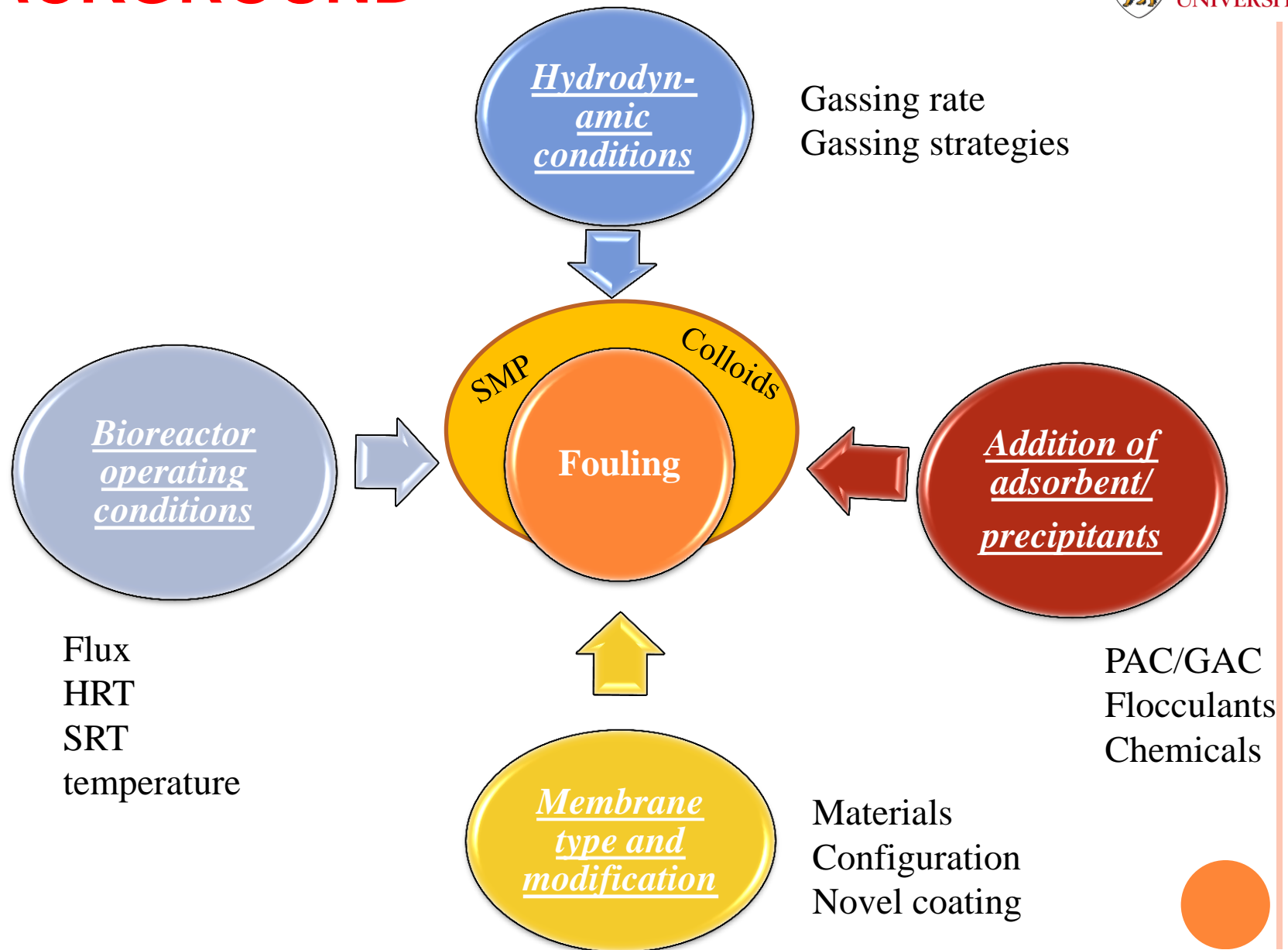
2) What fouls membrane? -soluble microbial products (SMPs) and colloids. SMPs dictate COD removal.

$$[SMP = COD_{\text{soluble effluent}} - COD_{\text{soluble VFAs + feed}}]$$

3) What are SMPs/colloids in terms of composition?
How are they produced? How does reactor operation affect [SMP] and composition, and how does this influence fouling?

Objective of this work is to measure the “critical flux” over different times and “critical gassing rate” analyse membrane fouling layer and correlate this to fouling

BACKGROUND

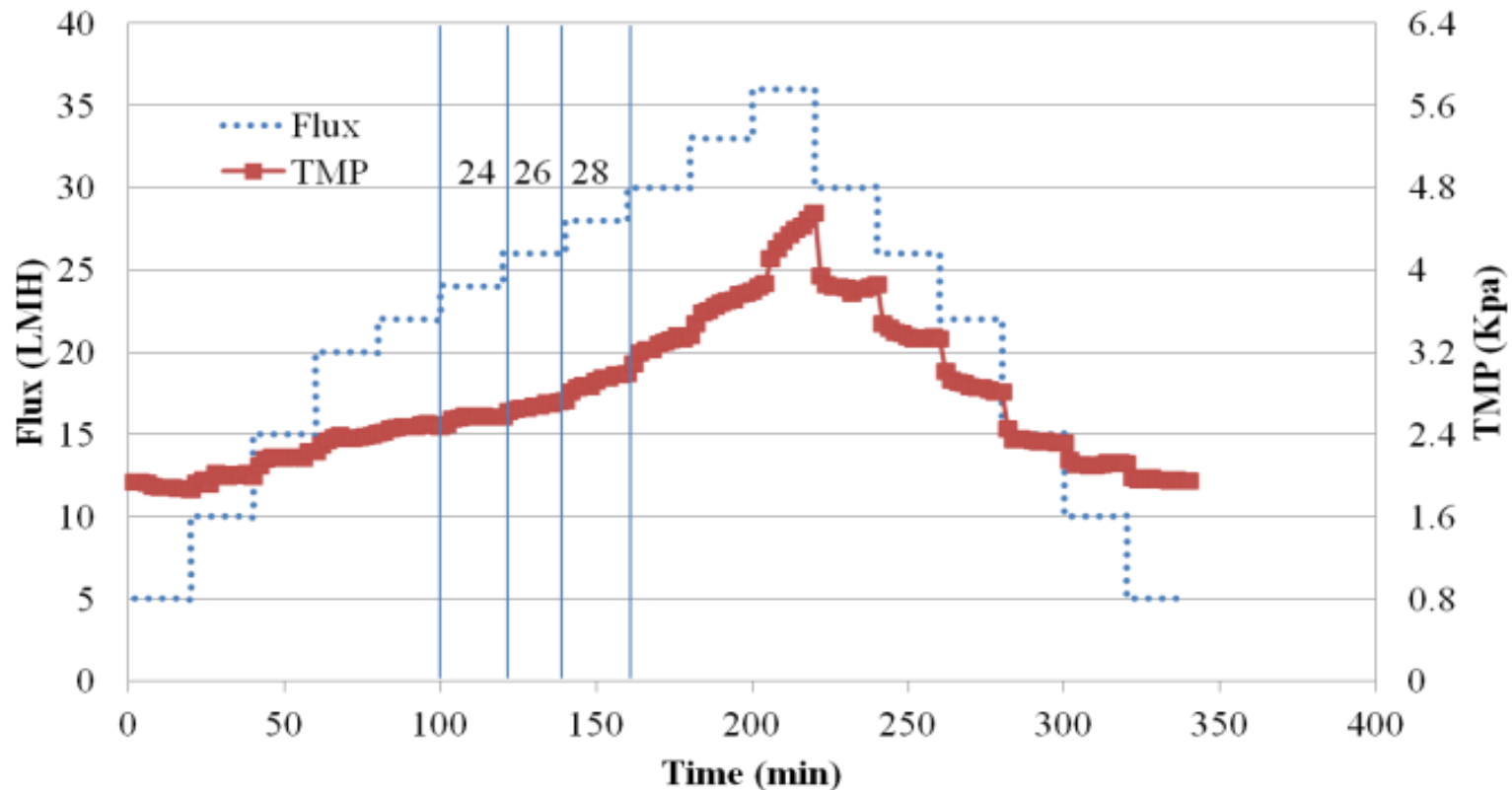


Analytical parameters	Analytical methods
COD	K ₂ Cr ₂ O ₇ method
Protein	BCA standard kits
Polysaccharide	Phenol-sulphuric acid method
TMP	Data logging
CH ₄	Gas Chromatography (Shimadzu)
SMP-SEC	High performance liquid Chromatography (Shimadzu)
Zeta potential	Malvern Zetasizer Nano Series
Particle Size	Malvern Zetasizer Nano Series
Sludge floc size	Malvern Mastersizer 2000
Membrane autopsy	Field emission scanning electron microscope (JSM-7600F)
Element autopsy	FESEM(JSM-7600F)

Assays
virtually
useless
for WW
analysis!
(see later
in A2!)

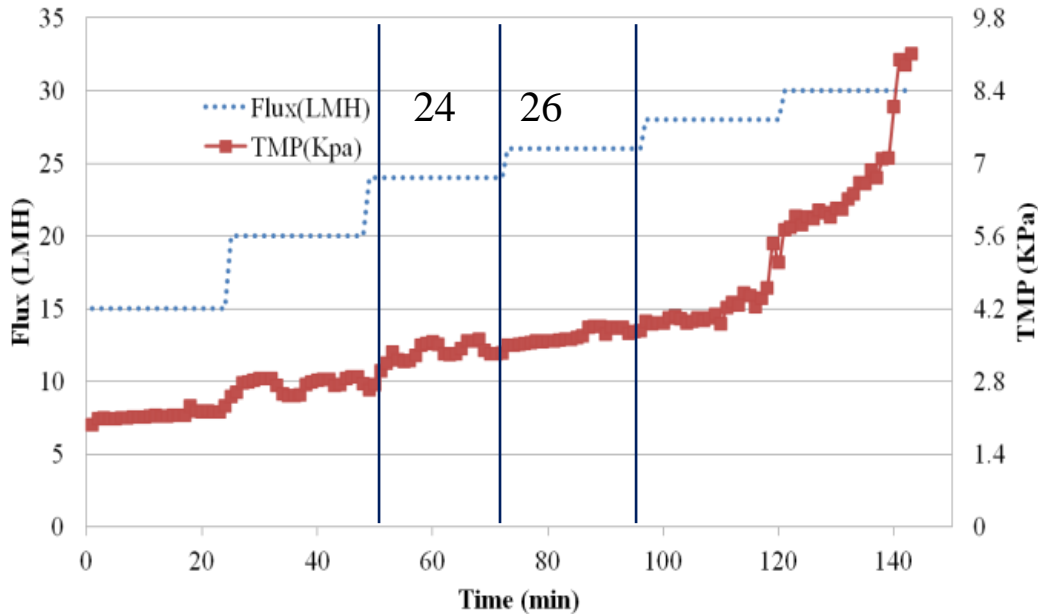
(HRT=6h, SRT>150d, 5 LPM)

1) Determine the critical flux (flux at which $\Delta\text{TMP} \sim 0$) - STRATEGY 1: 20 MIN PER STEP



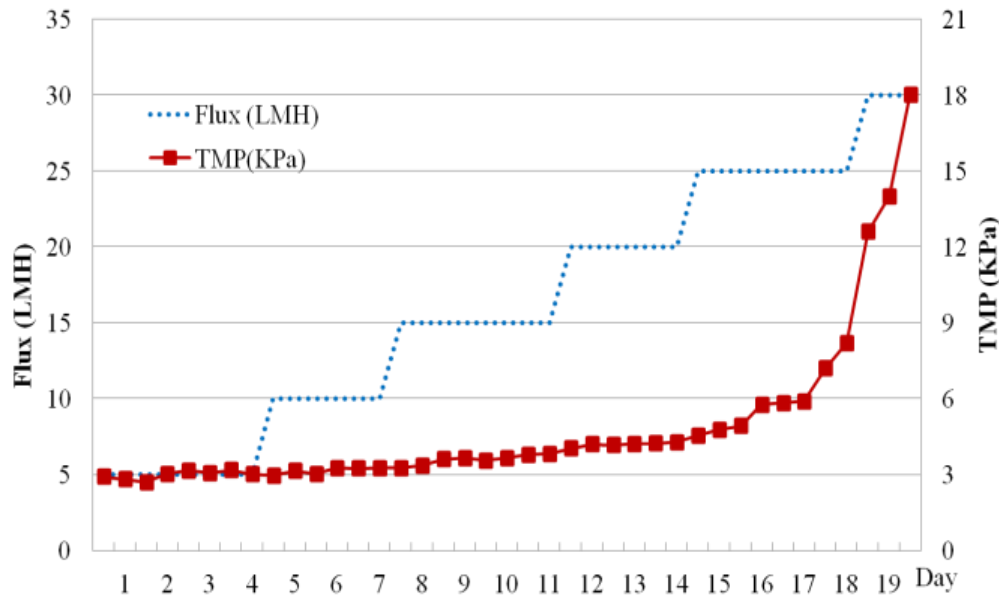
Flux	22	24	26	28
dTMP/dt (KPa/min)	1.1	0.6	5.3	9.1





Strategy 2: 24 hours

Flux	24	26
dTMP/dt (KPa/m)	0.13	1.0

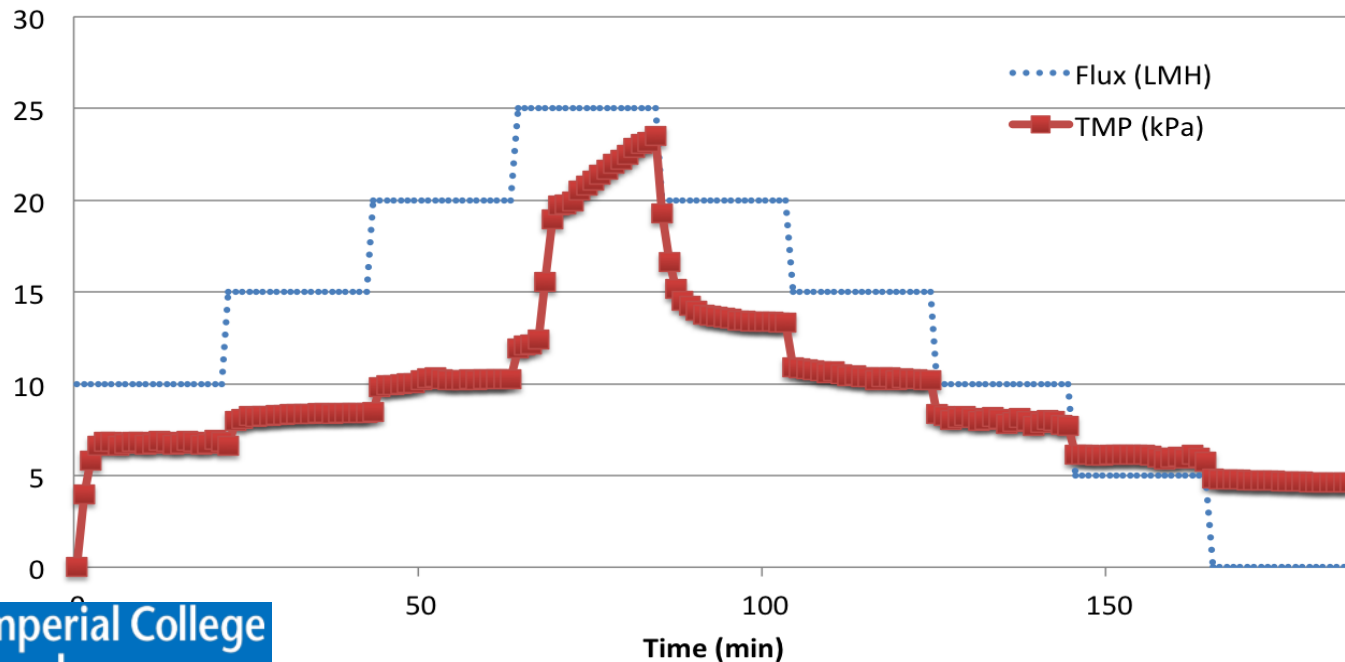
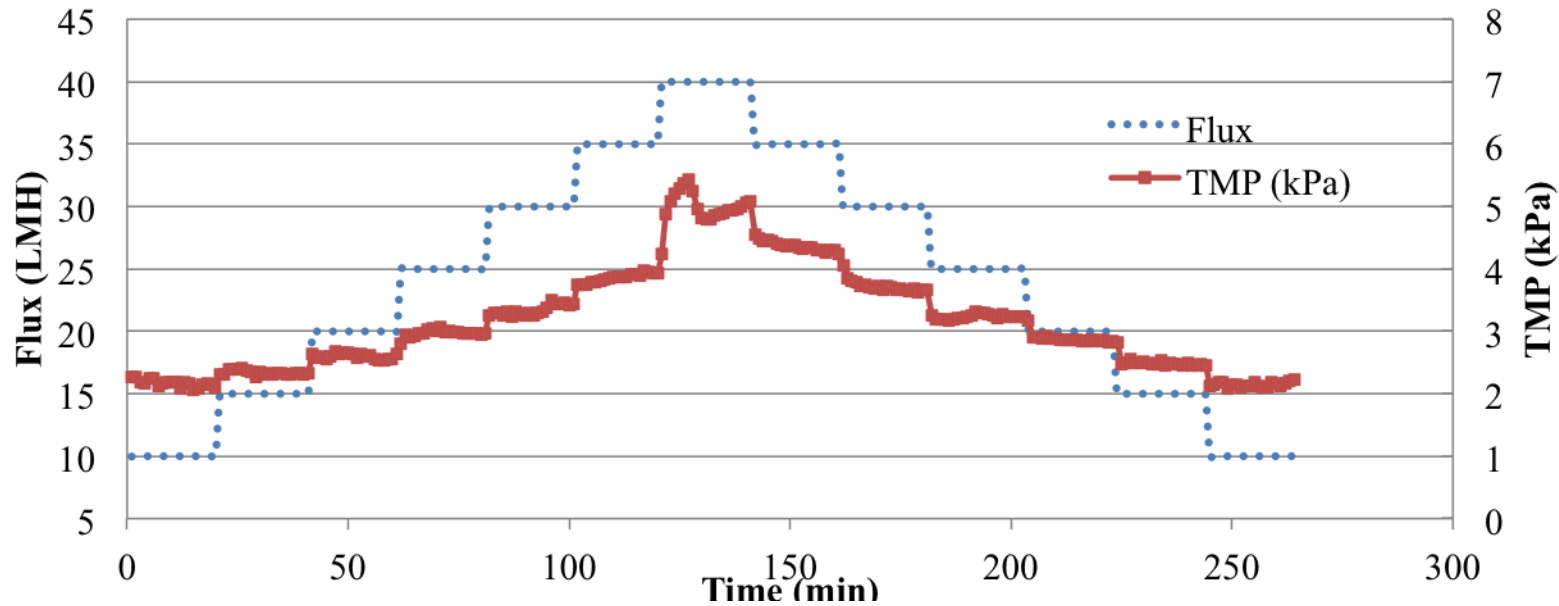


Strategy 3: 3 – 4 days

Since different time periods all give similar answers with fouling, the critical flux of the defined SAMBR is 24 LMH



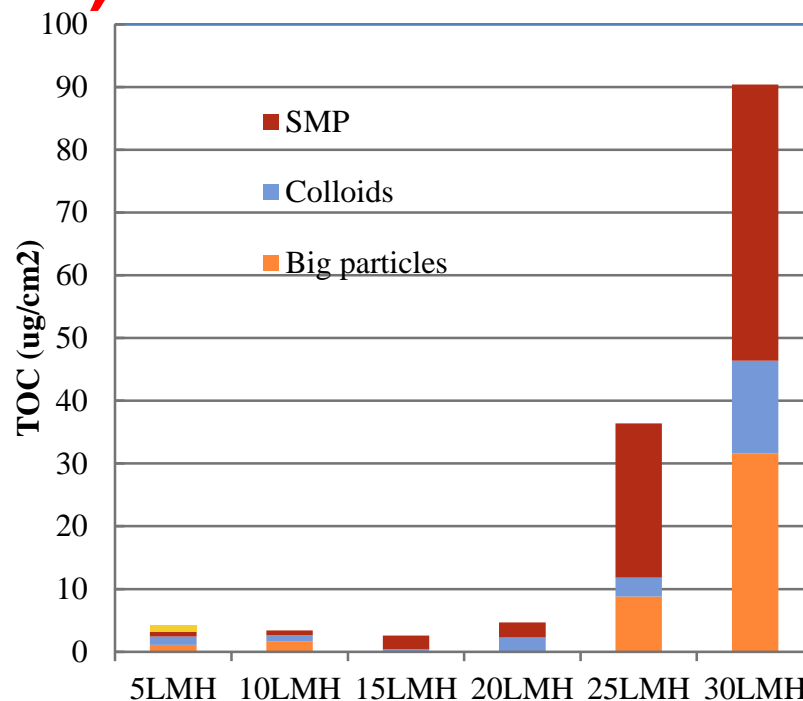
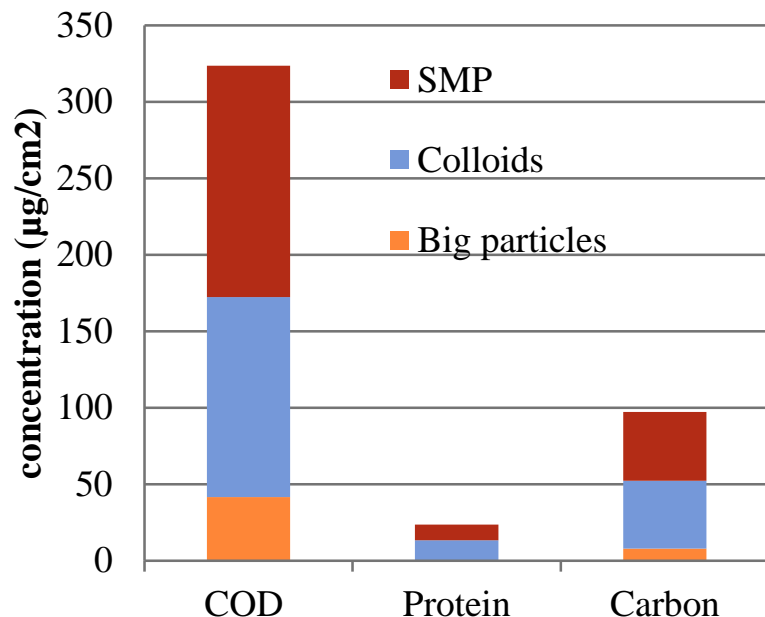
Critical Flux evaluation of hollow fibre reactor



Critical flux in hollow fibre between 25 and 30 LMH- maybe slightly higher than flat sheet.



Concentration of the MEMBRANE foulants (scrapped off surface)



At the end of strategy 2 (24 h at each flux)

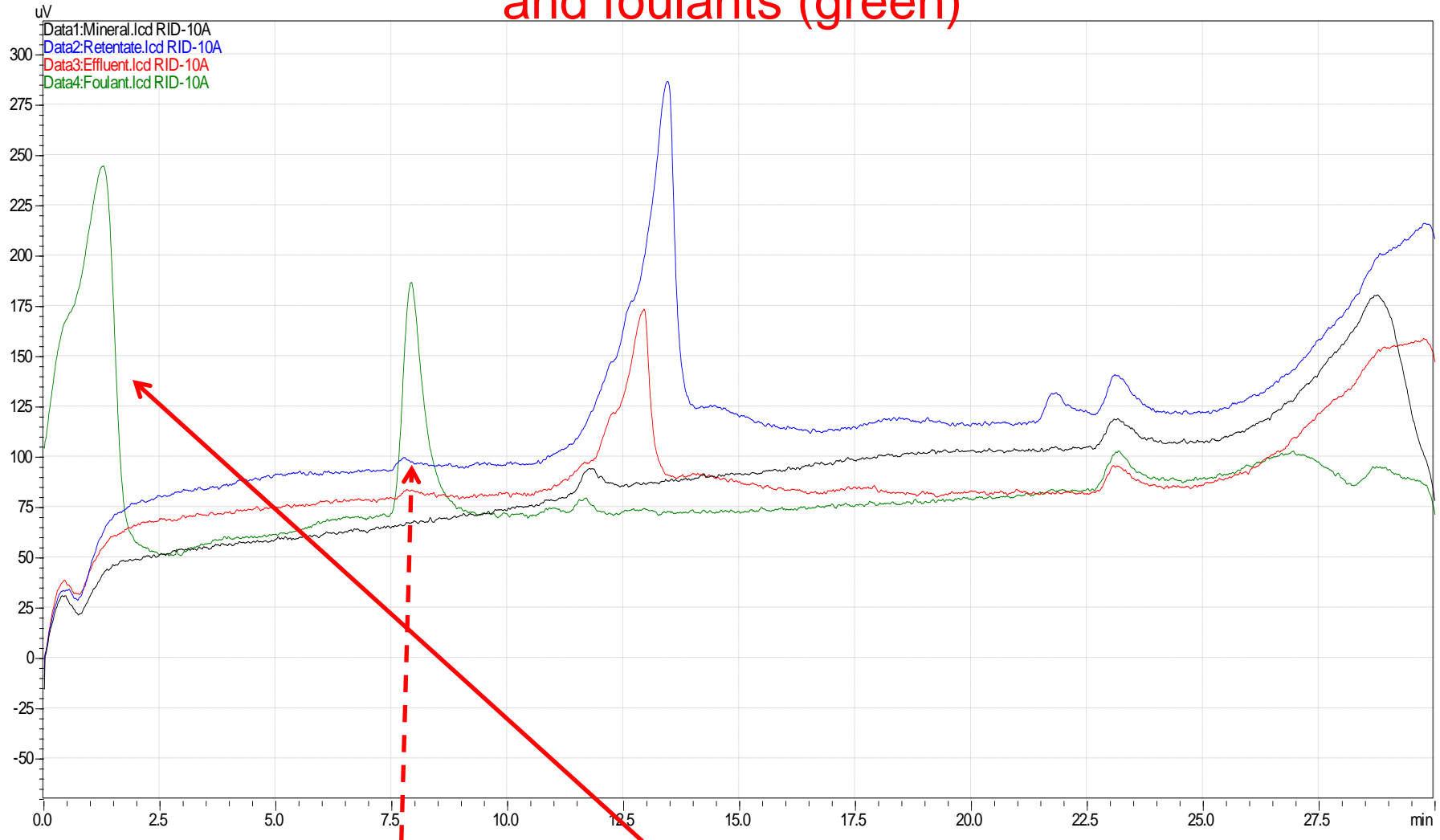
At the end of each flux of strategy 3 (2-3d)

SMPs: about half of the overall organics of the foulants

Colloids : a lot during short term operation, but reduced during longer term operation-hydrolysis?



2) SEC chromatography of the retentate (blue), effluent (red) and foulants (green)



SEC traces show mainly high MW fraction fouling the membrane, with some lower MW (~8min). However, even low MW is rejected by the membrane, and some ends up depositing on the membrane. Very low MW also seems to be rejected and fouling.



A2) Characterisation of proteinaceous materials and carbohydrates in wastewater systems

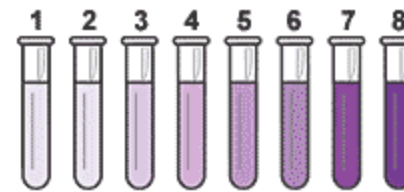
- What do the colourimetric methods actually measure with “proteins” and carbohydrates.

Colorimetric Methods – Problems

- Facile analytical process for the estimation of “protein” concentration
- Interaction with interfering substances



Standard BSA protein assay



Standard BSA protein assay
with interfering substances

- Highly dependent on the protein composition, ie. AA composition

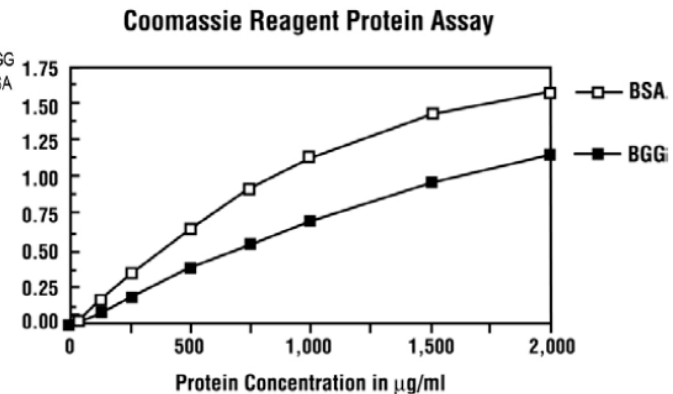
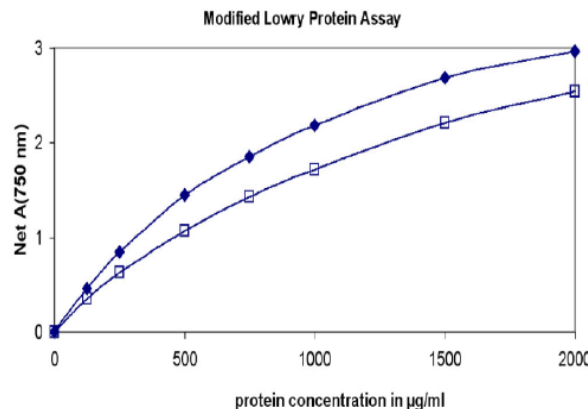
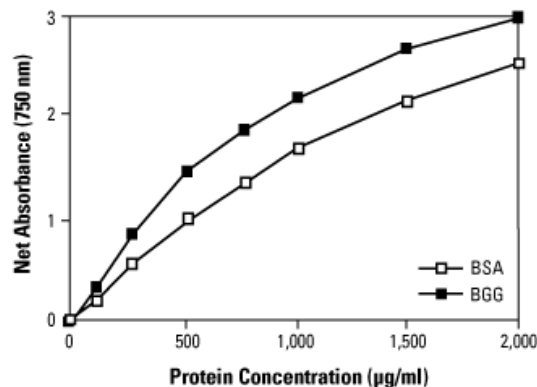


Image courtesy of Thermo Fischer Scientific Inc.

Colorimetric Methods – Case Study

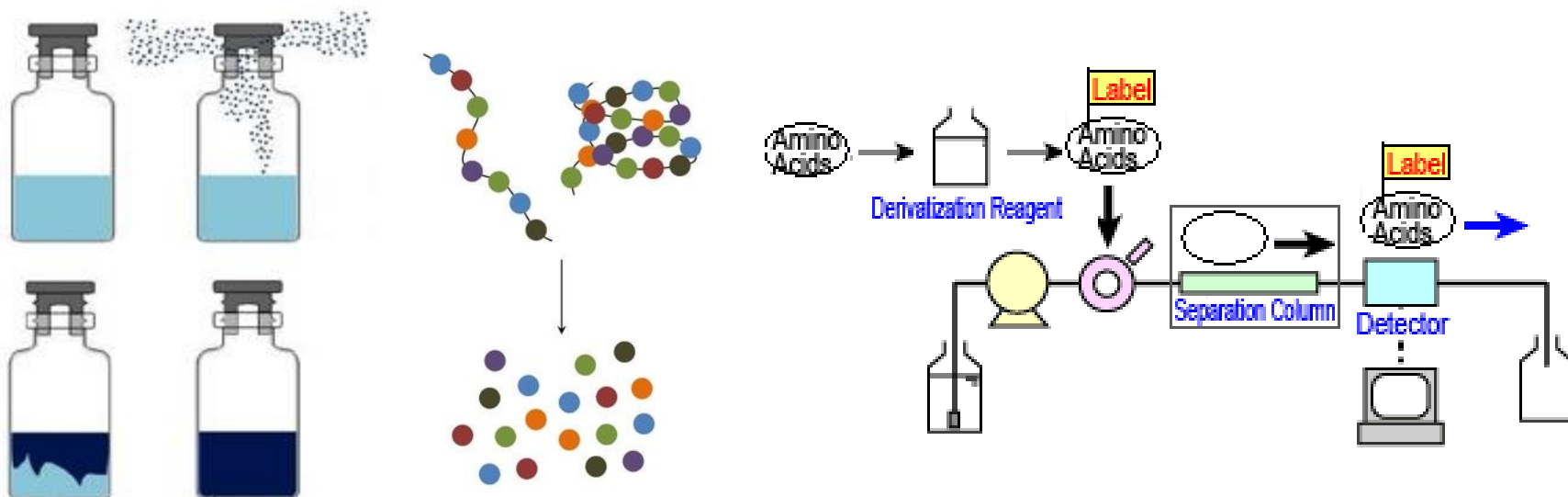
- Effluent samples at 3 different HRTs were characterised by measuring their COD and "protein" content.
- **Poor correlation between and across methods-need a better method!**

HRT (h)	COD (mg/L)	Reading (mg/L) \pm SD				
		Modified Lowry	Micro BCA	Pierce BCA	Bradford (Coomassie)	Bradford (Coomassie Plus)
6	19.12	5.6 \pm 0.8	6.8 \pm 0.1	6.5 \pm 1.8	1.9 \pm 2.0	15.2 \pm 1.8
4	31.16	8.3 \pm 0.5	10.3 \pm 0.3	16.5 \pm 2.1	3.0 \pm 1.2	16.6 \pm 0.8
2	40.65	9.5 \pm 0.5	23.7 \pm 0.8	31.5 \pm 1.4	3.5 \pm 0.7	18.7 \pm 2.4
6 (SA*)	-	24.6 \pm 1.4	19.2 \pm 0.1	369 \pm 22	379 \pm 15	373 \pm 5.4
4 (SA*)	-	32.9 \pm 1.1	20.4 \pm 0.2	373 \pm 24	384 \pm 9.9	375 \pm 4.4
2 (SA*)	-	33.1 \pm 2.8	25.9 \pm 0.5	388 \pm 20	386 \pm 11	380 \pm 5.2

*: Standard Addition of BSA at 10 mg/L for Modified Lowry and Micro BCA, and 300 mg/L for the others.

HPLC Analysis

- New standard method needs to be developed to accurately quantify and identify proteinaceous materials in wastewater using advanced instrument such as HPLC.





HPLC HPLC Analysis

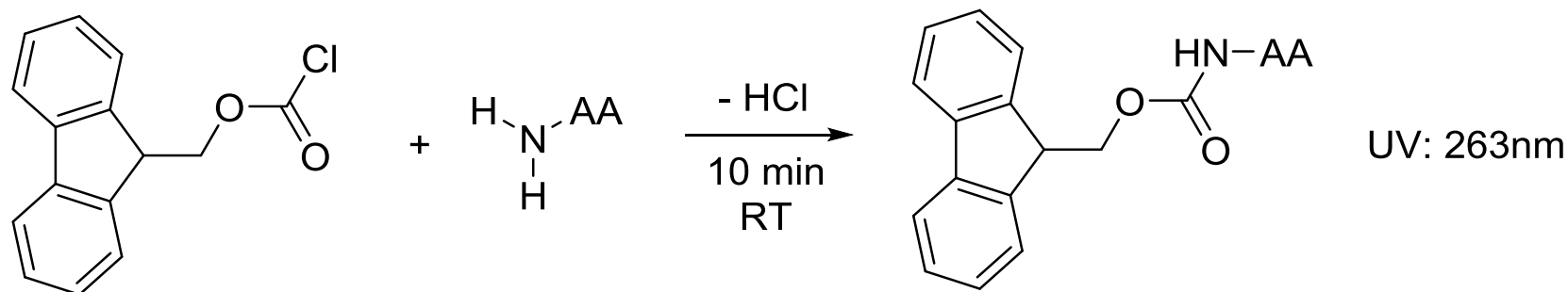
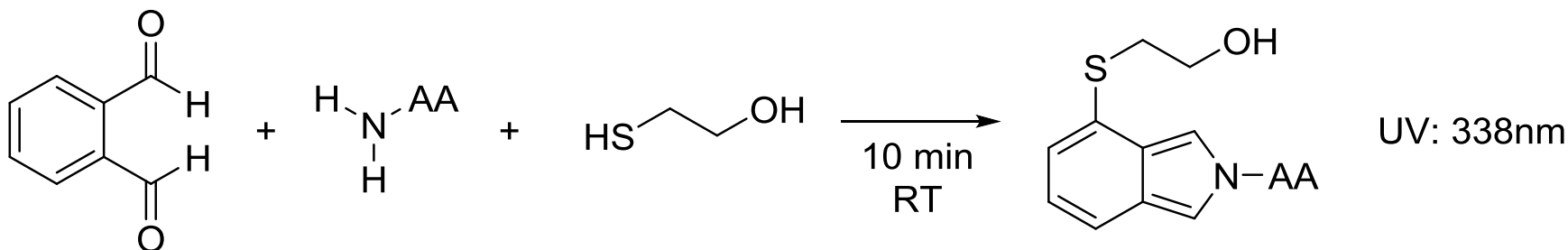
Lyophilisation

Acid
hydrolysis

Derivatization

HPLC
detection

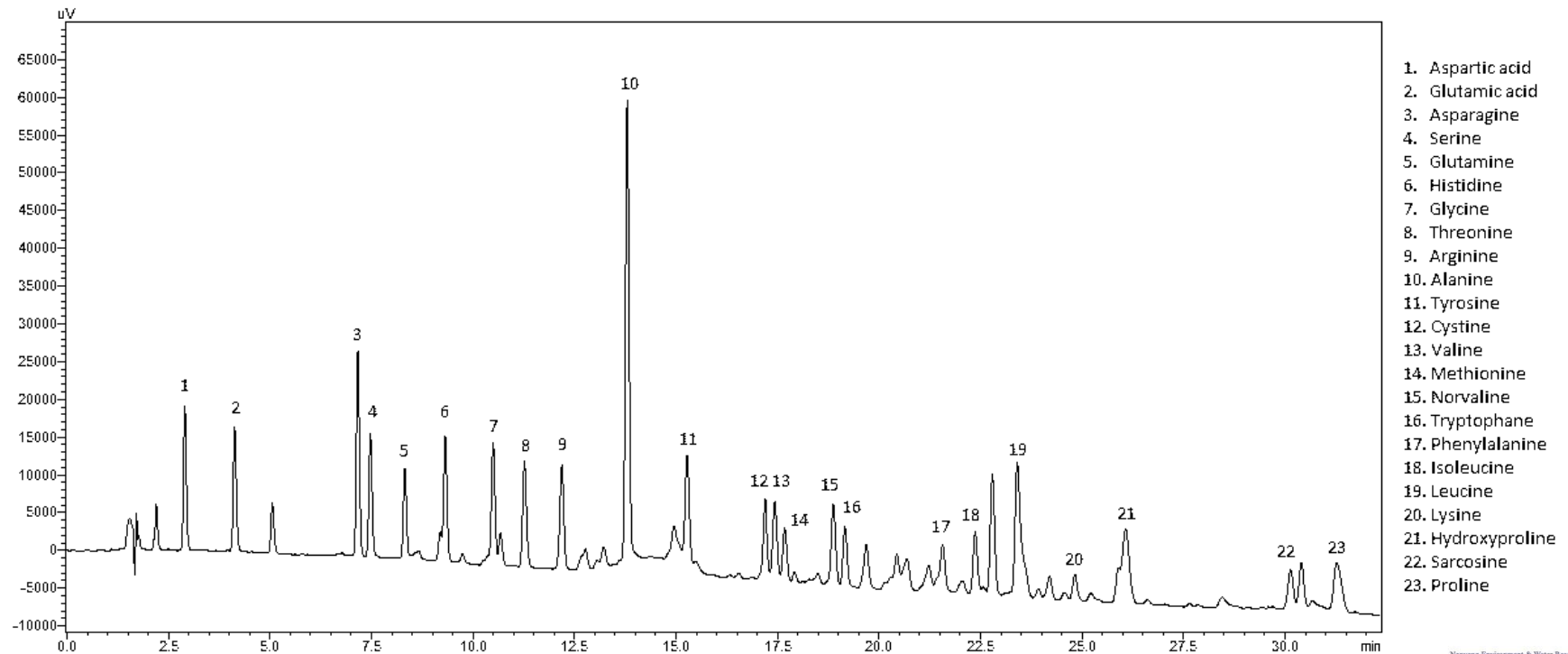
• Derivatization using OPA + FMOC-Cl



HPLC



• Successful separation of 23 amino acids using HPLC



HPLC

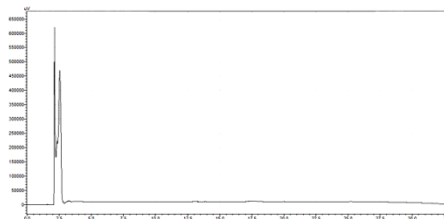
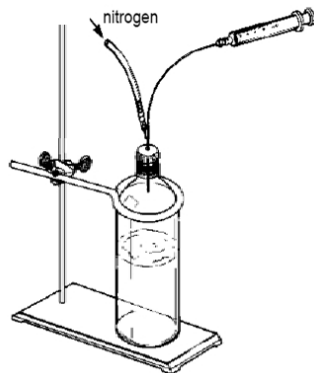
Lyophilisation

Acid
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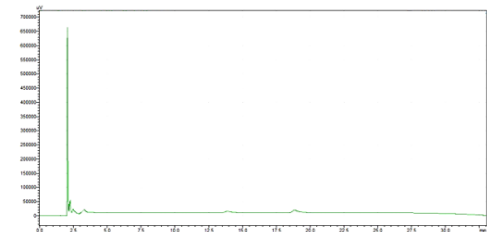
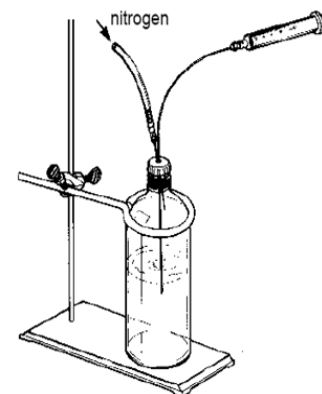
Derivatization

HPLC
detection

- Conducting hydrolysis in an oxygen-free environment enhances the recovery
- Sparging the sample with an inert gas



Headspace

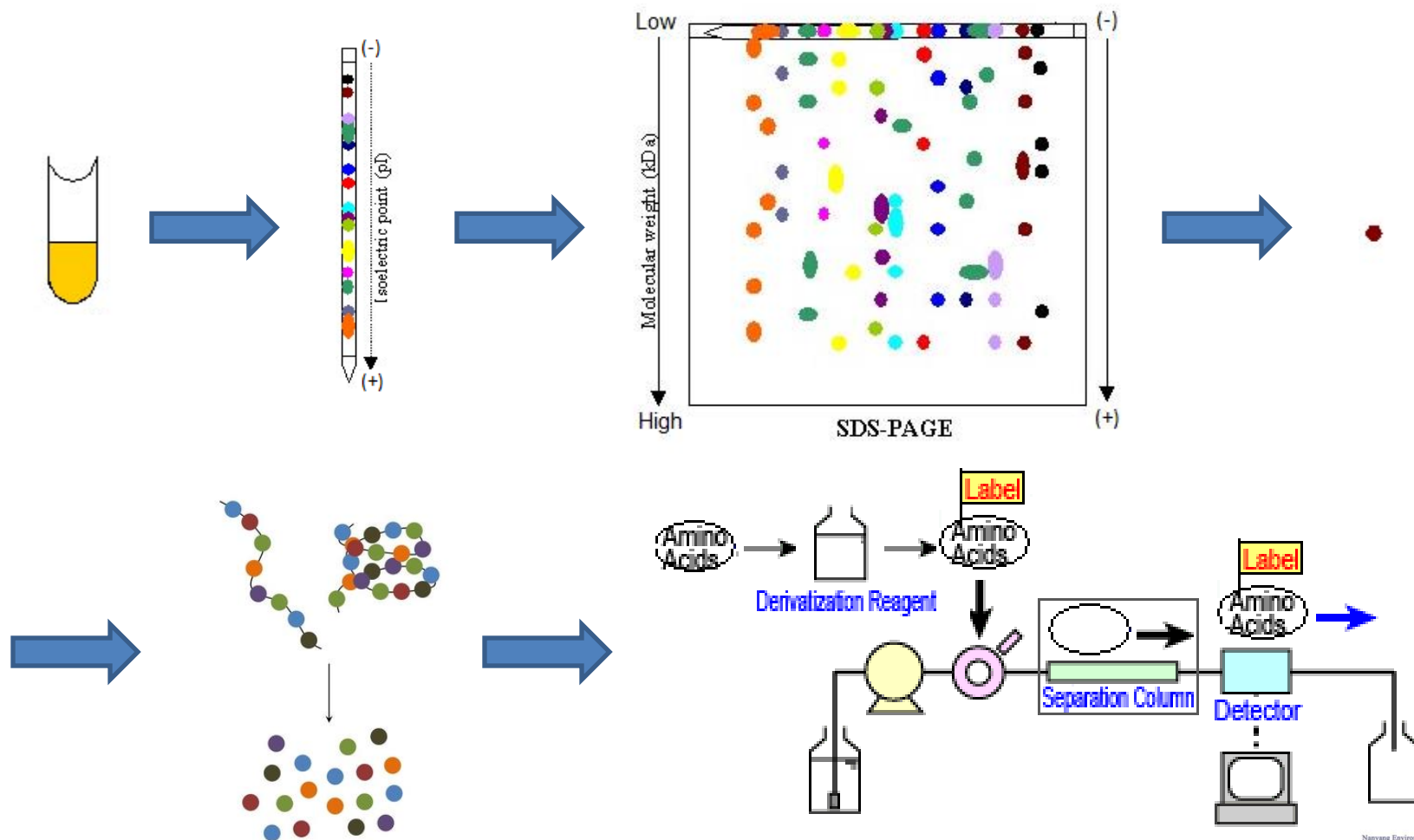


Bubbling

- Sparging with an inert gas and degassing under vacuum

HPLC

● Pre-treatment using 2D-SDS-PAGE

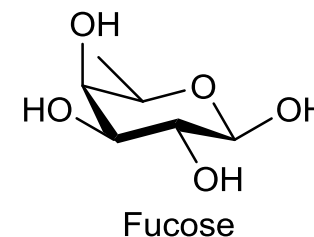
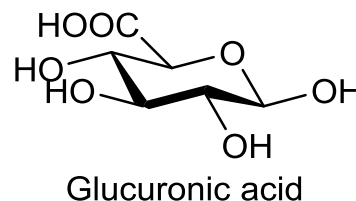
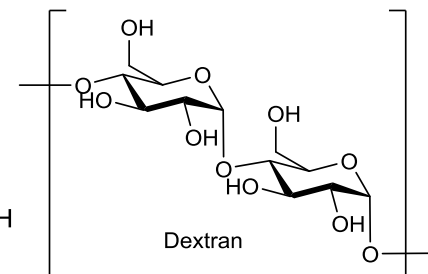
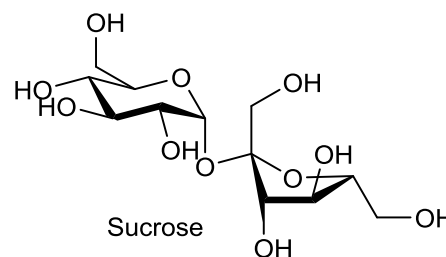
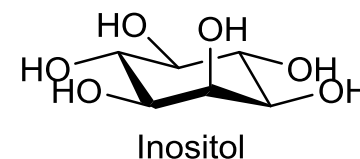
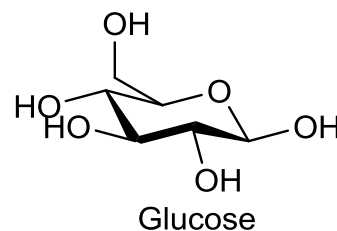




Colorimetric Carbohydrate Methods – Problems

- Non-specificity for a whole class saccharides

Class of saccharides	Samples
Monosaccharide	Glucose
Disaccharide	Sucrose
Polysaccharide	Dextran
Sugar alcohol	Inositol
Sugar acid	Glucuronic acid
Deoxy sugar	Fucose



- Possible interaction with interfering substances

Colorimetric Methods – Case Study

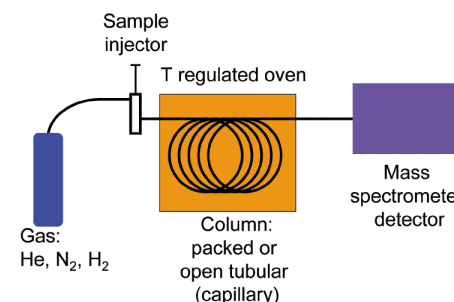
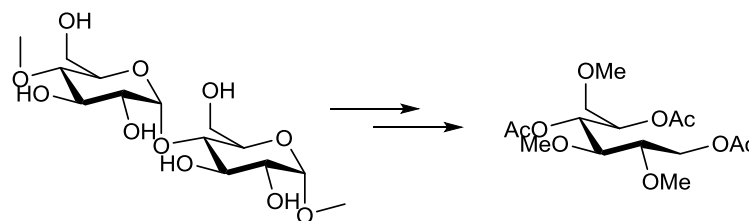
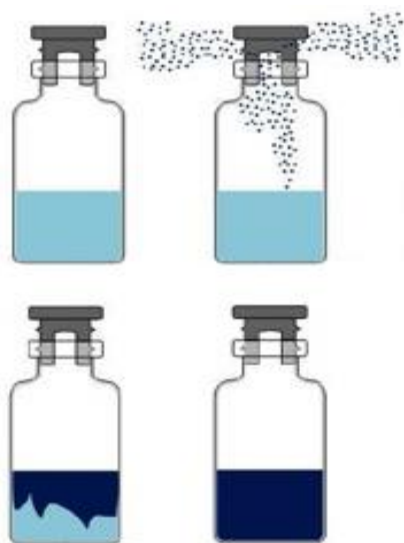
- Supernatant samples at 3 different HRTs were characterised by measuring their COD and carbohydrate content.
- **Again, poor correlation between and across methods**

HRT (h)	COD (mg/L)	Reading (µg/well) ± SD						
		Anthrone (0.1%)	Anthrone (0.2%)	Phenol (5%)	Phenol (8%)	S-A kit	Abnova kit	Glycoprotein kit
6	60.36	1.5 ± 0.4	0.7 ± 0.1	0.9 ± 0.4	1.4 ± 0.4	0.6 ± 0.2	0.4 ± 0.1	0.4 ± 0.1
4	73.12	1.8 ± 0.1	0.8 ± 0.2	0.9 ± 0.6	1.3 ± 0.4	0.8 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
2	91.49	2.1 ± 0.1	0.9 ± 0.1	0.9 ± 0.2	1.0 ± 0.2	0.7 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
6 (SA*)	-	10.2 ± 1.3	9.4 ± 0.5	9.7 ± 0.1	4.3 ± 1.3	9.9 ± 0.3	10.3 ± 0.3	16.4 ± 0.1
4 (SA*)	-	10.5 ± 0.1	10.3 ± 0.6	10.5 ± 1.1	4.1 ± 1.4	10.1 ± 0.2	12.1 ± 0.2	16.3 ± 0.1
2 (SA*)	-	10.9 ± 2.4	10.6 ± 0.4	10.1 ± 2.6	6.1 ± 1.7	10.2 ± 0.7	11.6 ± 0.9	17.8 ± 0.7

*: Standard Addition of glucose at 10 µg/well.

GCMS Analysis

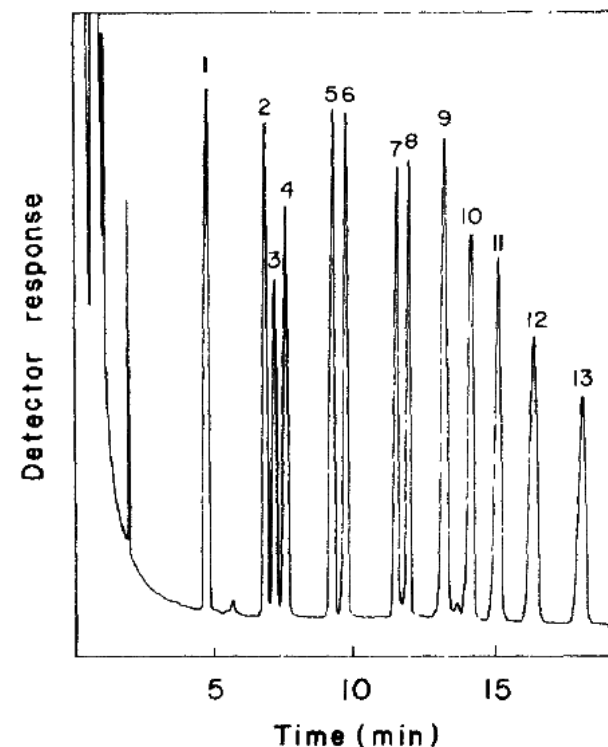
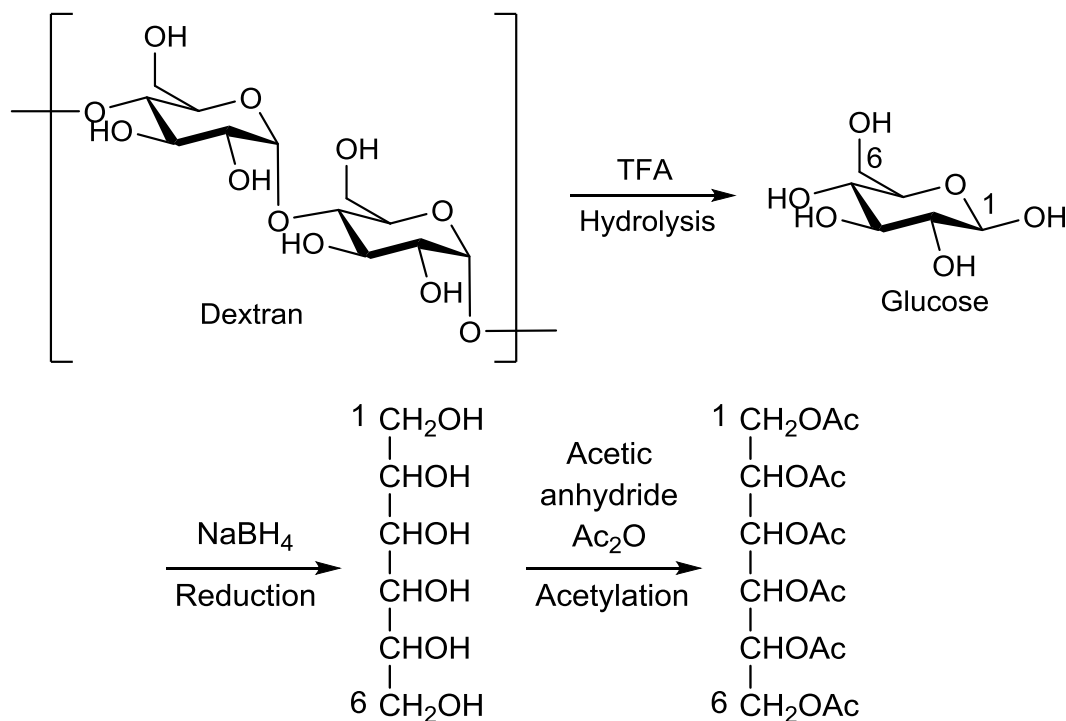
- New standard method needs to be developed to accurately quantify and identify carbohydrates in wastewater using advanced instrument such as GC-MS.



GCMS



- **Blakeney *et al.*, 1983**
- **Excellent monosaccharide analysis using GC**

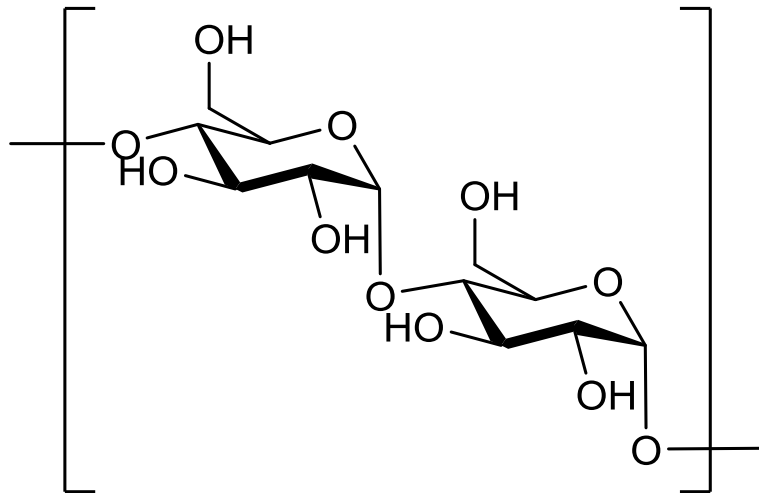




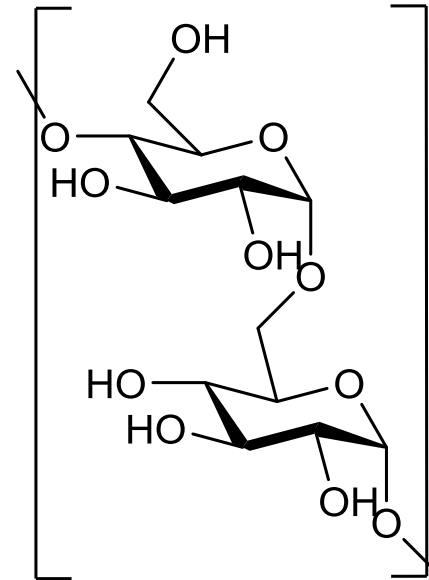
GCMS



- No structural information of polysaccharide will be provided



1,4 glycosidic linkage

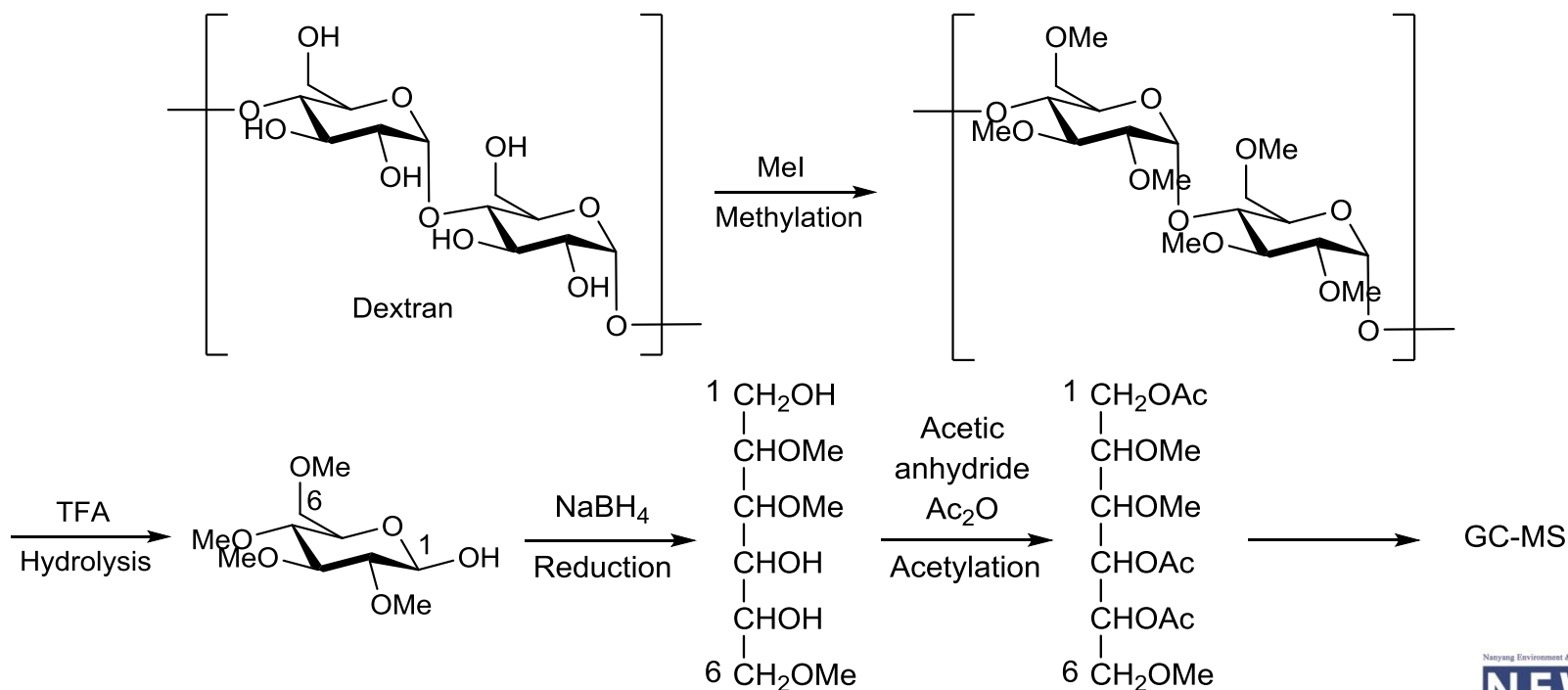


1,6 glycosidic linkage

GCMS



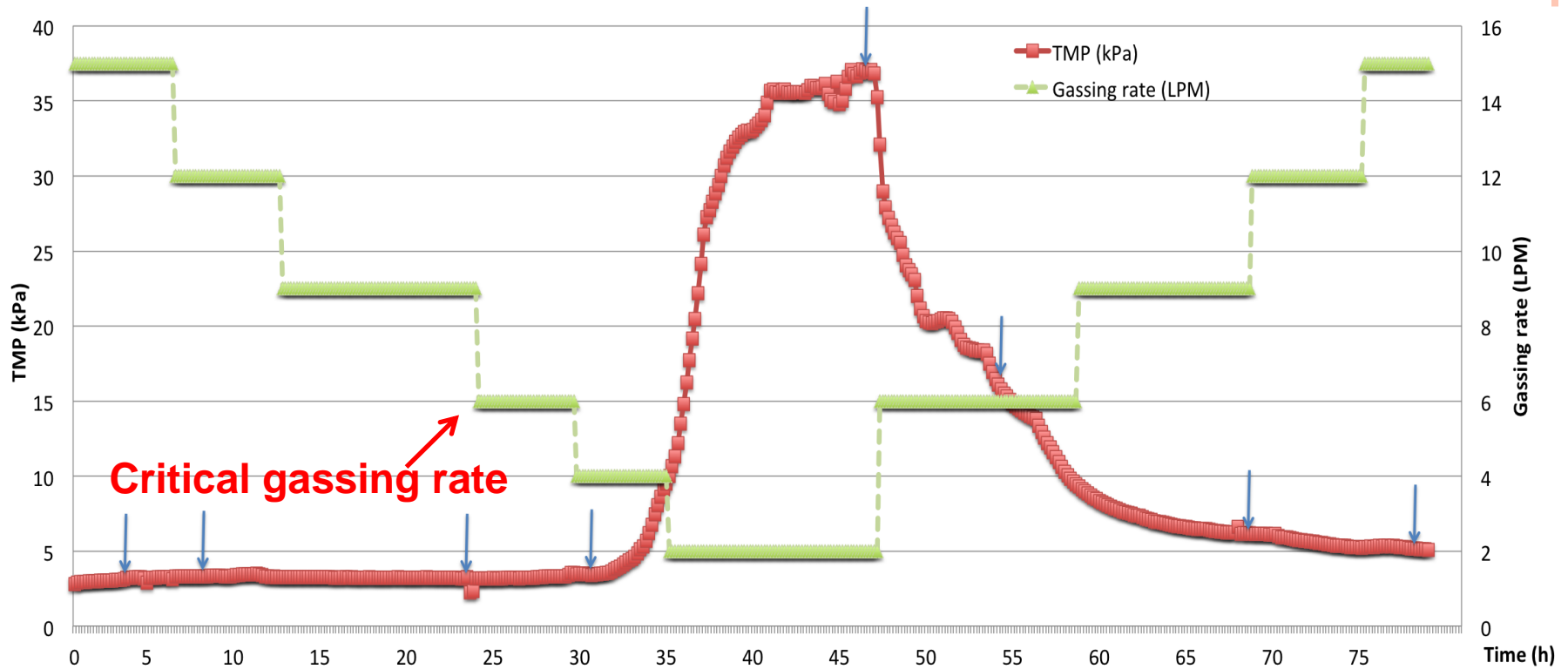
- **Determination of the polysaccharide composition in wastewater sample is developing**



A3) EFFECT OF CRITICAL GASSING RATE AND HRT ON REACTOR SUPERNATANT COMPOSITION/VISCOSITY/FLOC SIZE, AND MEMBRANE FOULING LAYER

- How does the reactor gassing rate influence composition in the supernatant, effluent, and fouling layer?
- Do these changes influence viscosity, floc size or Zeta potential?

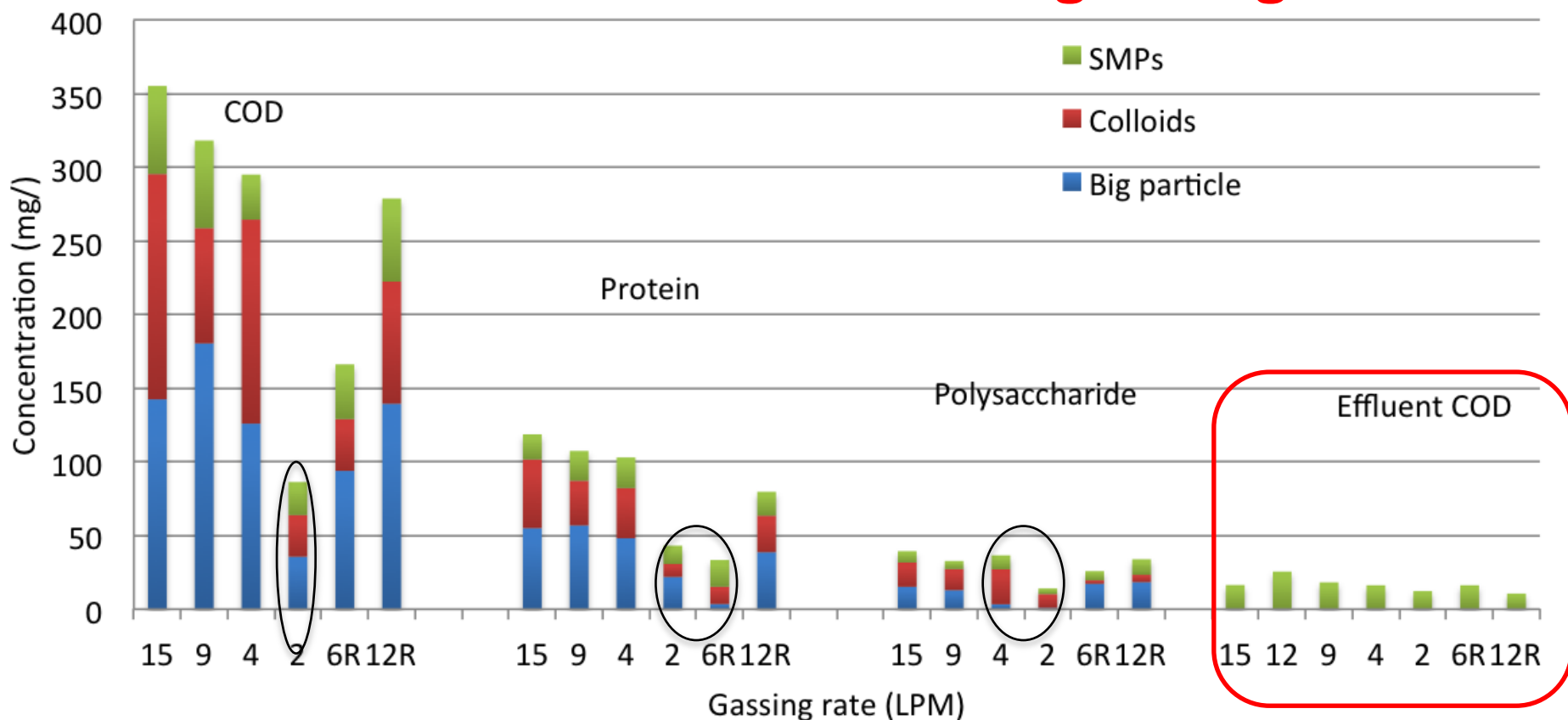
TMP PROFILE WITH CHANGES IN GASSING RATE- DETERMINATION OF “CRITICAL GASSING RATE”



Most research focuses on “critical flux”, but gassing rate (turbulence) just as important-can lead to trade-offs between energy and effluent quality.

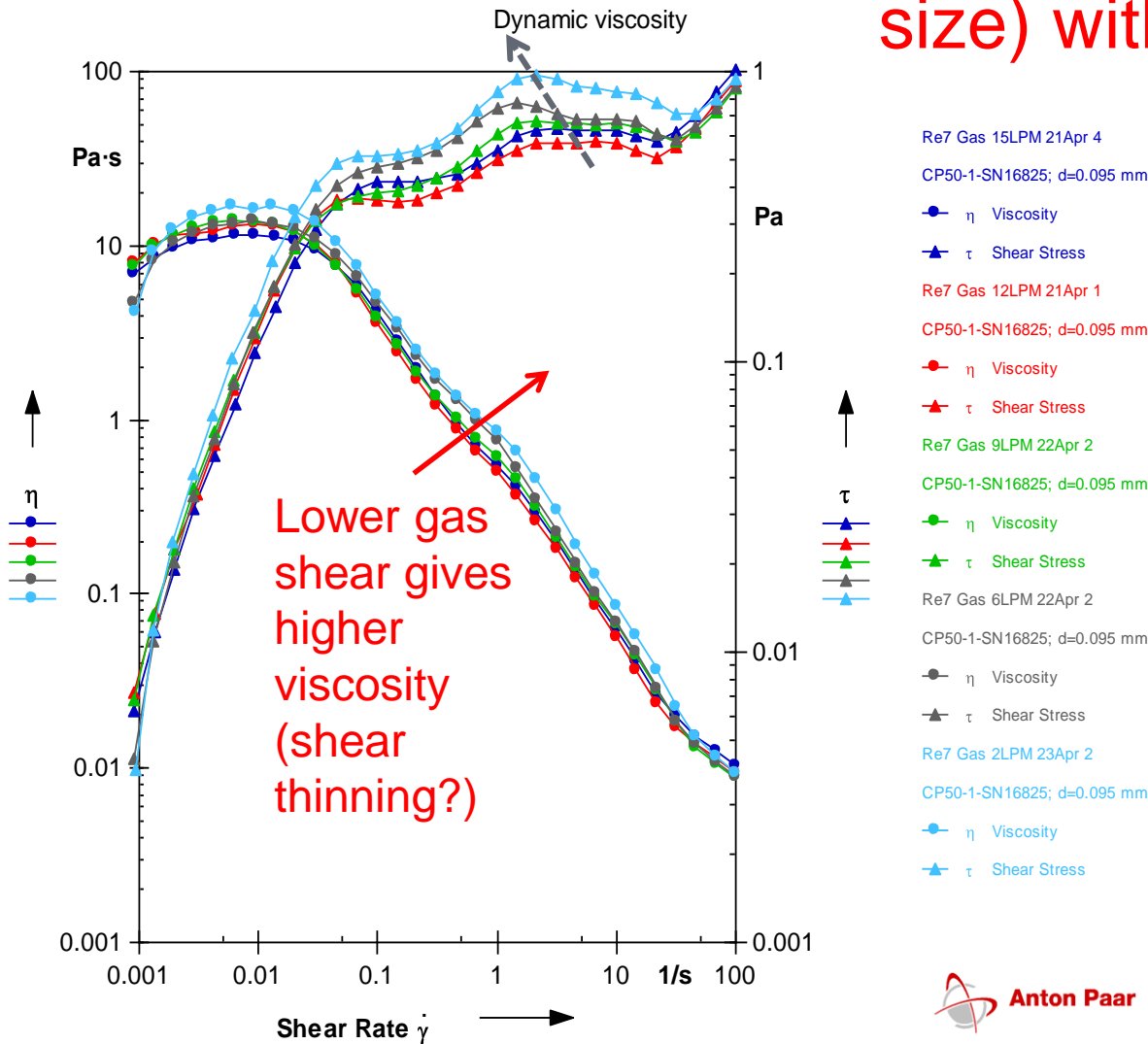
In this case the “critical gassing rate” is the rate below which we get a rapid increase in TMP, ~6LPM

The organic concentration of the supernatant and the effluent with different gassing rates



Interestingly, as the gassing rate increased, the supernatant COD increased substantially, BUT the fouling rate did not increase. **With low gassing, COD low but fouling high!**

Sludge characterization (viscosity and floc size), and organic characterization (zeta potential and particle size) with gassing rate

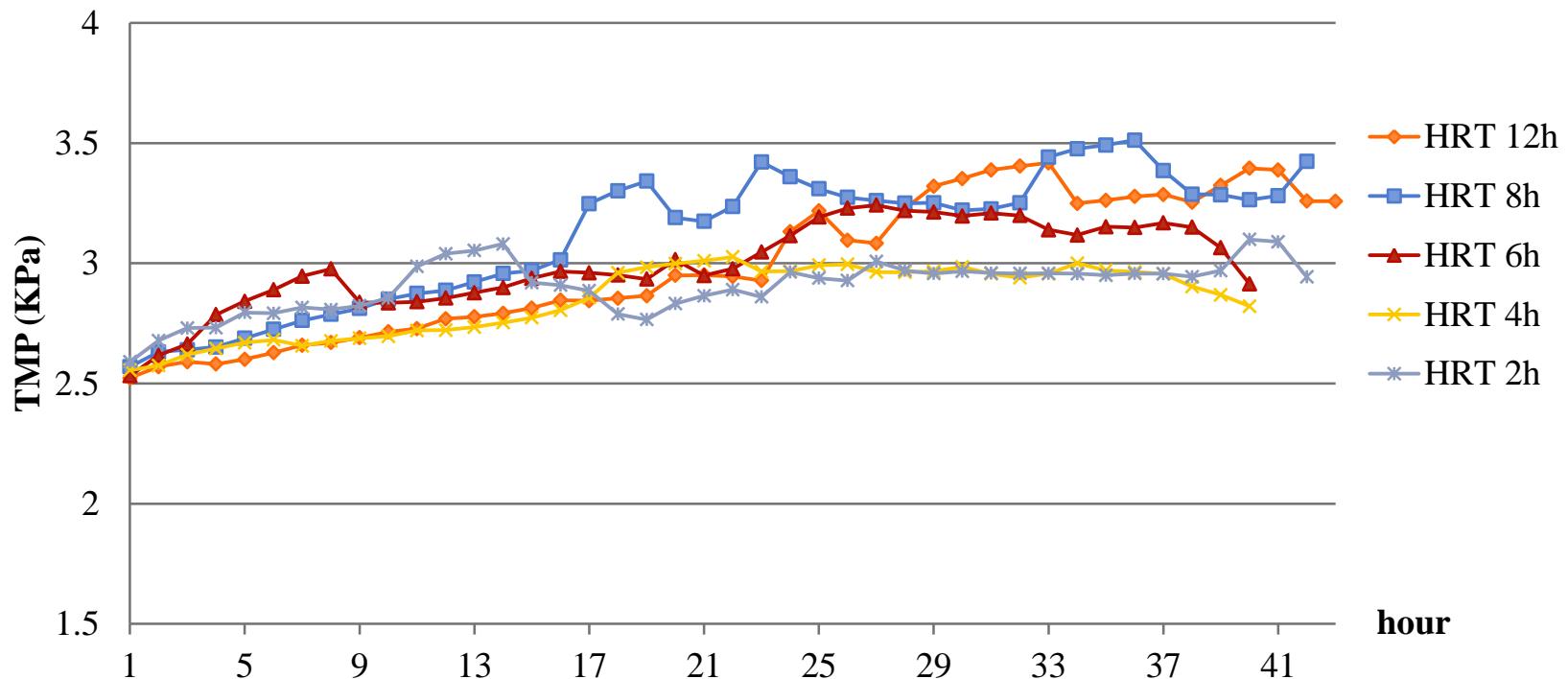


	Floc size (μm)	Zeta Potential	Particle size (nm)
15LPM	32.9	-16.7	350
9LPM	30.1	-17.6	296
4 LPM	30.3	-17.5	318
2 LPM	31.4	-17.8	326
6LPM R	33.1	-16.5	463
12LPM R	27.2	-16.5	356

Surprisingly, no effect of gassing rate on floc/particle size, OR zeta potential

Effect of operating conditions-HRT

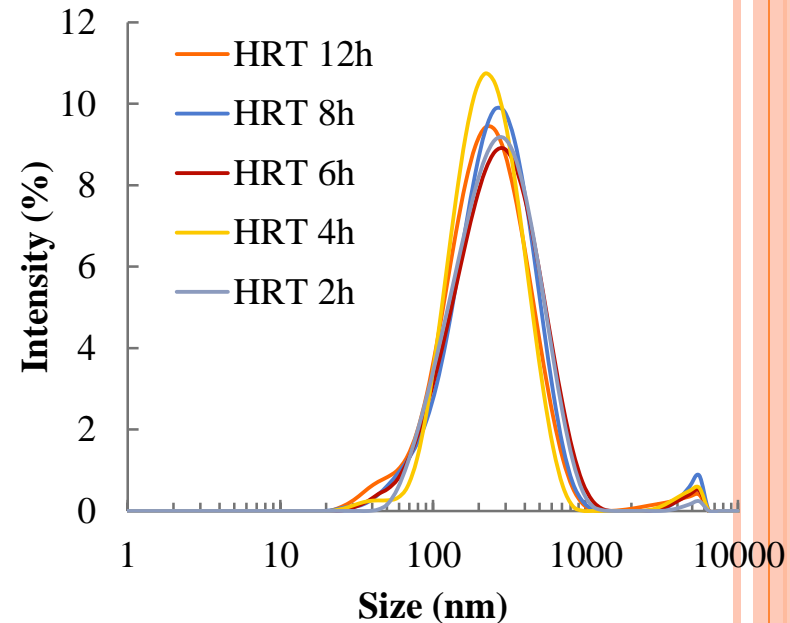
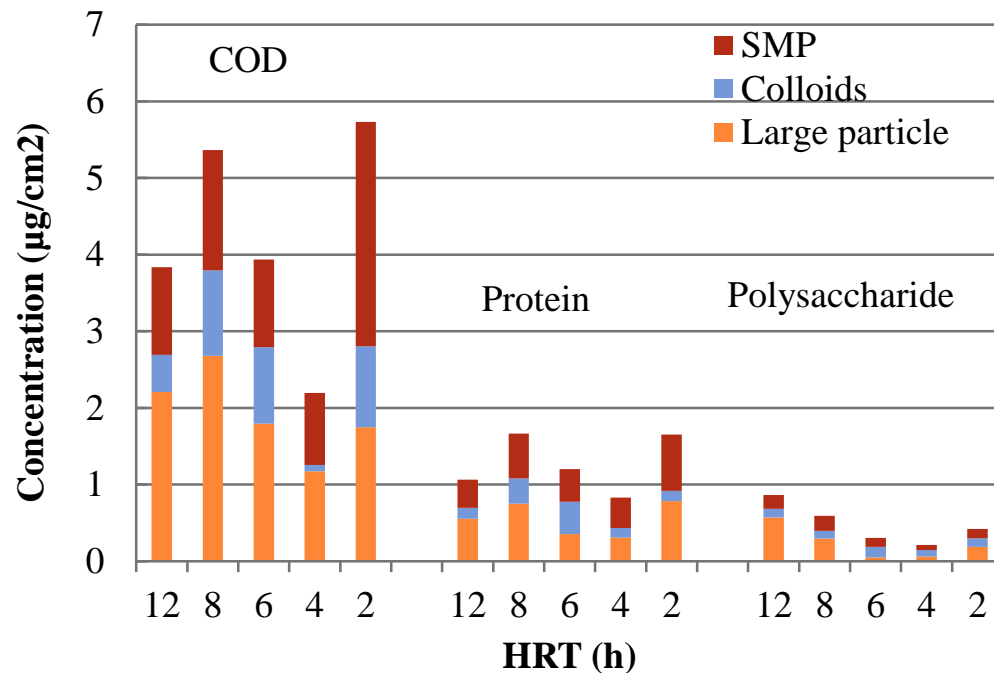
CHANGES IN TMP UNDER DIFFERENT HRTS - SAMBR WAS OPERATED AT 28 LMH



TMP in the SAMBR decreased slightly with shorter HRTs-maybe less SMP production?



SMPS AND COLLOIDS IN THE FOULANT LAYER



Foulant mass per membrane area was lowest at 4h HRT.



SLUDGE PROPERTIES, SMPs/COLLOIDS, VFA AND METHANE PRODUCTION

	HRT 12h	HRT 8h	HRT 6h	HRT 4h	HRT 2h
Sludge floc size -D(4,3)(μm)	43.8	45.3	46.4	46.6	47.1
Zeta potential - average (mV)	-19	-23.2	-17.1	-16.9	-16.3
Supernatant size - average(nm)	265	189	205	344	204
Colloids (mg/L)	11.9	6.9	13.6	14.3	19.1
SMP (mg/L)	25.5	29.9	56.6	46.6	114.5
Retentate VFA (mg/L)	0	38.3	33.4	9.1	97
Effluent VFA (mg/L)	0	0	4.6	7.4	38.3
Foulants VFA (mg/L)	0	4.4	0	0	21

Floc size seems to get bigger with decreasing HRT (more ECPs?), while Zeta potential seems to decrease with decreasing HRT (due to +ve charged ECPs?).



CONCLUSIONS

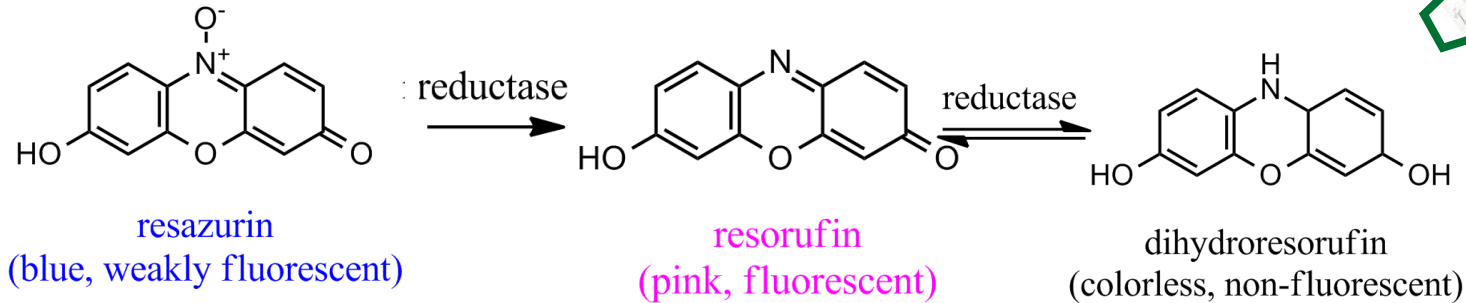
- The period over which we test for “critical flux”, which we varied from 20 min-4 days, does not seem to affect the value of the critical flux.
- **SMPs**: about half of the overall organics of the foulants. **Colloids**: a lot during short term operation, but reduced during longer term operation by hydrolysis.
- For every flux there is an **optimum gassing rate** which is critical to prevent membrane fouling. However, low gassing rates can lead to good effluents and low fouling.
- Lower HRTs (4h) can lead to less fouling on the membrane but decreased COD removals.



B) RAPID TOXICITY MEASUREMENT USING A FLUORESCENT ASSAY, AND TOXICITY AMELIORATION

- How do we quickly and reliably measure toxic pollutants, eg. Organics/heavy metals entering AD so that we can take remedial action?
- What remedial action can we take to ameliorate toxic events, and hence prevent digesters becoming “sour”, ie very high levels of VFAs?

RESAZURIN REDUCTION ASSAY*

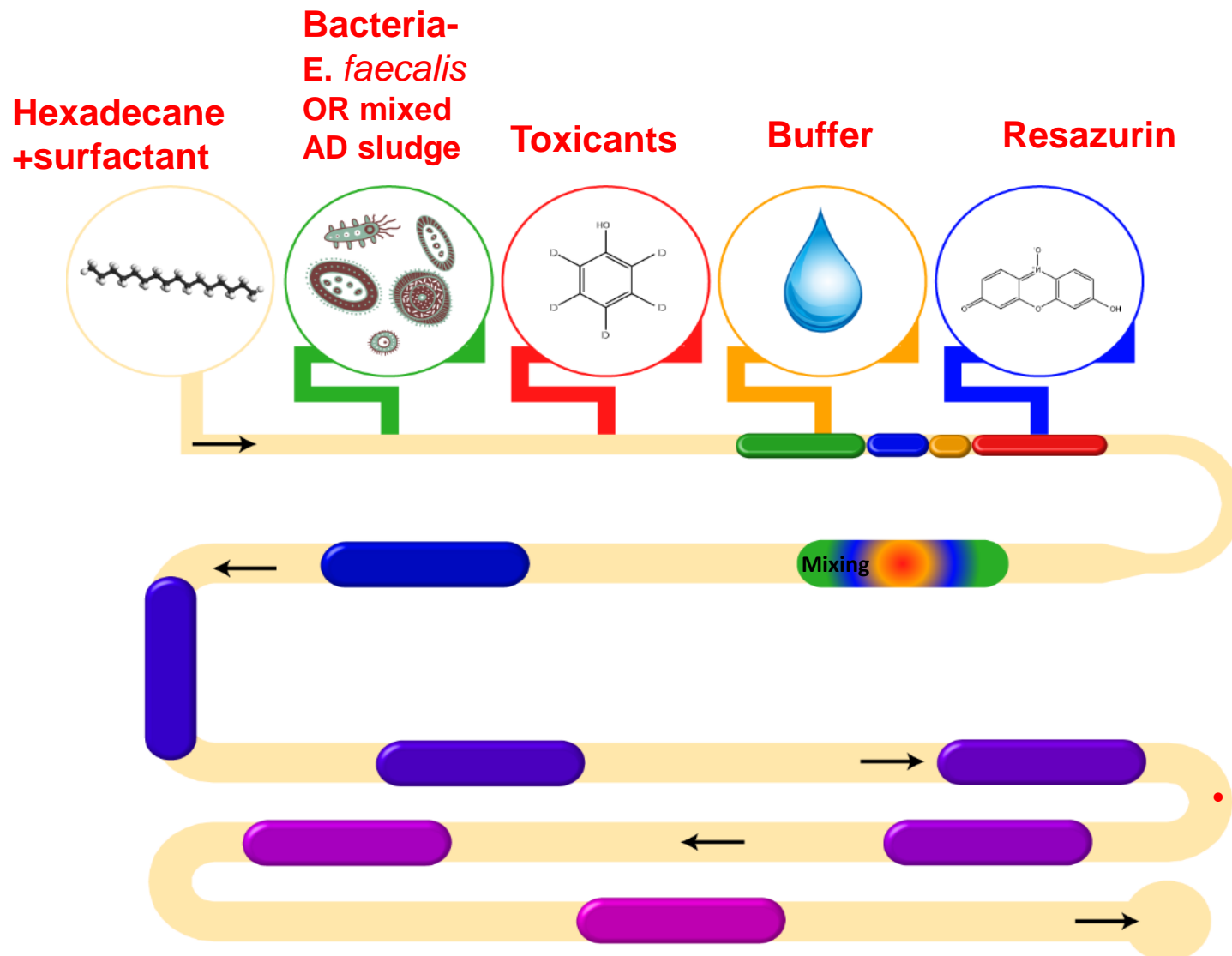


- Fast (few minutes)
- Compatible with anaerobic conditions
- Minimally toxic (kinetic)
- Flexible
- High fluorescence intensity = nontoxic

*Chen J.L., Ortiz R., Xiao Y., Steele T.W.J., Stuckey D.C., Rapid fluorescence-based measurement of toxicity in anaerobic digestion. Water Research, 75, (2015), 123-130. &

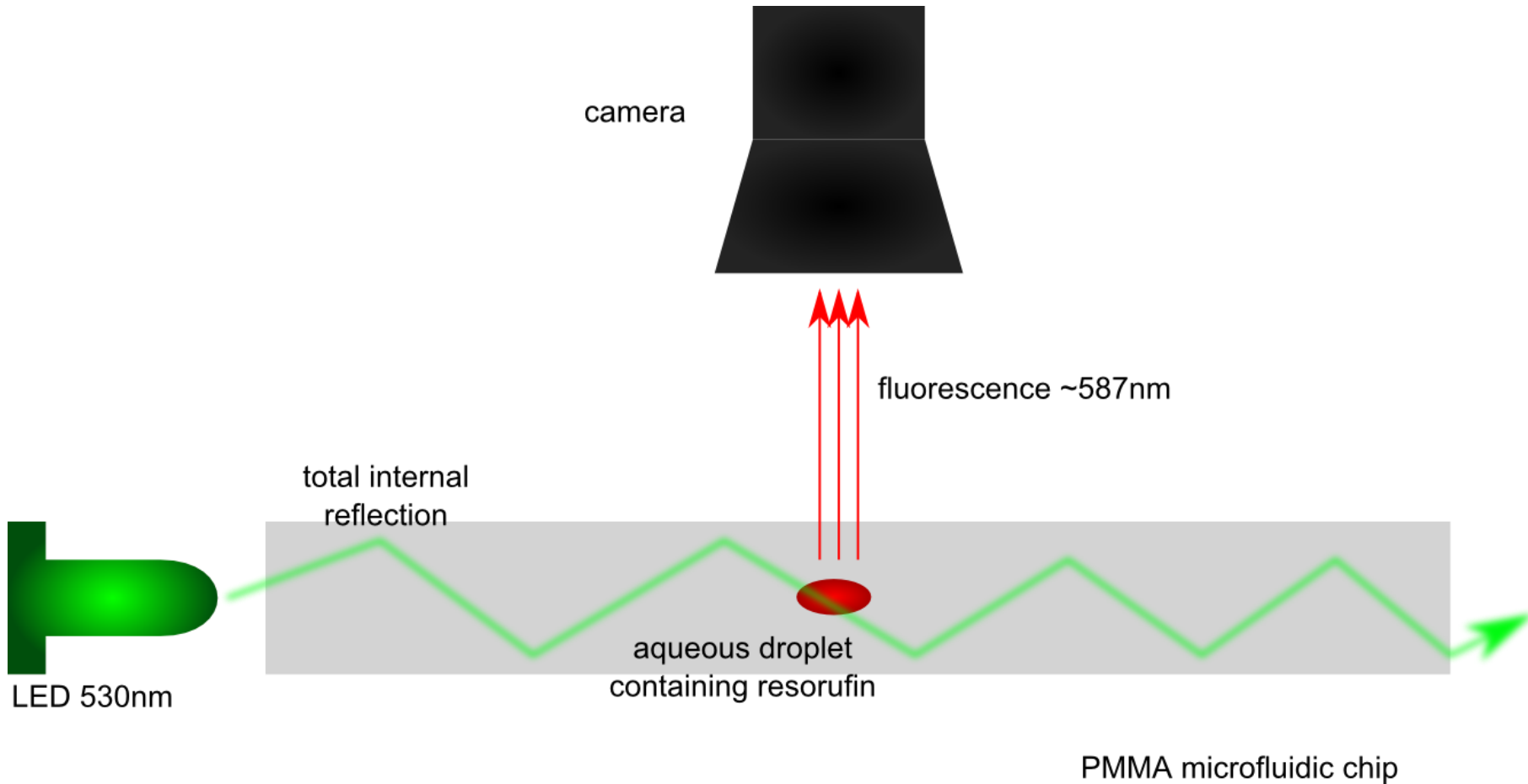
Chen J.L., et al. Modeling and Application of a Rapid Fluorescence-Based Assay for Biotoxicity in Anaerobic Digestion, ES&T, in press.

MICROFLUIDIC DEVICE - OVERVIEW

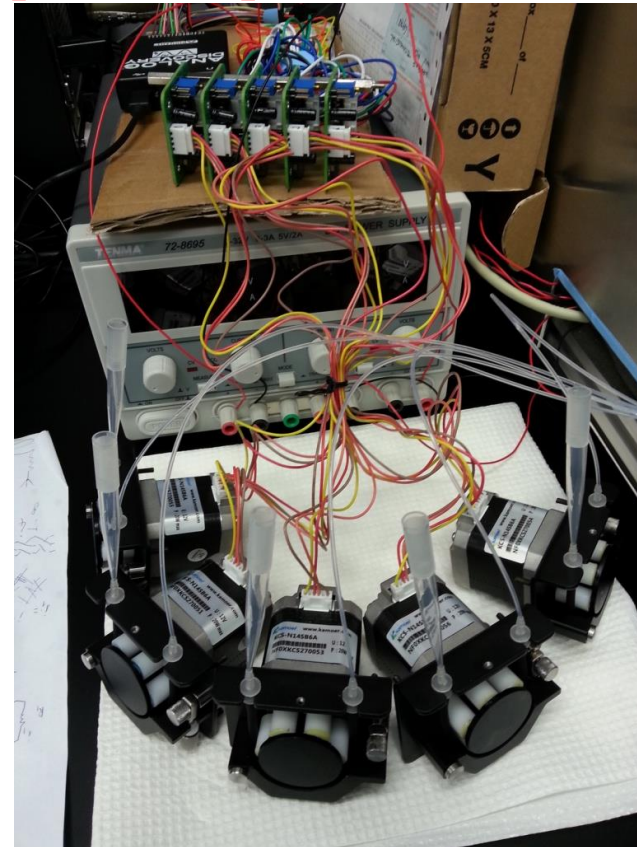
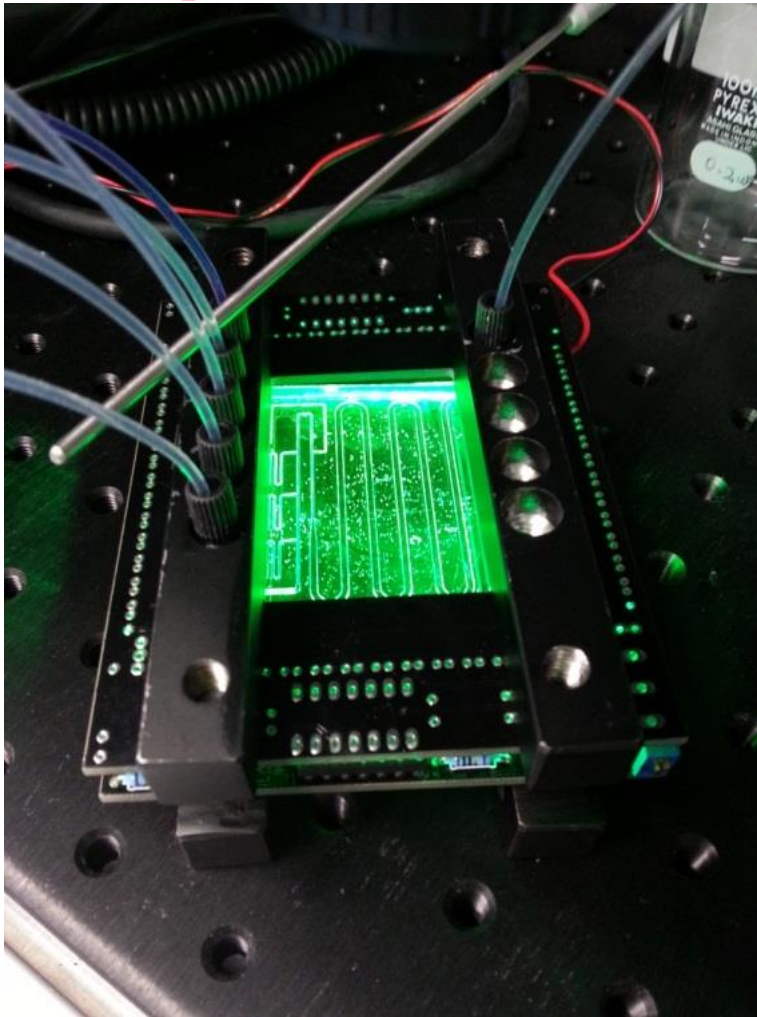


Incubation channel
• Fluorescence signal is continuously recorded by a camera

Experimental setup



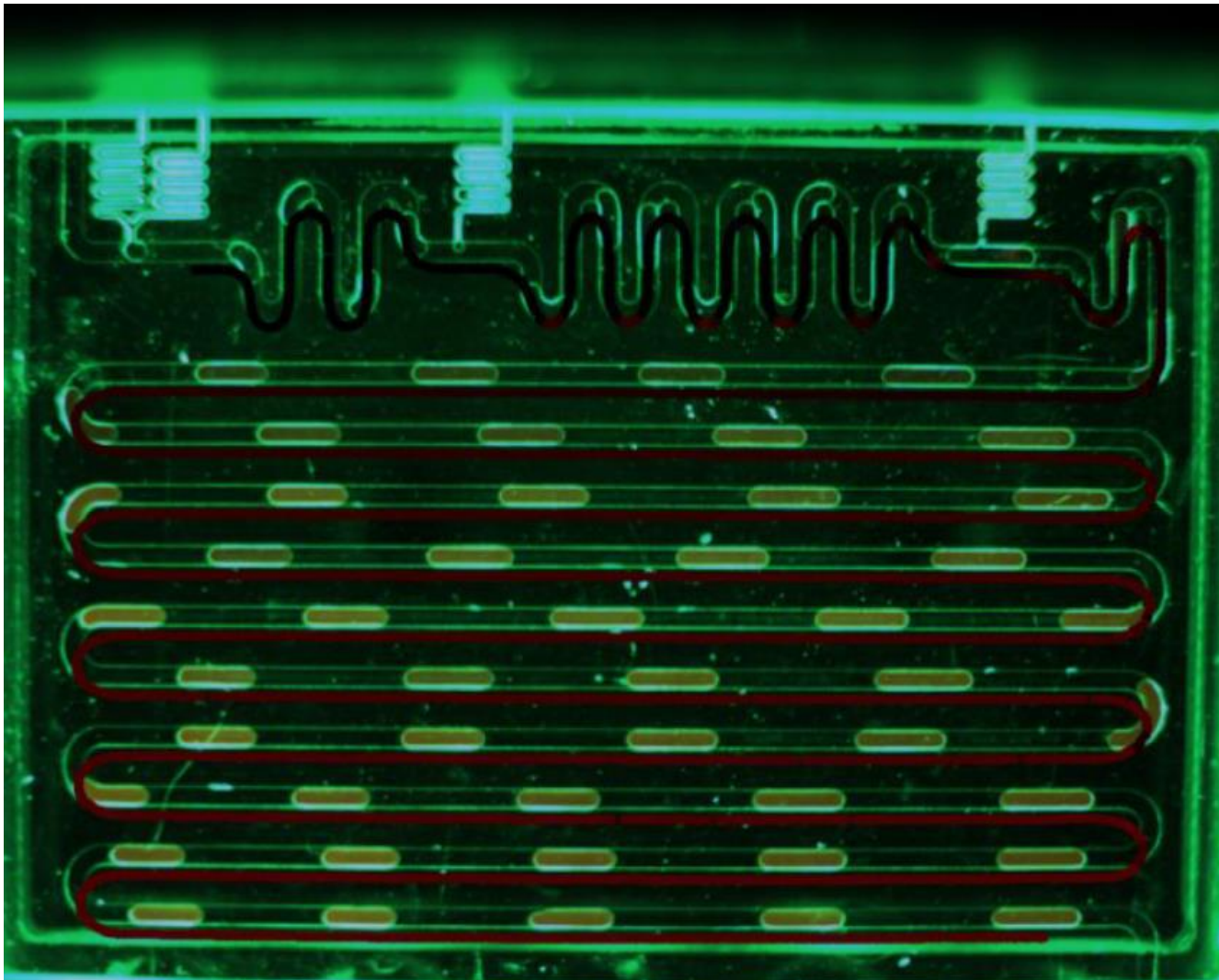
Experimental setup: in practice



- C++ program control the camera and pumps
- Video feedback compensate for peristaltic pump inaccuracy

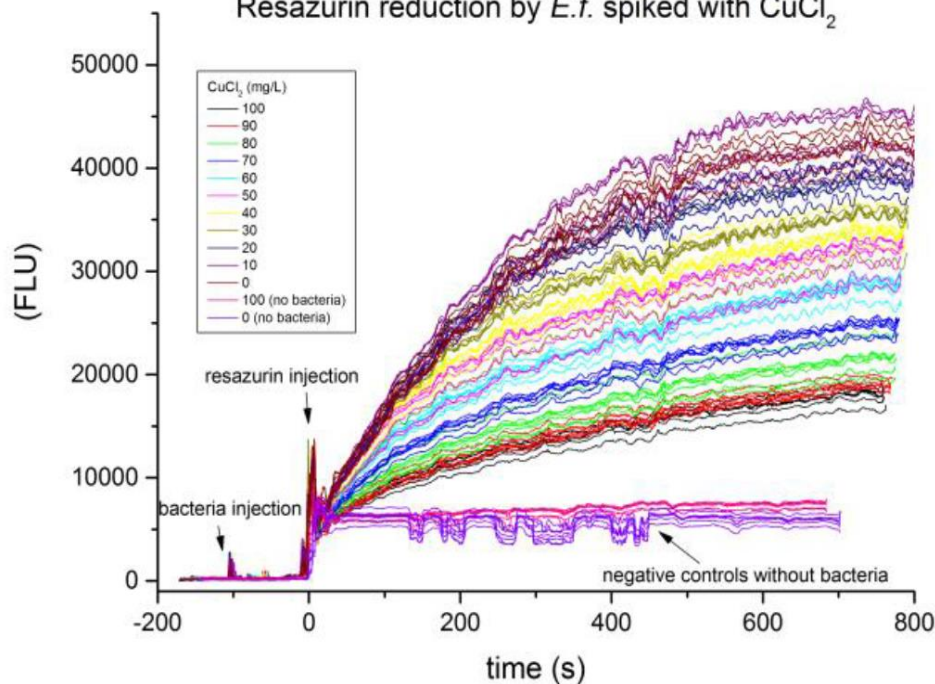
MICRO-FLUIDIC DEVICE WITH HRT= 10 MIN

Strain: *Enterococcus faecalis* (without toxicants), Resazurin: 25 ppm, Total aqueous flow rate = 1 $\mu\text{l}/\text{min}$.



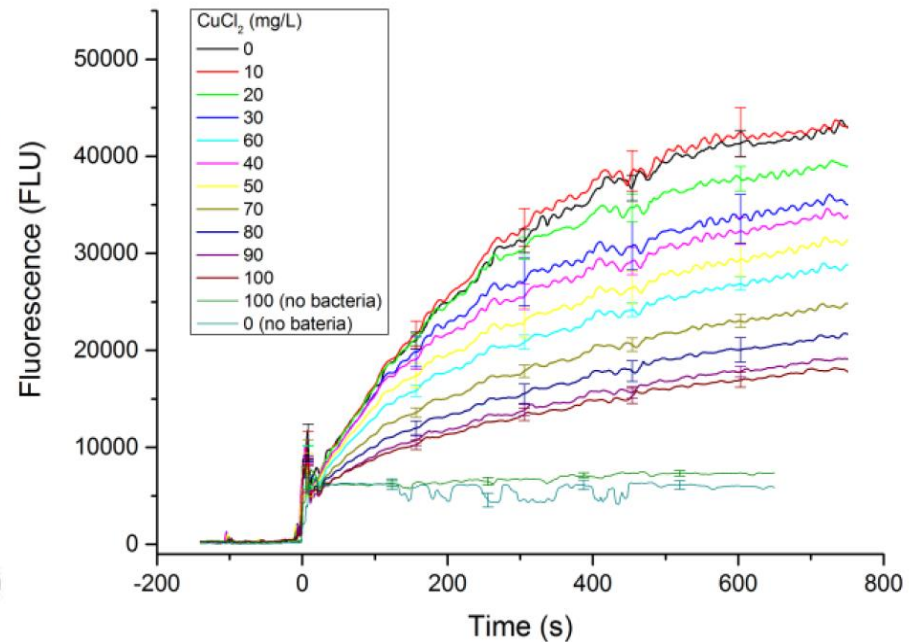
Toxicity of Copper on *E. faecalis*

Resazurin reduction by *E.f.* spiked with CuCl_2



Kinetic of resazurin reduction. 7 replicates for each concentration of copper

Resazurin reduction by *E.f.* spiked with CuCl_2 (average)

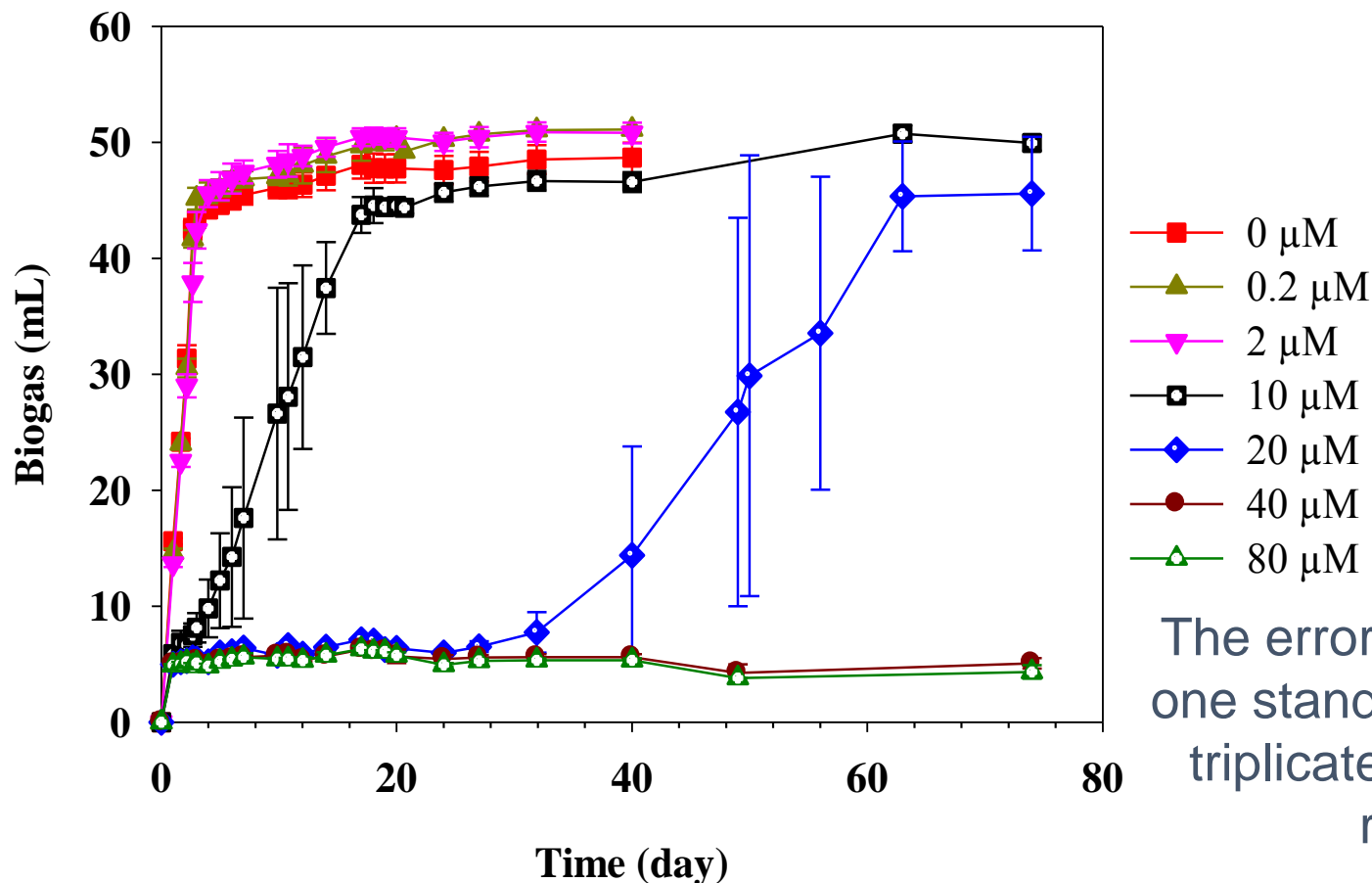


Average curves from 7 replicates for each copper concentrations. Error bar represent standard deviation.

Lowest copper concentration significantly different (Tuckey test) from positive control -decreases with time from 50 mg/L after 1 min ($p=0.0025$), 40 mg/L after 2 min ($p=0.0389$), 30 mg/L after 5 min ($p=3\text{E}-7$) and 20 mg/L after 10 min ($p=0.0012$).

C) CONTROL OF ORGANIC AND METAL TOXICITY IN ANAEROBIC DIGESTION USING Powdered Activated Carbon (PAC) AND EDTA (chelating agent)

EFFECTS OF PCP ON BIOGAS PRODUCTION OF METHANOGENIC SLUDGE*.

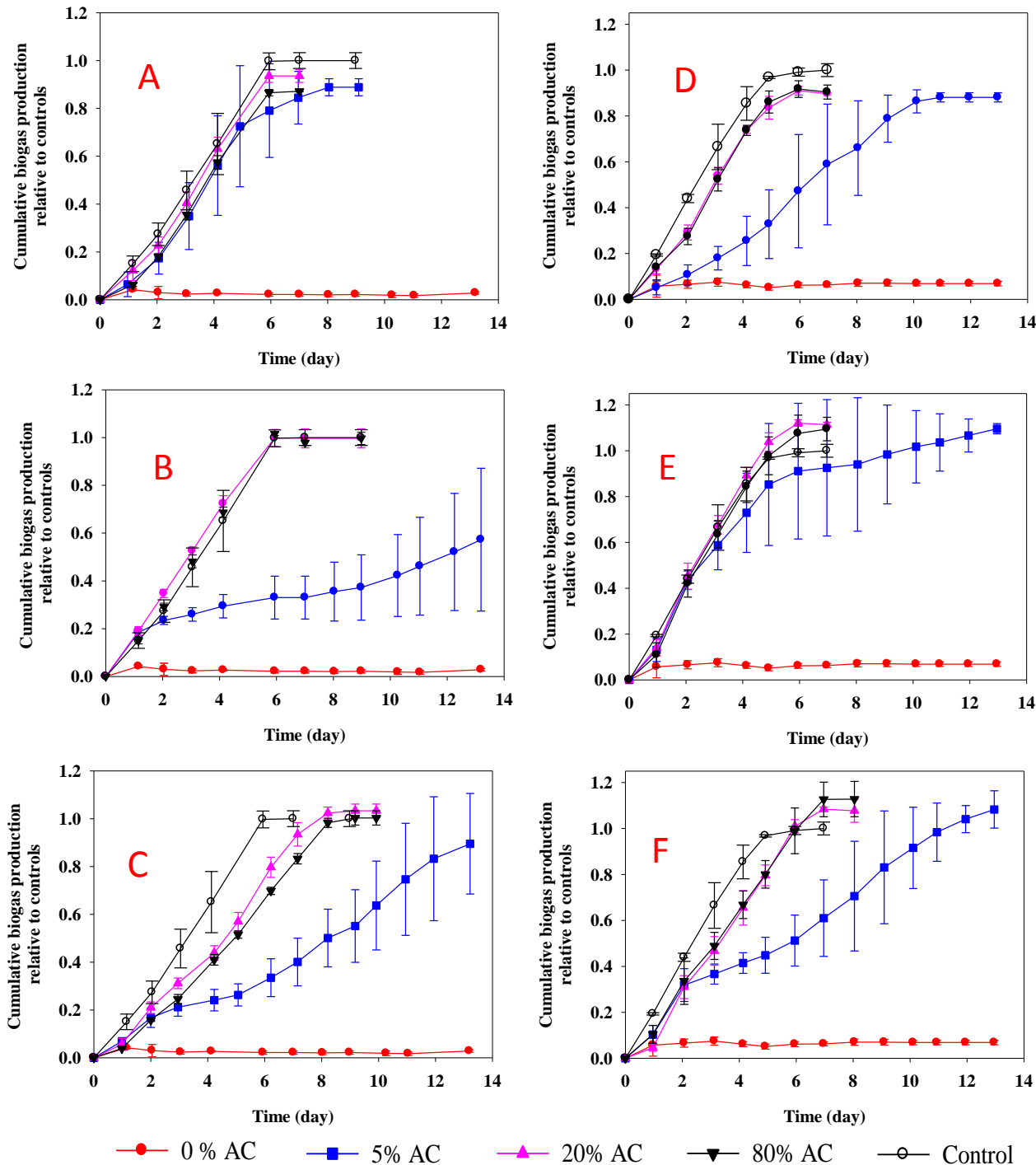


The error bars represent one standard deviation of triplicate experimental results.

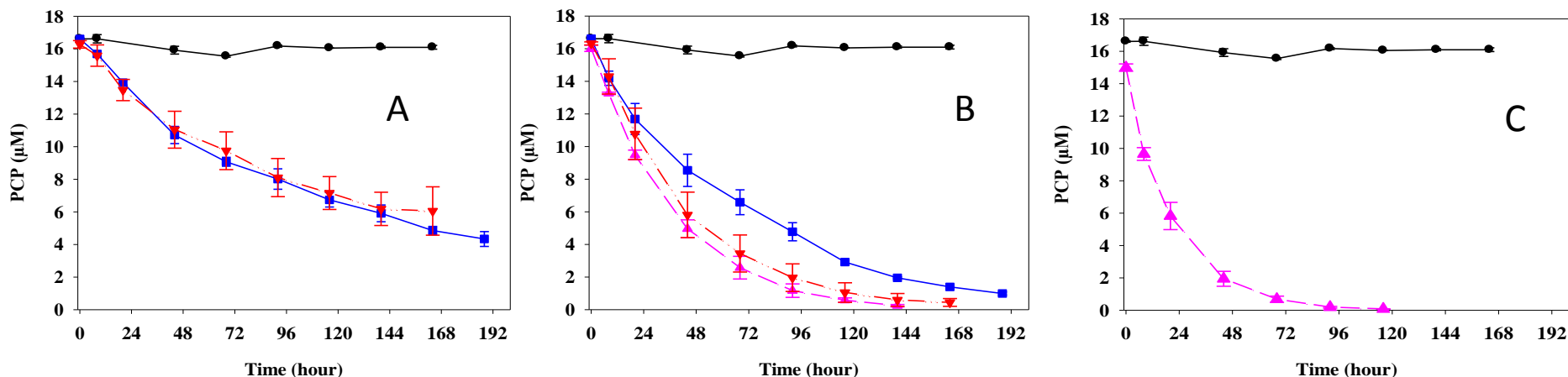
*Xiao Y., De Araujo C., Sze C.C., Stuckey D.C. Controlling a toxic shock of pentachlorophenol (PCP) to anaerobic digestion using activated carbon addition. *Bioresource Technology*.181 (2015) 303–311.

EFFECT OF PAC ADDITION ON METHANE PRODUCTION WITH PCP

Cumulative biogas production relative to controls for sludge incubated with 15 μM PCP at different doses of PAC (SAE2 on the left and WP-AO on the right) added A) and D) *simultaneously with*, B) and E) *prior to*, and C) and F) *after the addition of PCP*. The control had no PCP in it. The error bars represent one standard deviation of triplicate measurements.



SOLUBLE PCP AFTER PAC ADDITION



PCP concentration change after addition of A) 20%, B) 80% and C) 320% of GAC. A control (No GAC) with only sludge and PCP was included.

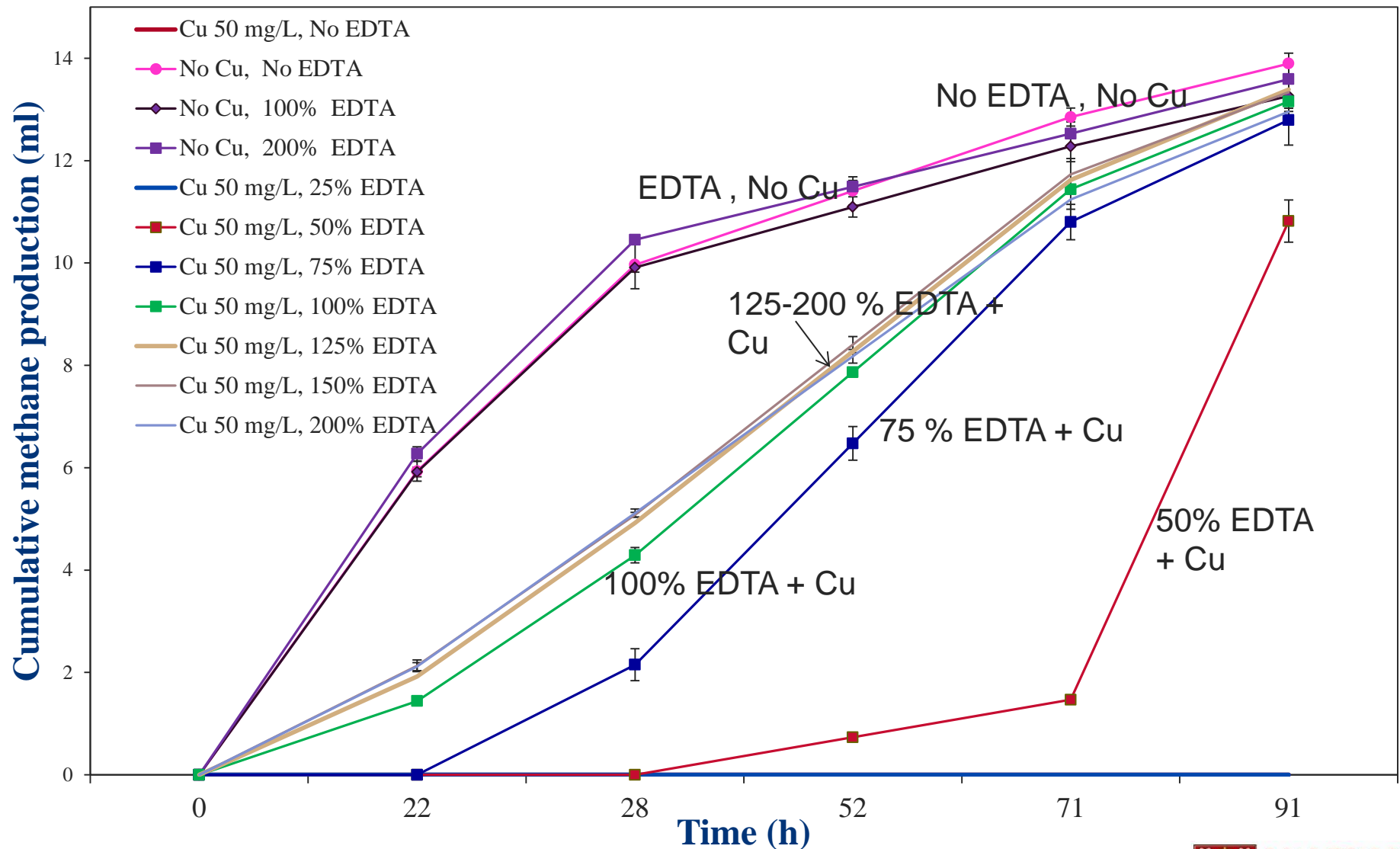
The error bars represent one standard deviation of triplicate measurements.

As PAC dose increases the solubility of PCP decreases, and the toxicity reduces in line with this reduction.

Using chelating agents (EDTA/NTA) to control heavy metal toxicity

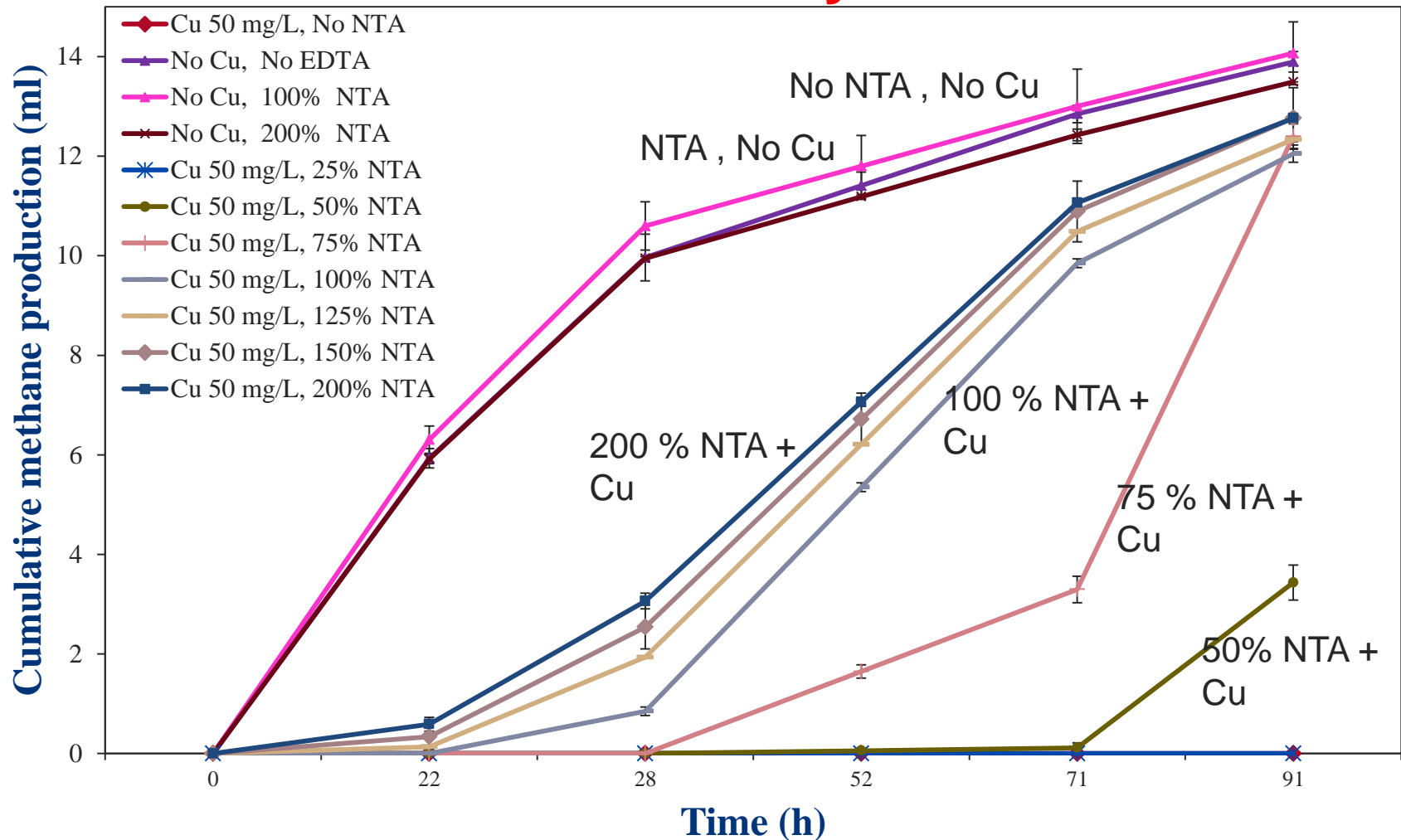
- EDTA-Me, NTA-Me complexes can reduce the bioavailability of metals in AD
- Equimolar addition of EDTA/NTA can prevent heavy metal toxicity without adverse effects
- Heavy metals tested: Cu, Ni, Zn
- 100 mg/L Zn and 50 mg/L Cu resulted in irreversible inhibition to methanogens
- 50 mg/L Ni caused transitory inhibition to methanogens and retarded methane production

Effects of stoichiometric addition of EDTA on Cu toxicity



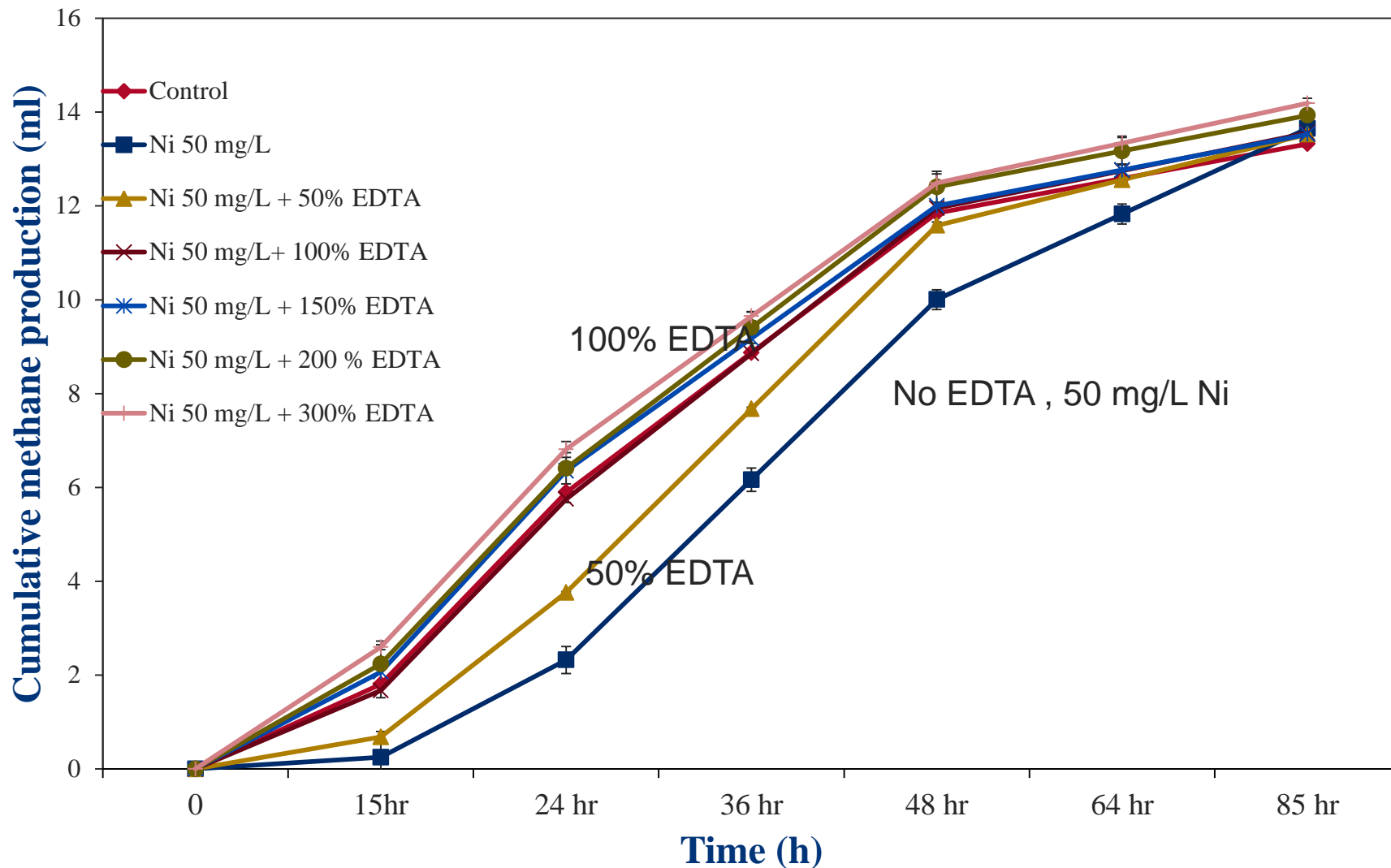
EDTA reduces toxicity over time, but not completely until 4 days

Effects of stoichiometric addition of NTA on Cu toxicity



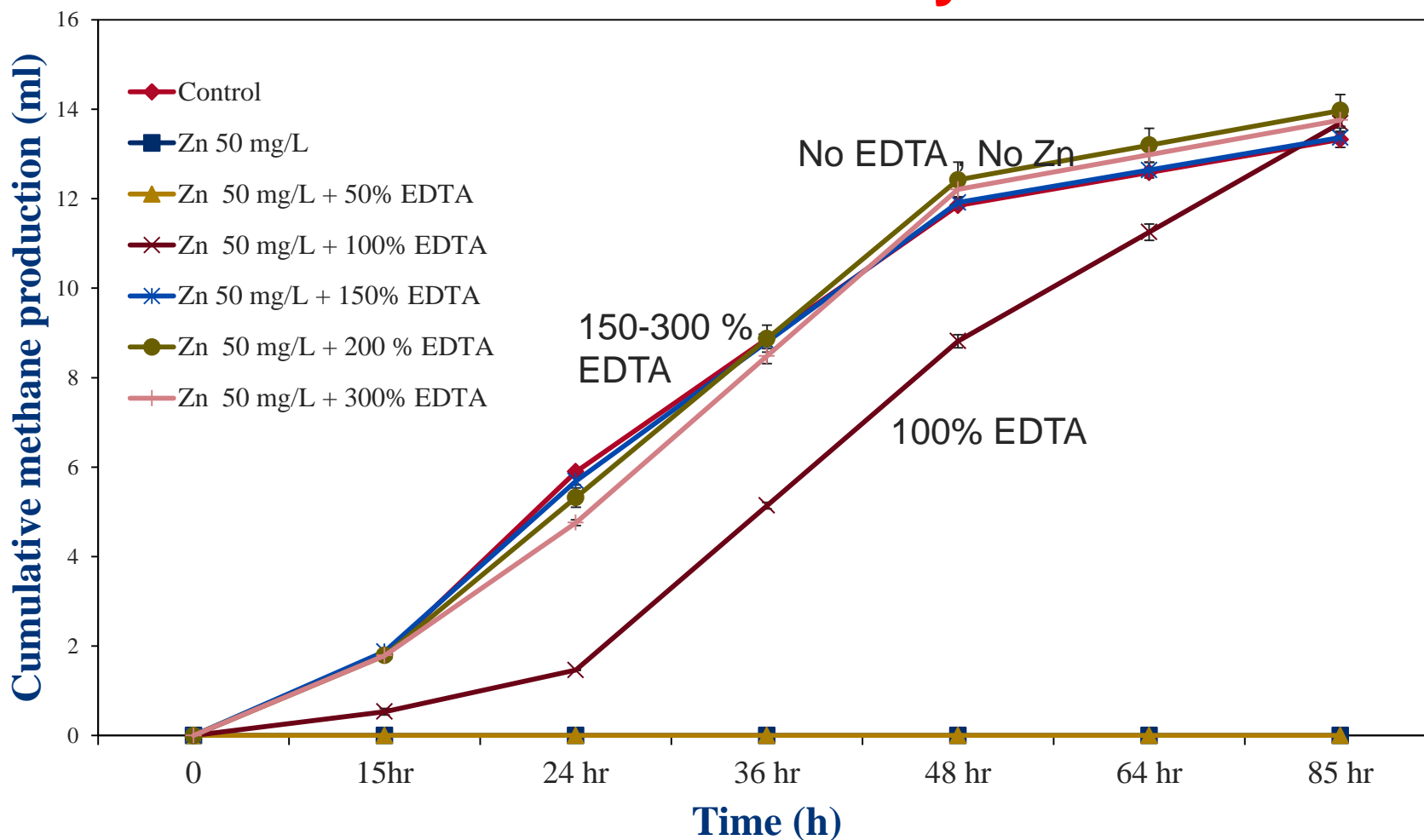
Clearly NTA not as effective as EDTA due to lower binding capacity

Effects of stoichiometric addition of EDTA on Ni toxicity



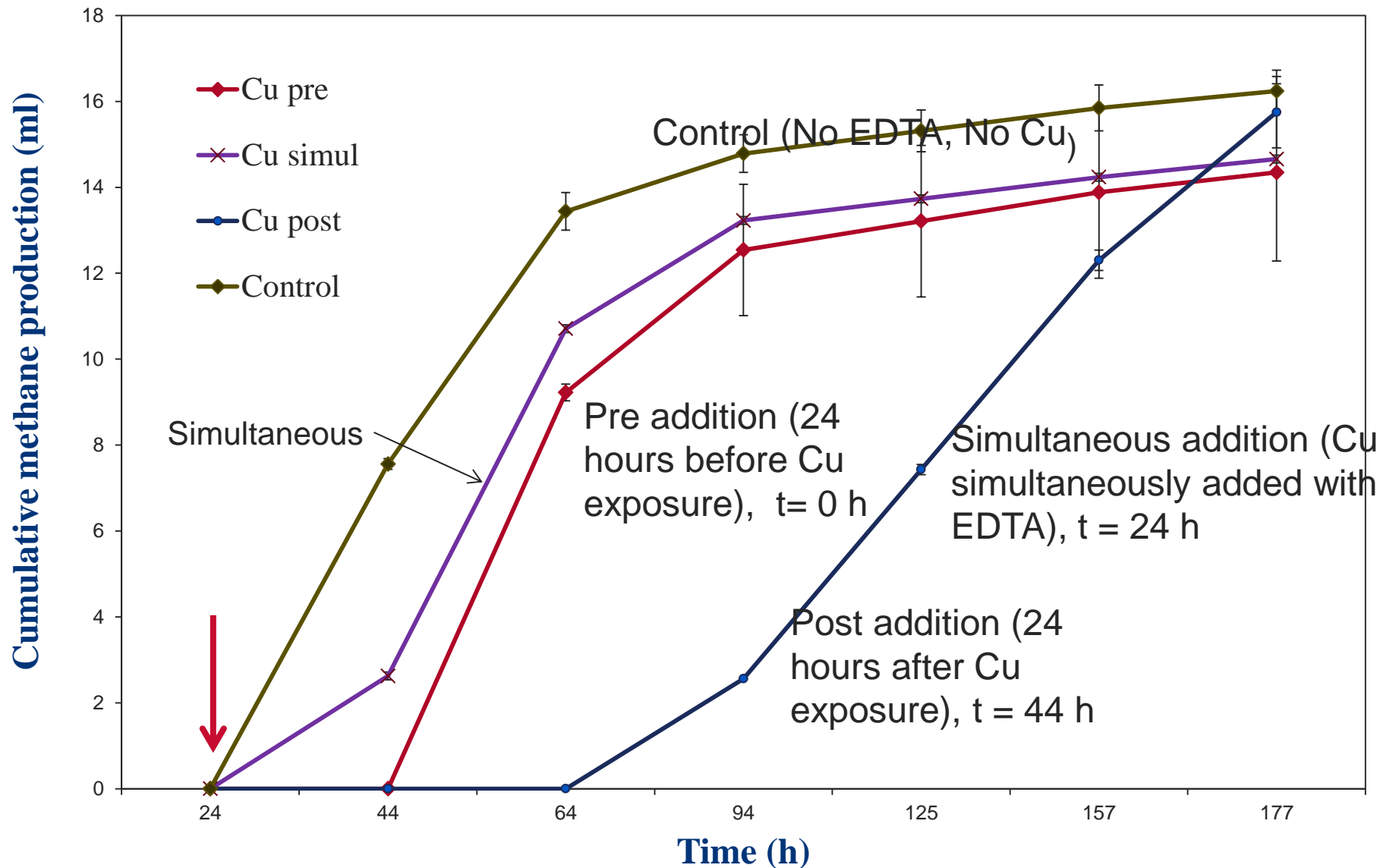
EDTA very effective reducing Ni toxicity

Effects of stoichiometric addition of EDTA on Zn toxicity



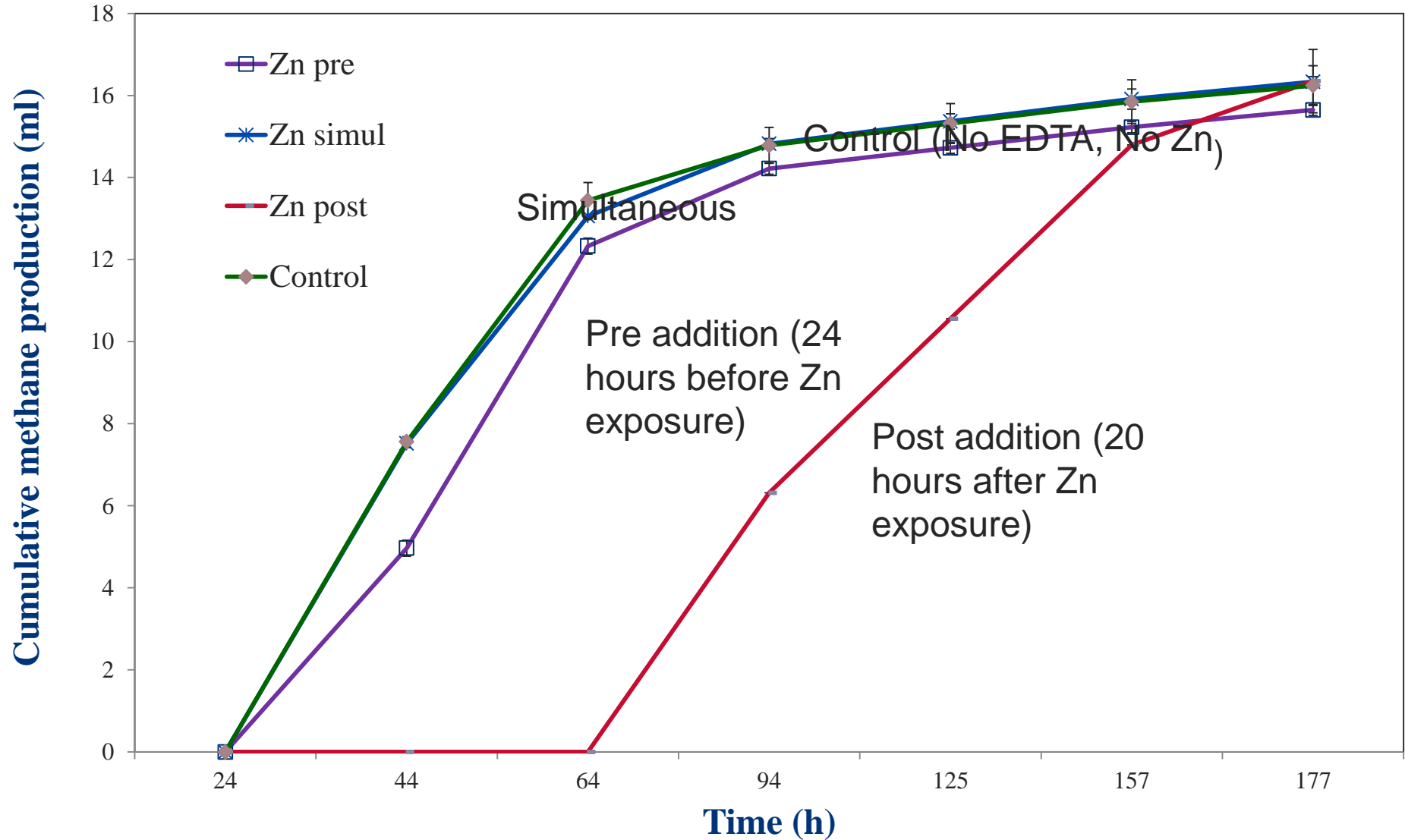
EDTA effective for reducing Zn toxicity

Time of EDTA addition – Cu



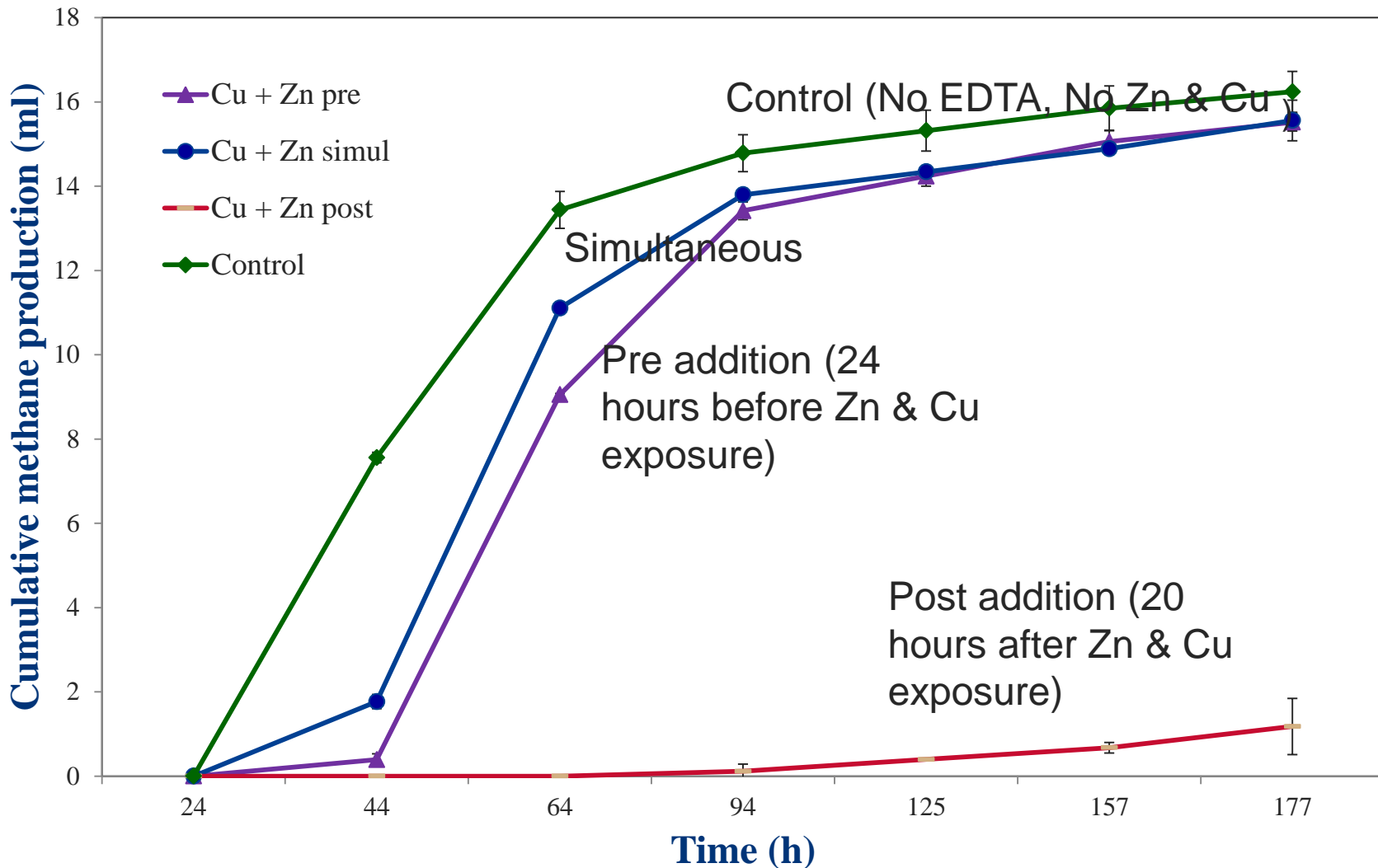
Even nearly 2 days after a toxic shock EDTA can “recover” stuck digesters within 6 days

Time of EDTA addition –Zn



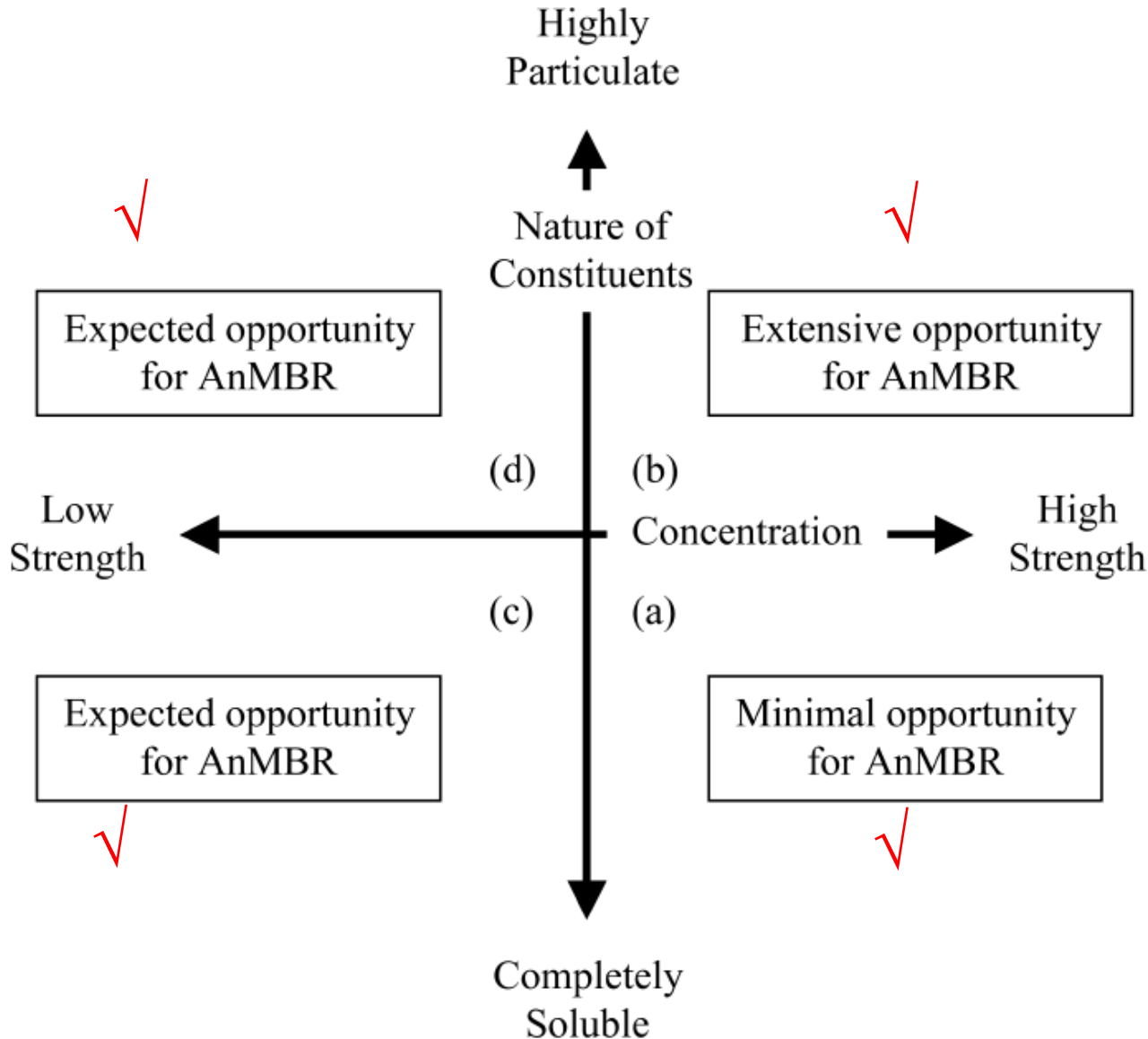
With simultaneous or pre-addition we can ameliorate toxicity, even 20 hours afterwards

Time of EDTA addition –Zn & Cu



Toxic synergism between Zn and CU to exacerbate toxicity

What is the scope and application of anaerobic membrane bioreactors (AnMBRs) in wastewater treatment?



- High COD removals
- Short HRTs (1 hr)
- Long SRTs (200 d)
- Can tolerate toxic shocks and we can now monitor them
- Can ameliorate toxins in AD with EDTA and PAC
- Understand membrane fouling by SMPs and colloids, and their composition
- Better understand metal speciation in SAMBR

CONCLUSIONS

- “Critical Flux” about the same whether measured for 20 min OR 24 h.
- For every critical flux there is a critical gassing rate-influences energy input.
- Composition of fouling layer is partially SMPs and partly colloids.
- Gassing rate does not seem to strongly influence Zeta potential or particle size.
- Metal and organics (PCP) toxicity can be monitored in a rapid assay (<10 min) using fluorescent resazurin reduction in a micro-fluidic cell.
- Toxicity can be ameliorated of organics using PCP, while metals can be ameliorated using EDTA complexation.
- AnMBRs are treating a wide variety of WWs to very high levels with small footprints.

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- Chengcheng LE



Thank you for your
attention-
QUESTIONS?