Rheological Monitoring of Cell Lysis in *E. coli* Fermentation using acoustic sensors

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Introduction

- Bacterial cell lysis is seen in late-stage fermentation, causing product loss and additional complexities in downstream processing. Monitoring the physical or chemical properties of cell broths to infer cell status is often challenging due to the complex nature of the broth.
- Post-induction cell-wall strength tend to be weaker & although technically "viable" the cells leak product to extracellular space (Fig.1)
- Flow curves (fig 4) show an increase in viscosity as the fermentation progressed and an increase in shear thinning behaviour

We report the rheological examination of an industrially relevant *E. coli* fermentation system producing antibody fragments (Fab'), and demonstrate the utility of rapidly monitoring the physical properties of fermentation broths as a tool for process development to determine the optimal harvest time, in order to minimise product loss and maximise intracellular product concentration.

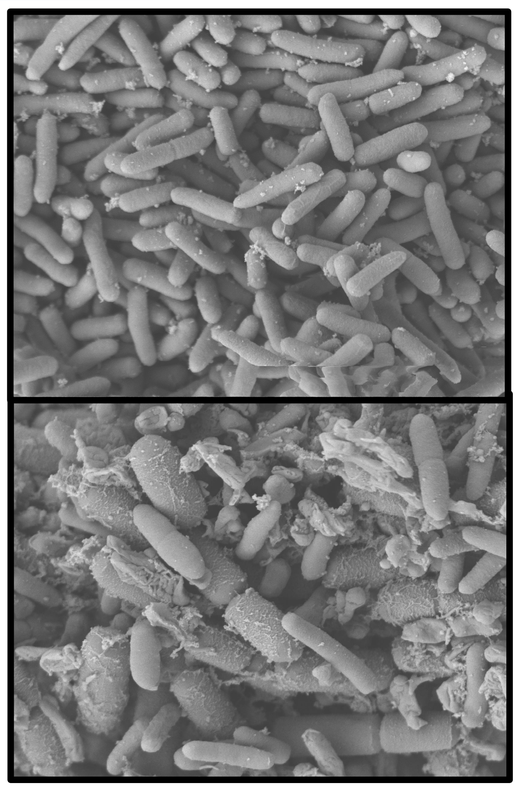

Figure 1: Scanning Electron Microscopy (SEM) images at x10,000 magnification, at early and late stage stationary phase.

Real-time Rheological Monitoring of Cell Lysis using acoustics

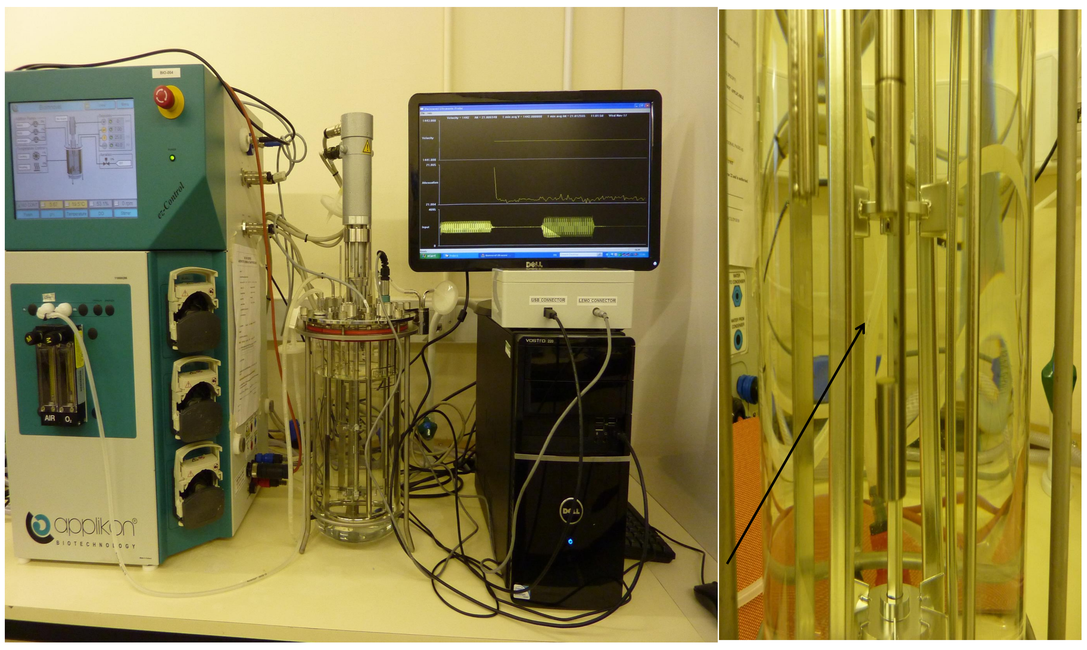

Figure 2: Procellia's RHEO probe monitoring fermentation *in-situ.* Ultrasonic pulses at 10 MHz are propagated in real-time as the broth flows through a transmitter & receiver. The attenuation & velocity of the waveform are recorded during the process. The inverse problem of ECAH model is used to infer the concentration of cells in terms of their volume fraction from attenuation & velocity .

We model the live cells in a bio-culture as colloidal particles, hence, we apply fundamental rheology to track changes in the bio-broth during growth as shown in Figure 3. The *E.coli* broths at low volume fraction levels (<5% cells)behave as Newtonian suspensions at shear rates <500 s^{-1}. (Fig.5)
We use *in-situ* ultrasonic probes to propagate waves through the culture in real-time as a non destructive technique, and calculate colloidal parameters such as volume fraction, viscosity & effective radius of growing cells.

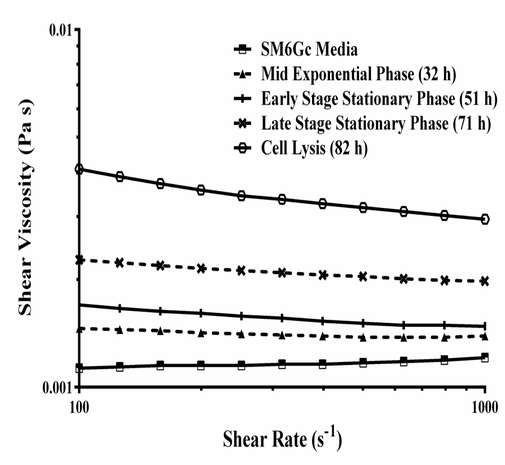

Figure 5: Flow curves of *E. coli* cell broth at various times during fermentation. Behaviour flow index was greater than 0.95 for all samples.

Future Work

- Calibration of the RHEO- sensor & determination of cellular parameters for the acoustics model.
- Obtain profiles of attenuation & velocity during *E. coli* fermentation & calculate viscosity from acoustics measurements.
- Adapt the acoustics model to accommodate poly-dispersity & visco-elasticity for the detection of cell lysis.

Acknowledgements

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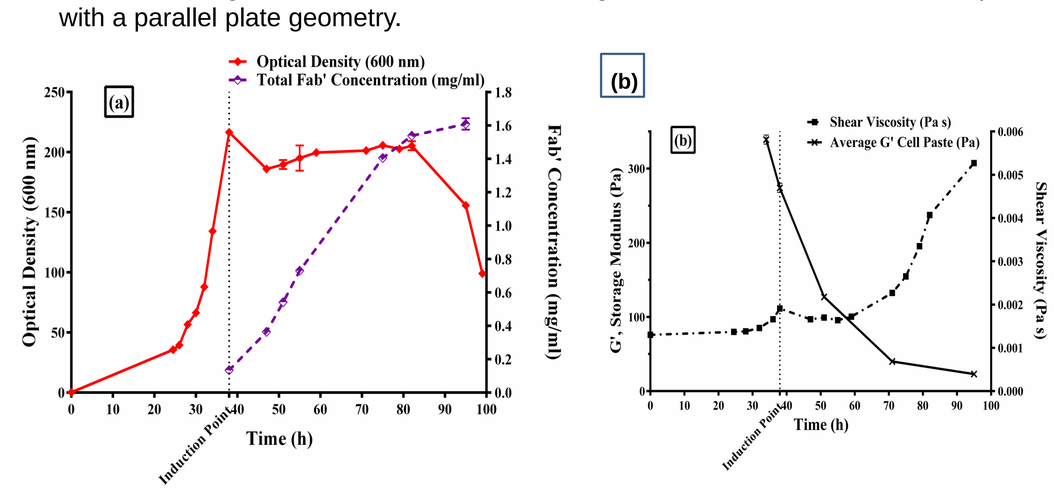
E. coli Fermentation

Figure 3: Rheological profiles depictive of cell lysis in an *E. coli* (Fab') fermentation. (a) Optical density and total Fab' concentration (b) Shear viscosity of broths & storage modulus of harvested cell paste over time . Fermentations carried out in 5L Applikon fermenter using defined SM6Gc media. Off-line rheological measurements taken using a controlled strain rheometer (Kinexus by Malvern) with a parallel plate geometry.



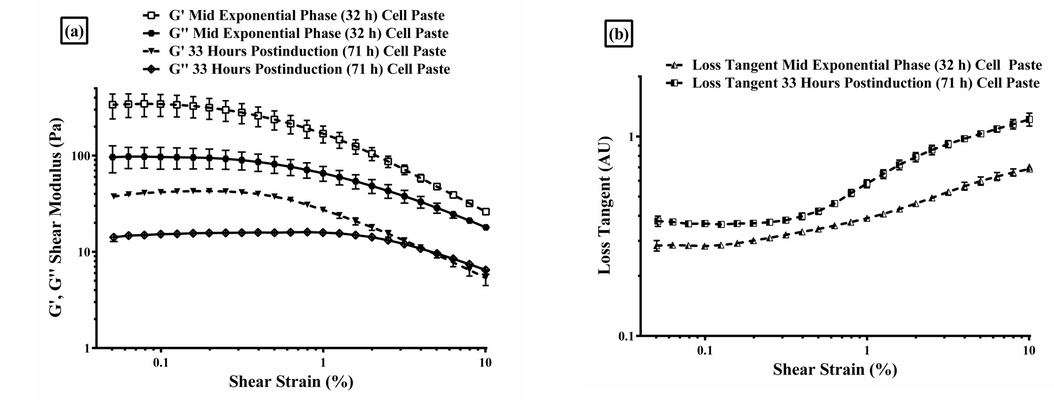


Figure 4a.: Storage (G') & Loss modulus (G") of harvested cells over a range of strain values showing the linear visco-elastic region at strains below 1%.
Figure 4b. Loss tangent of harvested cells from exponential & post-induction phases as a measure of the "fluidity" of the cells , i.e. loss of cell wall strength during lysis

Acoustics theory for the determination of volume fraction (live biomass)

The ECAH Model is used to calculate the attenuation of a wave as it moves through a suspension of particles in a fluid, where $\beta(\omega)$ is the waveform, and $k_{c/T/s}$ the wave-numbers for the compression/tensile, thermal & shear waves respectively.

$$\beta(\omega) = \frac{\omega}{c(\omega)} + i\alpha(\omega)$$

$k_c = \omega/c + i\alpha_L$
$k_T = (1+i)\,(\omega/2\sigma)^{1/2}$
$k_S = (\omega^2\rho/\mu)^{1/2}$

c= speed of sound & α =intrinsic attenuation

The model considers energy loss of compression wave incident on a particle due to a number of loss mechanisms such as

1) Viscous losses – due to density contrast between particle and liquid which produces viscous damping.
2) Thermal loss – due to irreversibility of heat exchange at the interface.
3) Scattering – portion of compression wave which is reflected at the particle interface
4) Intrinsic attenuation – Attenuation due to the liquid and particles on their own.

We calculate the viscosity of the broth as a function of the inferred volume fraction using the Einstein equation. The selection of the appropriate equation for the calculation of viscosity is based on off-line rheological characterisation of broths & harvested cell paste using both shear viscometry (Fig.5), and dynamic oscillatory testing (Fig.4).

Conclusions

- Clear correlation between product loss, and increase in viscosity (post-induction).
- 25% increase in post-induction viscosity correlated to 10% product loss
- Continuous viscosity monitoring using ultrasonic wave propagation can be carried out in-line & *in-situ* to enable rapid decision-making about cell harvesting
- Cells can be modelled as colloidal particles so rheological principles of dilute suspensions can be applied (Einstein equation).
- Viscosity monitoring can detect cell lysis earlier than several other common monitoring techniques (OD_{600}, HPLC, flow cytometry, capacitance measurements, DNA assay.

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