

Understanding Flocculation: Particle Size, Filterability

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Amgen WA

Investigation Goals:

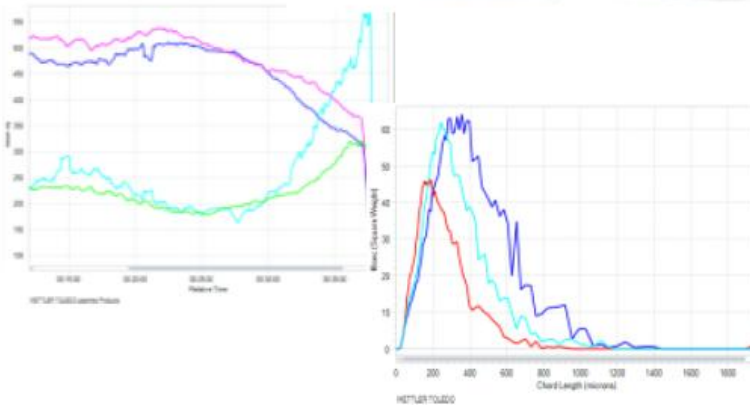
- Understand how flocculation works in our processes
- Could particle distribution analysis help optimize flocculation?
- Is there a direct correlation between particle distribution and filterability?

Methods

- FBRM -focused beam reflectance
- SHC filtration as an analytical method
- Lipid assays (sieving and adsorptive properties of filters)

FBRM

In Situ Floc Characterization Tools

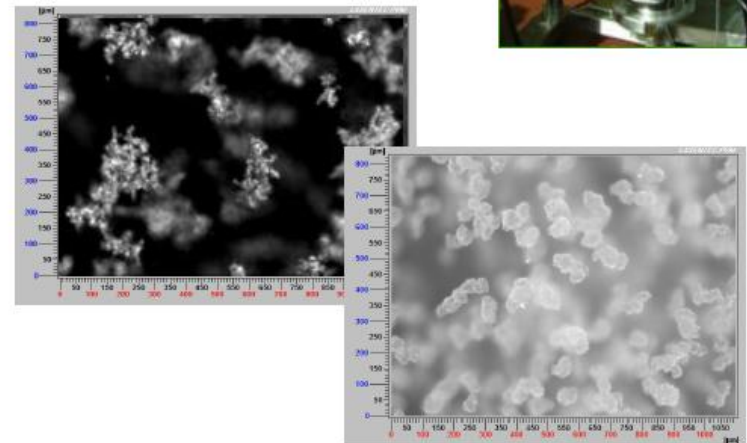
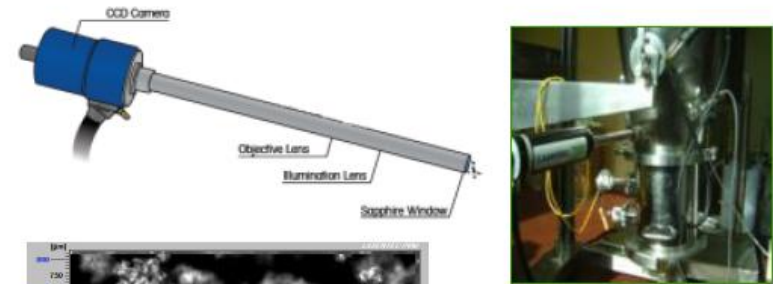


FBRM® Technology

Focused Beam Reflectance Measurement

Track real-time changes in floc density, floc size and morphology directly in the process.

- Characterize flocs and their constituents from 0.5µm to 1mm



PVM® Technology

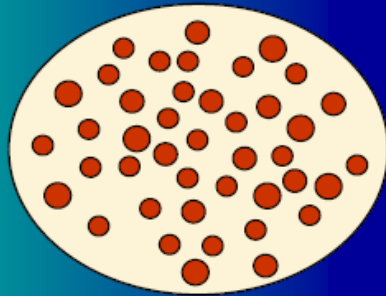
Particle Vision Microscopy

Microscope quality images, in-process and in real time.

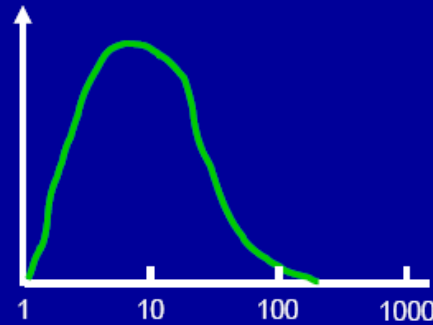
- Characterize flocs and their constituents from 2µm to 1mm

Eric Dykus
Mettler Toledo

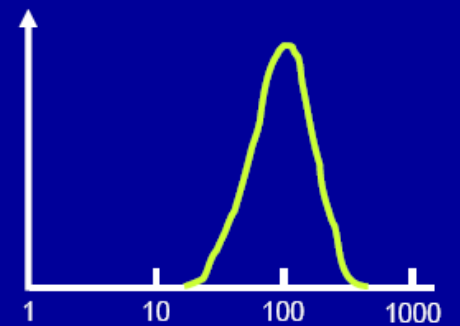
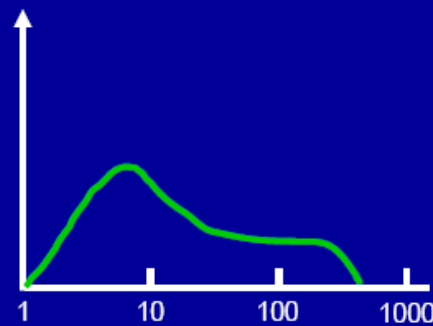
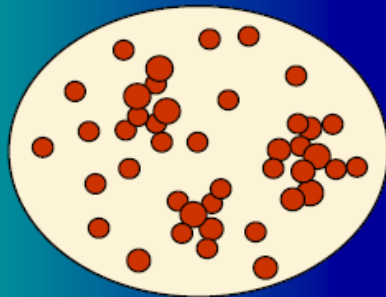
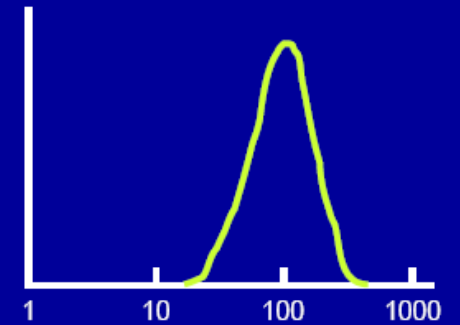
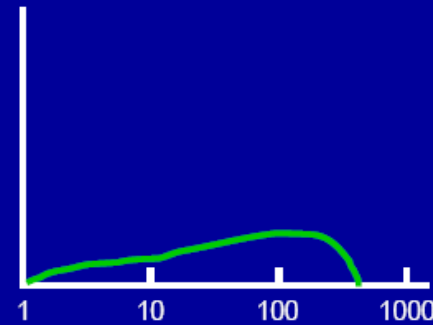
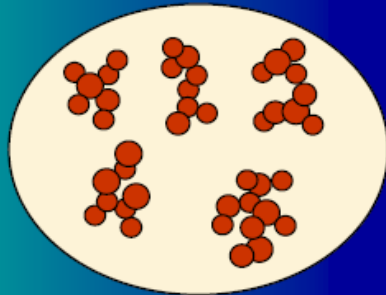
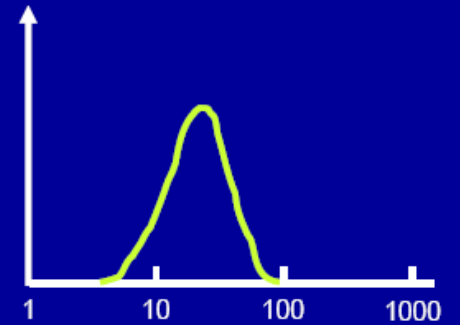
FBRM response to flocculation



Unweighted



Square-weighted



General FBRM interpretation

Eric Dykus
Mettler Toledo

Mean chord length

Mean square-weighted chord length

Behaviour



Efficient flocculation (good fines capture and significant aggregation)



Large aggregates but inefficient fines capture



Flocculation predominantly through fines capture - no large aggregates



Dispersion or rupture of aggregates with no re-aggregation

Important aspects

- Mean Square

Count	Size
15,000	1 μm
3,000	5 μm
50	50 μm
Simple mean	2.1 μm
Squared weighted mean	8.2 μm

- Why are big particles so important in this analysis?

- 1 sphere of 100 μm diameter = 525,000 μm^3

- 1 sphere of 2 μm diameter = 4.2 μm^3

- One 100 μm sphere \sim 125,000 2 μm spheres

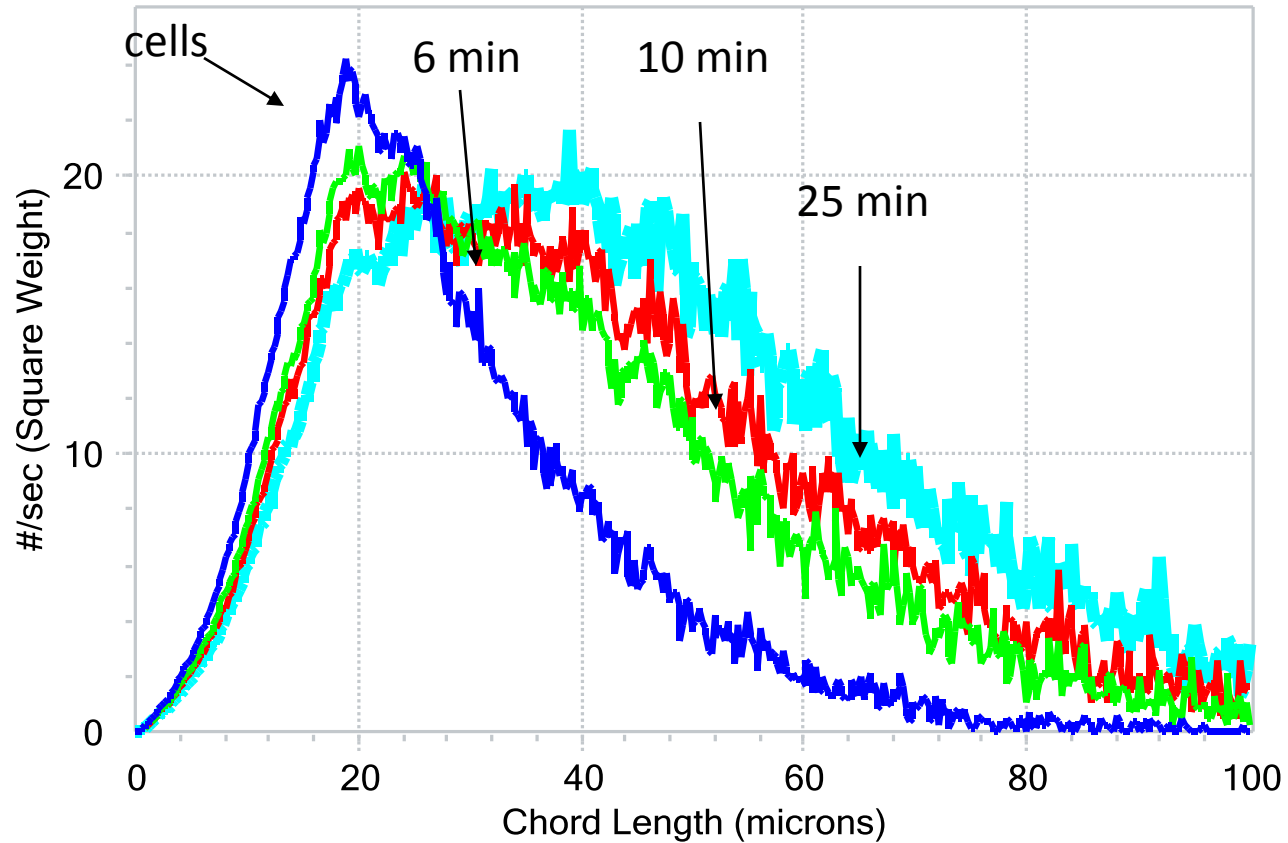
Analysis of two sets of data per molecule

- Flocculation by Settling- small scale experiments
 - **Flocculation reaction in 1L cylinder**
 - Tested cell culture with flocculants and decanted supernatants
 - Control: untreated sample bench top- centrifuged (non-ideal)
 - **Settling V_{max} = SHC filter (3.5cm²)**
- Flocculation followed by centrifugation
 - Centrate from the **main centrifuge**
 - Filterability based on Anne Thomas' experiments= **DF +0.2 μ m**
(Depth Filter Throughput=DFT)

FBRM Analysis

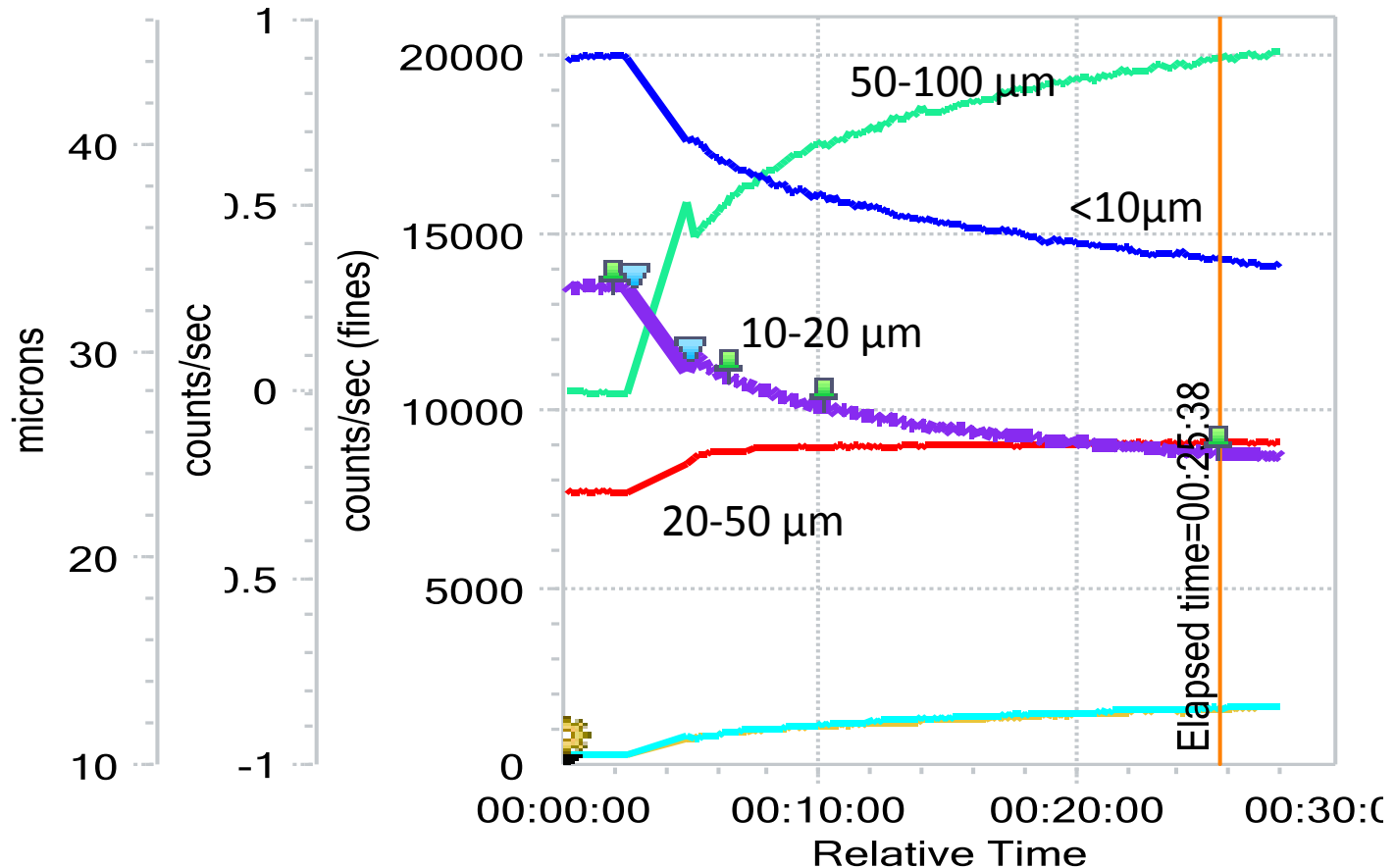
- “Raw data” - observation of cell culture with flocculant
- Settling supernatant= SHC filter feed
- Centrate=DF feed

Example: AB 1 PDADMAC 0.08%- Size Distribution- Flocculation Reaction Time Course



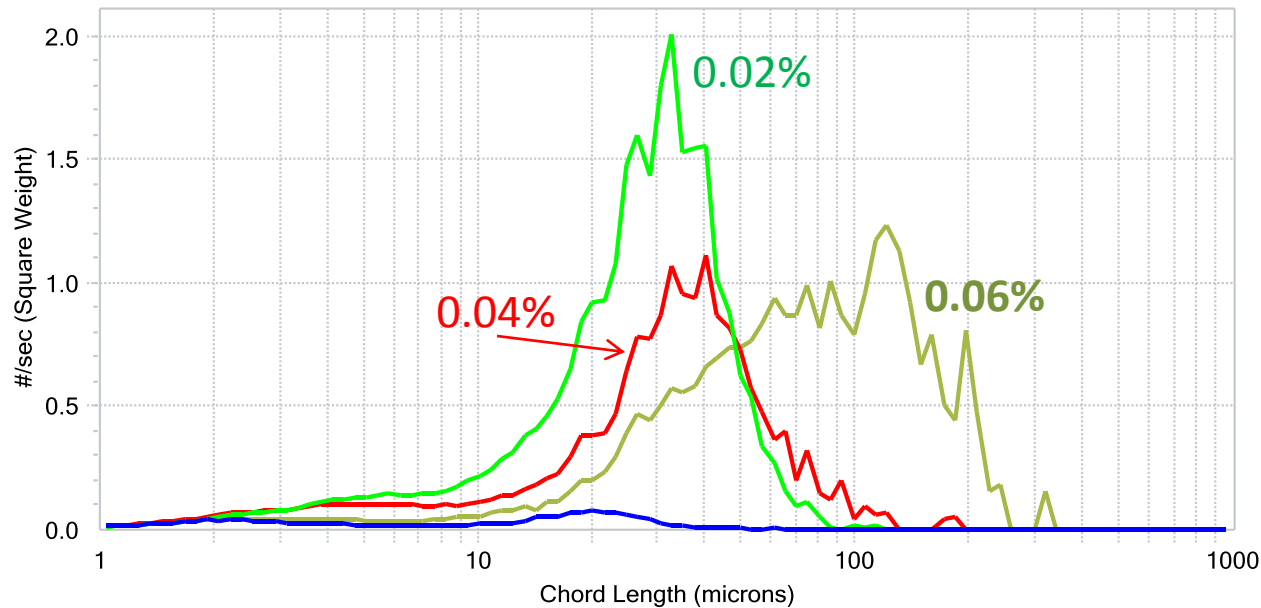
PDADMAC – 0.08% Time Course

looking at specific particle size groups



Settling Supernatant

Visualize the change in particle distribution dependent on flocculant concentration
AB2 Flocculation with PDADMAC



METTLER TOLEDO

Increasing concentration of flocculant



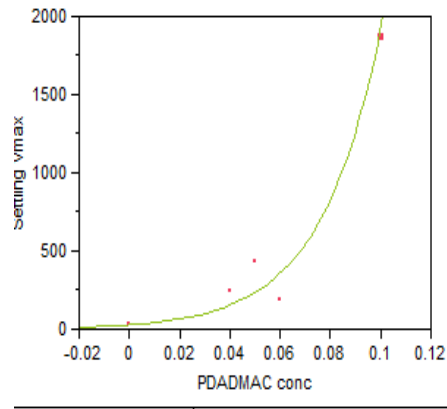
Increase in particle size

Small Scale Flocculation

- Explore the effect flocculant concentration on SHC- V_{max}
- Correlate mean particle size and turbidity to V_{max}

Settling with Pdadmac- SHC as an analytical tool

**AB3 at 15°C
CDM**



— Transformed Fit Log

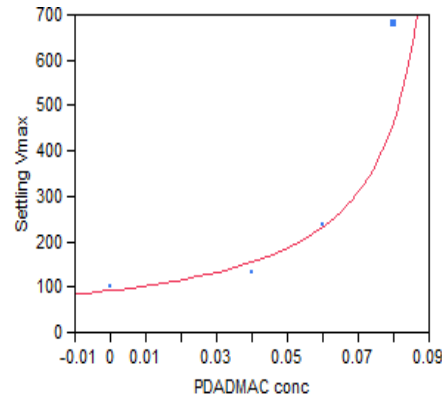
Transformed Fit Log

$$\text{Log}(\text{Settling Vmax}) = 3.3528337 + 42.103919 \cdot \text{PDADMAC conc}$$

Summary of Fit

RSquare	0.893524
RSquare Adj	0.858033
Root Mean Square Error	0.605112
Mean of Response	5.45803
Observations (or Sum Wgts)	5

**AB2 at 15°C
HYP**



— Transformed Fit Reciprocal

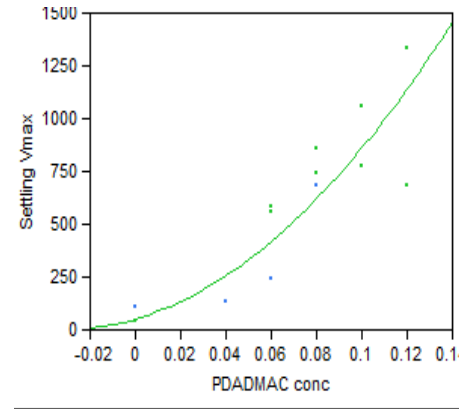
Transformed Fit Reciprocal

$$\text{Recip}(\text{Settling Vmax}) = 0.0107746 - 0.1078662 \cdot \text{PDADMAC conc}$$

Summary of Fit

RSquare	0.937179
RSquare Adj	0.905769
Root Mean Square Error	0.001168
Mean of Response	0.005921
Observations (or Sum Wgts)	4

**AB1 10°C
HYP**



— Transformed Fit Sqrt

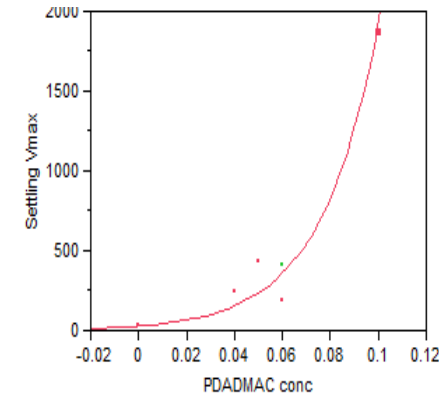
Transformed Fit Sqrt

$$\text{Sqrt}(\text{Settling Vmax}) = 7.200388 + 221.69558 \cdot \text{PDADMAC conc}$$

Summary of Fit

RSquare	0.856746
RSquare Adj	0.844808
Root Mean Square Error	3.929146
Mean of Response	21.45225
Observations (or Sum Wgts)	14

**AB1 15°C
HYP**



— Transformed Fit Log

Transformed Fit Log

$$\text{Log}(\text{Settling Vmax}) = 3.3528337 + 42.103919 \cdot \text{PDADMAC conc}$$

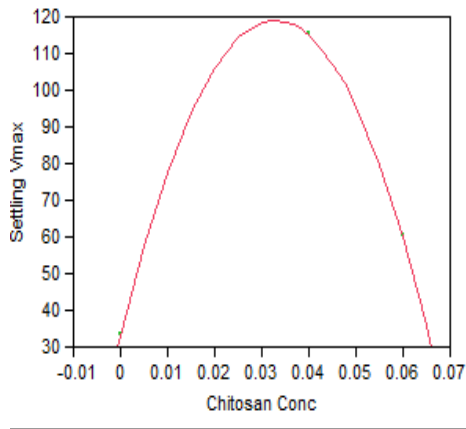
Summary of Fit

RSquare	0.893524
RSquare Adj	0.858033
Root Mean Square Error	0.605112
Mean of Response	5.45803
Observations (or Sum Wgts)	5

- Settled supernatant -Vmax with SHC is a very good indicator of flocculation
- Regardless of scale, media type and molecule increasing PDADMAC conc increases settling Vmax .
- Settling with PDADMAC will eliminate the need for centrifuge and depth filter

Settling with Chitosan

AB1 at 10°C



— Polynomial Fit Degree=2

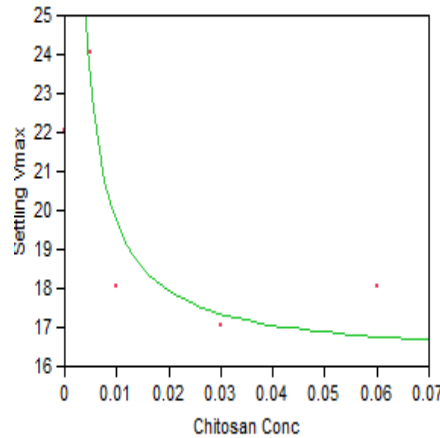
Polynomial Fit Degree=2

Settling Vmax = 121.88889 - 83.333333*Chitosan Conc - 800
Conc-0.033333)*2

Summary of Fit

RSquare	1
RSquare Adj	.
Root Mean Square Error	.
Mean of Response	69.33333
Observations (or Sum Wgts)	3

AB3 at 15°C



— Transformed Fit to Reciprocal

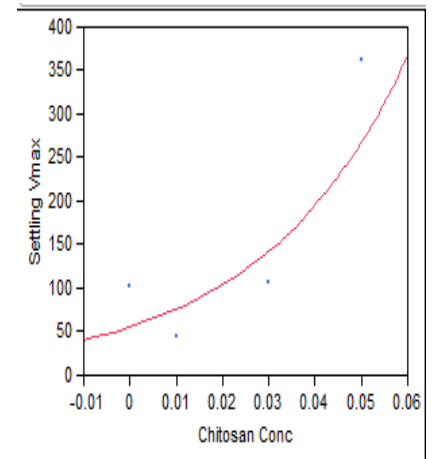
Transformed Fit to Reciprocal

Settling Vmax = 16.177258 + 0.0351171*Recip(Chitosan Ci

Summary of Fit

RSquare	0.832722
RSquare Adj	0.749082
Root Mean Square Error	1.603716
Mean of Response	19.25
Observations (or Sum Wgts)	4

AB2 at 10°C



— Transformed Fit Log

Transformed Fit Log

Log(Settling Vmax) = 4.0163271 + 31.487219*Chitosan Conc

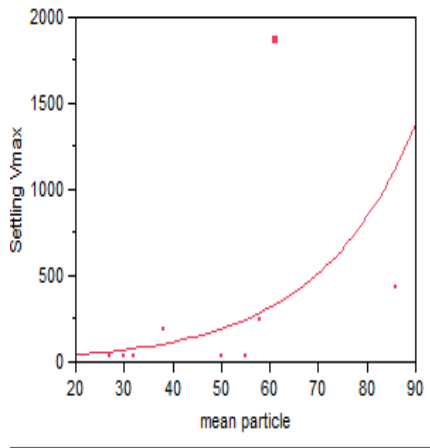
Summary of Fit

RSquare	0.634318
RSquare Adj	0.451478
Root Mean Square Error	0.649252
Mean of Response	4.72479
Observations (or Sum Wgts)	4

- Overall SHC Vmax values are very low compared to PDADMAC
- Trends are inconsistent for different molecules

Pdadmac: Settling Vmax and Mean Particle Size

AB1 at 15°C



— Transformed Fit Log

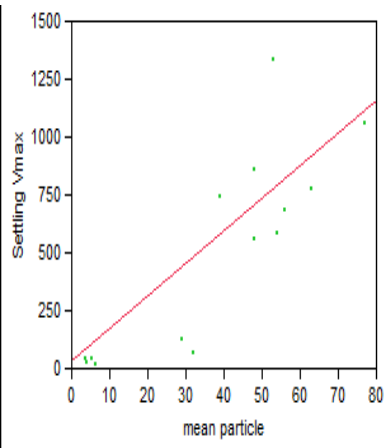
Transformed Fit Log

$$\text{Log}(\text{Settling Vmax}) = 2.7998484 + 0.0492256 * \text{mean particle}$$

Summary of Fit

RSquare	0.487134
RSquare Adj	0.316179
Root Mean Square Error	1.328044
Mean of Response	5.45803
Observations (or Sum Wgts)	5

AB1 at 10°C



— Linear Fit

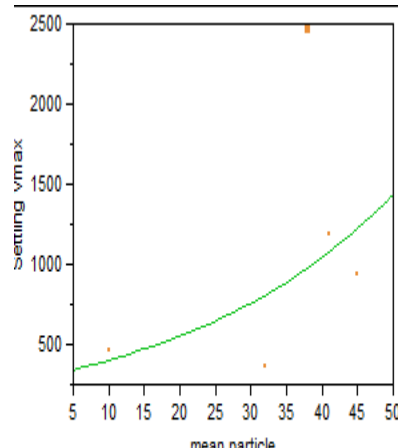
Linear Fit

$$\text{Settling Vmax} = 30.911627 + 14.077627 * \text{mean particle}$$

Summary of Fit

RSquare	0.677038
RSquare Adj	0.636668
Root Mean Square Error	242.221
Mean of Response	659.9
Observations (or Sum Wgts)	10

AB4 at 36°C

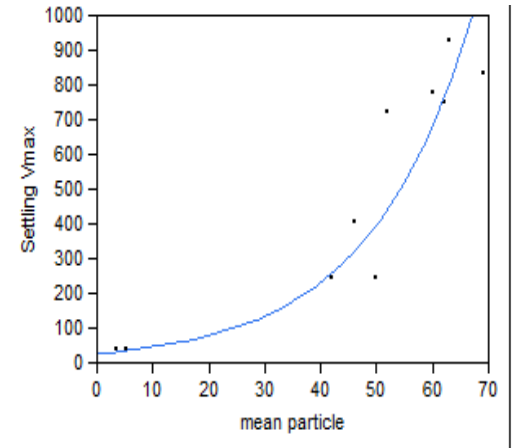


— Transformed Fit Log

Transformed Fit Log

$$\text{Log}(\text{Settling Vmax}) = 5.6770092 + 0.0318764 * \text{mean particle}$$

AB1 at 36°C



— Transformed Fit Log

Transformed Fit Log

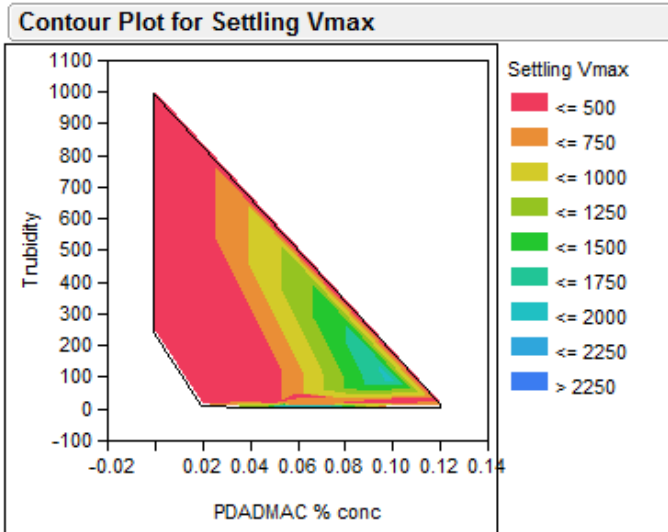
$$\text{Log}(\text{Settling Vmax}) = 3.2847202 + 0.0540951 * \text{mean particle}$$

Summary of Fit

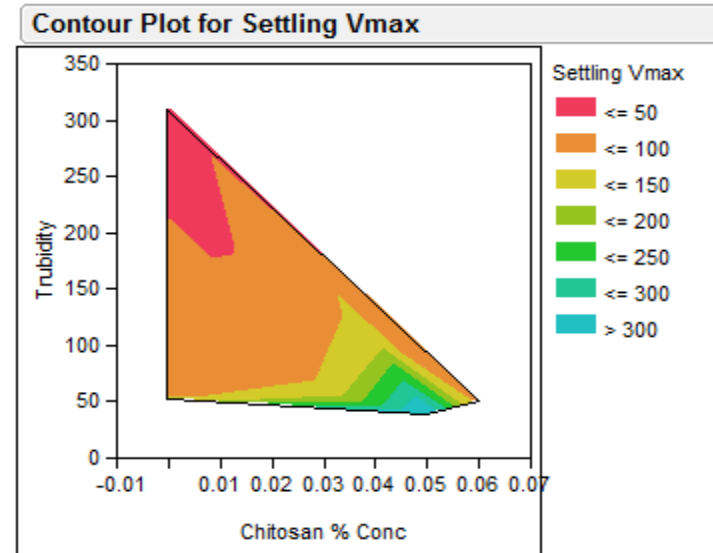
RSquare	0.955142
RSquare Adj	0.949534
Root Mean Square Error	0.287015
Mean of Response	5.734145
Observations (or Sum Wgts)	10

- Mean particle size explains SHC Vmax trend for settled supernatant

AB1-4: Settling Summary by Flocculant with Turbidity



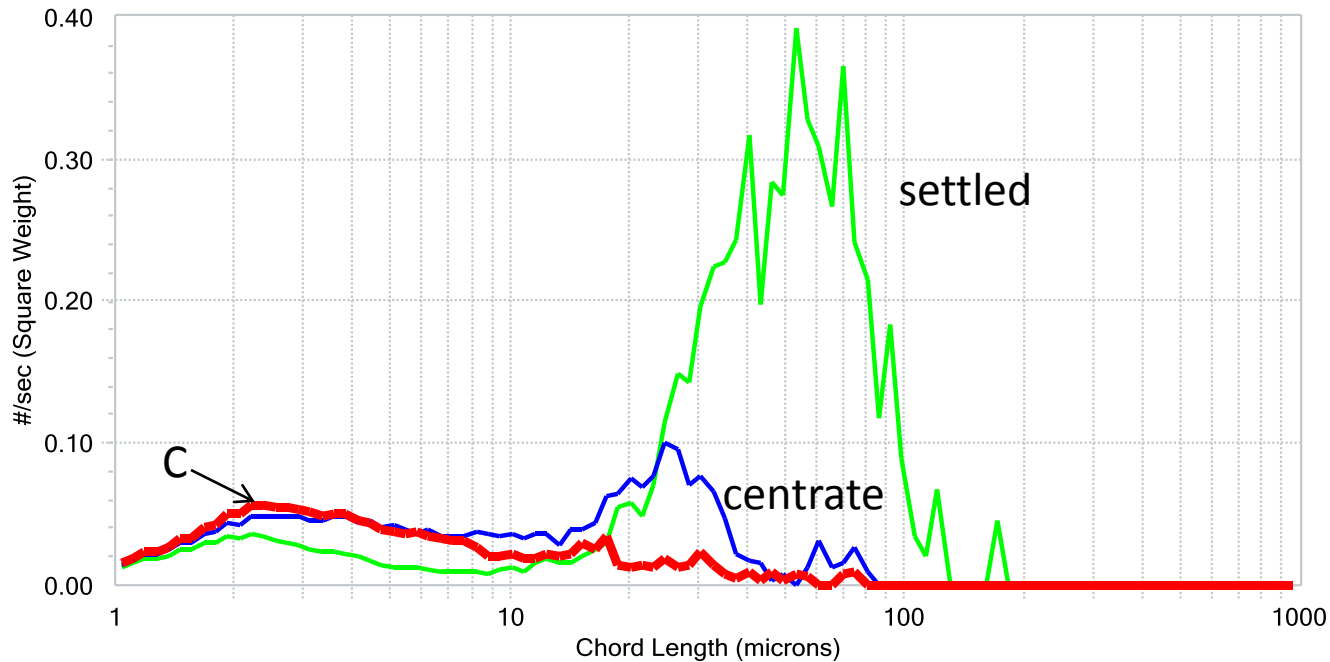
48 samples



14 samples

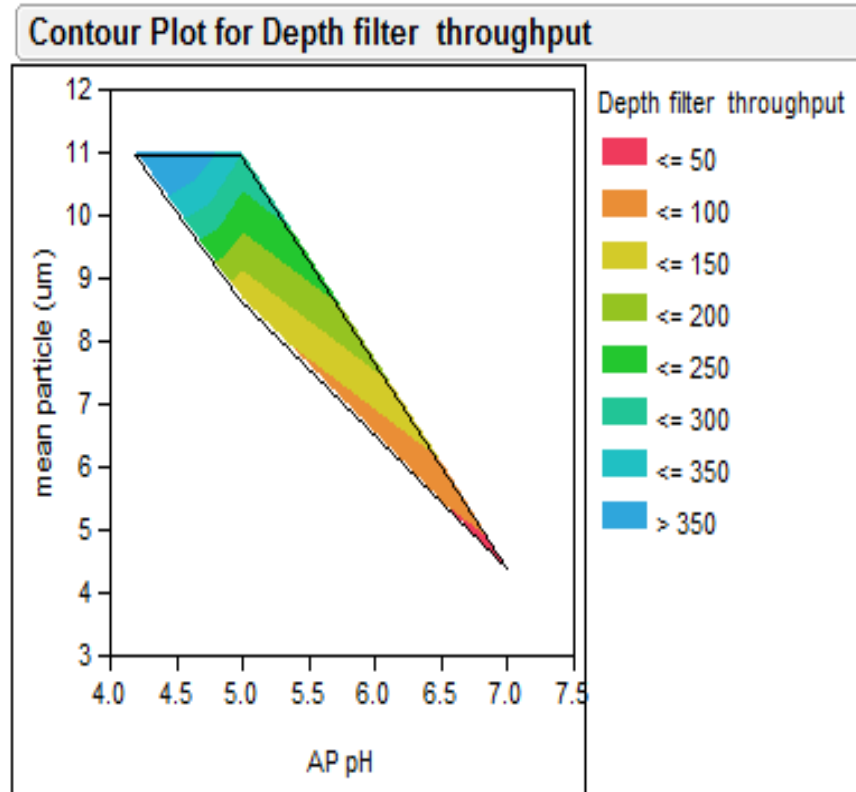
Centrates

Pdadmec Flocculation: Centrate and Settled Supernatant at 0.06% and 10°C



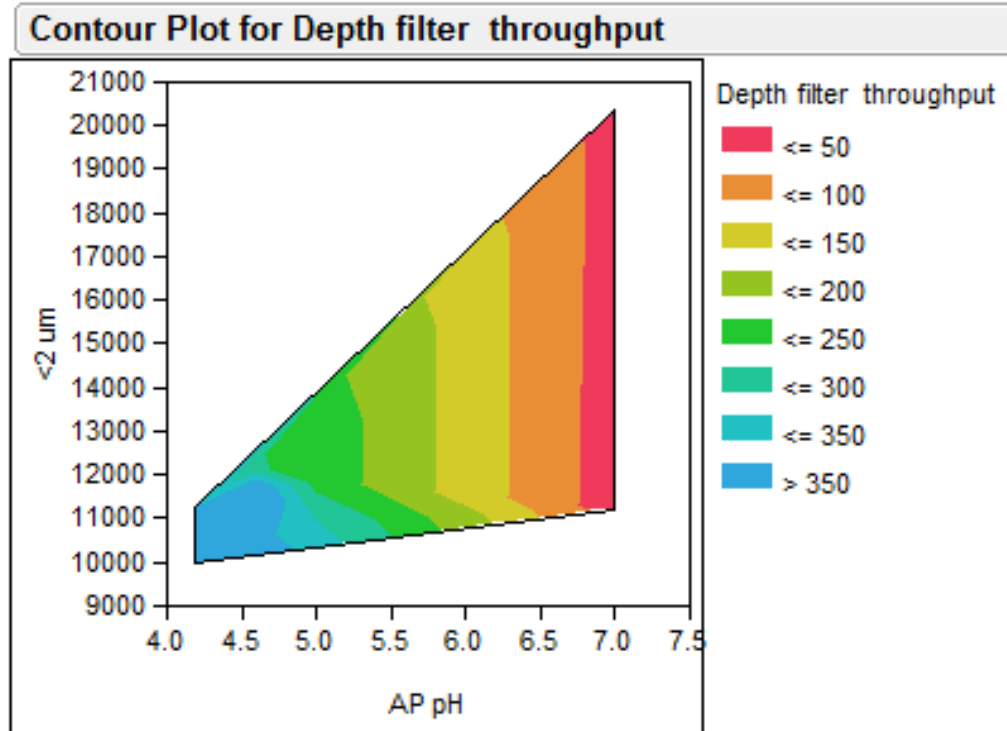
Large particles are more efficiently removed by centrifuge

Centrates: Depth Filter Throughput with AP (3 molecules, 14 samples)



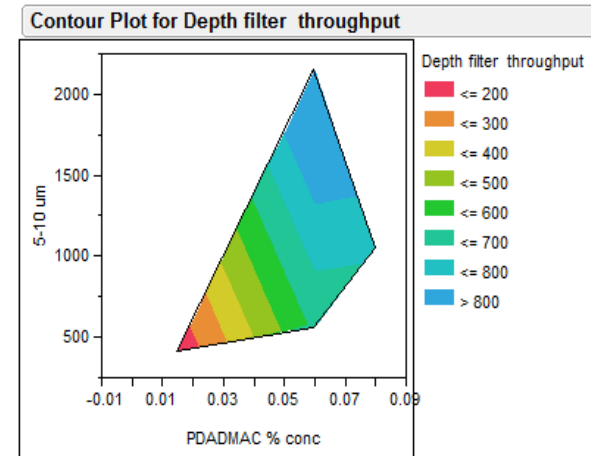
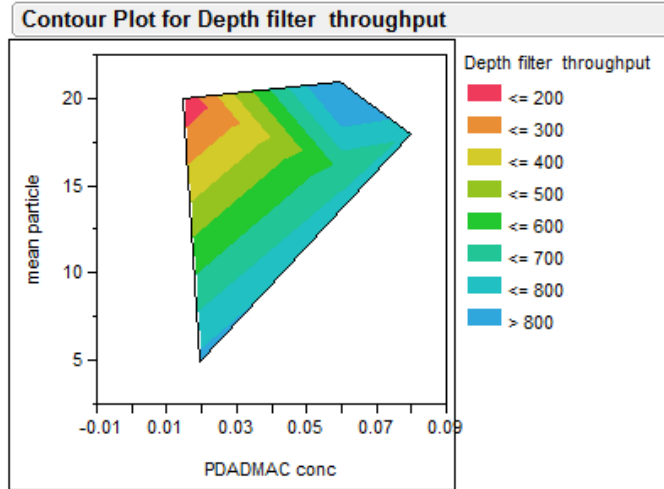
- Mean particle size of AP centrate poorly correlates with depth filter throughput
- With AP, the change in mean particle size is very small

Depth Filter Throughput with AP



- With decreasing pH:
 - Particles with size of <2 um decrease significantly
 - Depth filter throughput increases

Centrates: DFT with Pdadmec (2 molecules, 8 samples)



- mean particle size is not as predictive of SHC Vmax
- 5-10 µm particles seem to play an important role, contrary to settling
- more data needed

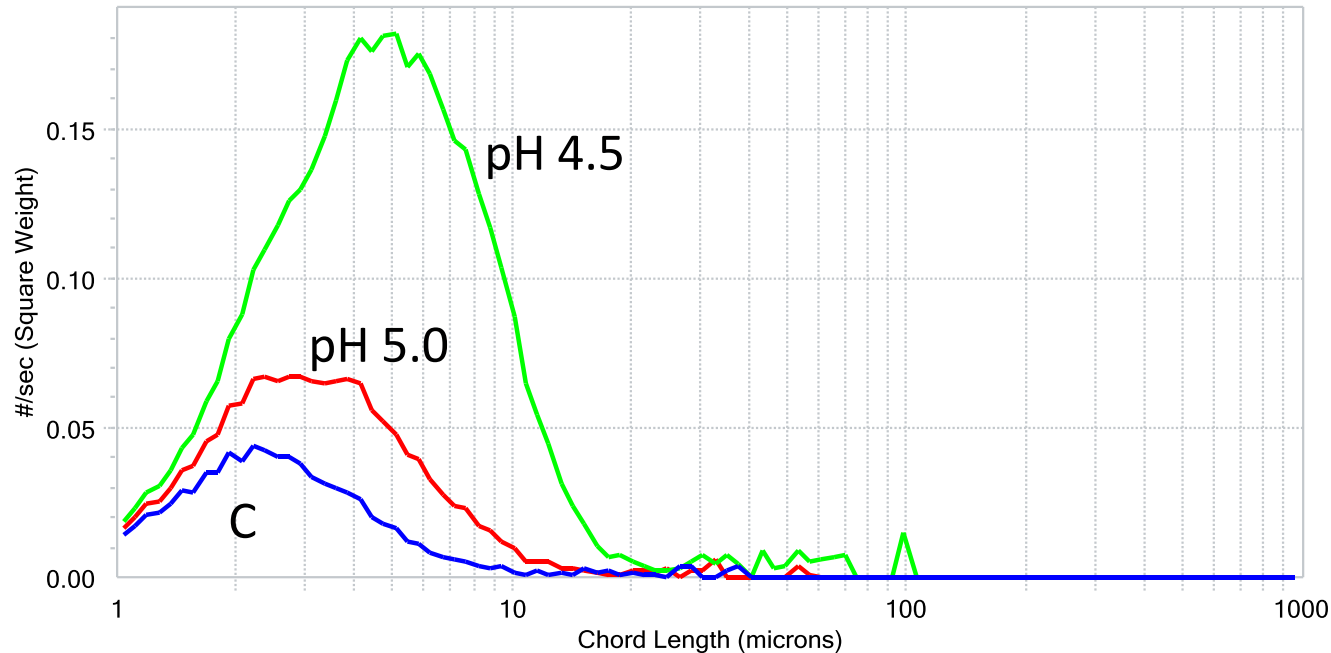
Conclusions

1. Mean particle distribution
 - Correlates with SHC V_{max} on settled samples of PDADMAC or chitosan flocculation
 - Poorly correlates with centrate depth filter throughput
2. Particles of $<2\mu m$ count
 - Correlate with AP centrate depth filter throughput
 - Pdadmact- Particles 5-10 μm correlate well with DFT
 - The best predictor of DFT is the overall shift in distribution by FBRM
3. AP- does not improve V_{max} on SHC; usually improves DFT but less efficiently than Pdadmact. AP may significantly worsen filterability.
 - When AP works, particle size increase is smaller than with Pdadmact, but seems to be changing the balance between particles in $<2\mu m$ size group

Acknowledgements

- Yinges Yigzaw, Tom McNerney, Anne Thomas, Rob Piper, Art Hewig, Ganesh Vedantham
- Mettler Toledo- Jack Shu, Eric Dykus.

AB1- AP Settling



Vmax- all samples below 35 L/m²

No size shift

SHC Vmax never improved by AP (unlike DFT)