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Investigating and modelling the effects of cell lysis on the rheological properties of fermentation broths

Joseph M. Newton [a], Joanna Vlahopoulou [b], Yuhong Zhou [a,*]

[a] Department of Biochemical Engineering, UCL, Torrington Place, London WC1E 7JE, UK
[b] Procellia Ltd., Netpark Incubator, Thomas Wright Way, Sedgefield, County Durham TS21 3FD, UK

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ABSTRACT

This article examines the rheology of an industrially relevant *E. coli* fermentation system producing antibody fragments (Fab′), to gain a deeper understanding of the physical properties of fermentation broths. Viscosity monitoring has been shown to be a useful tool to detect cell lysis and product leakage in late stage fermentation, and here we add to this work by characterising the rheological properties of an *E. coli* cell broth and its individual components, such as cell paste, supernatant, DNA and protein. Viscoelastic measurements have been carried out to provide novel insight into properties such as changes in cell strength, stability and robustness during fermentation, with ramifications for alternative process monitoring and control strategies.

Additionally, an empirical model has been created to determine the extent of cell lysis using viscosity measurements, based on DNA leakage in late stage fermentation. The model directly indicates product loss to extracellular space, as intracellular content (product, DNA and host cell protein) is released simultaneously during cell lysis in late stage fermentation. We envisage that this model, in combination with online viscosity monitoring, could be a valuable tool to monitor and detect cell lysis for both process development and industrial scale process operation.

1. Introduction

Biologics now dominate the list of top selling drugs in the world, with antibody fragments (Fab′) featuring heavily in this list [1]. *E. coli* is well established as a microbial host for recombinant protein production as its genome is well-understood, cells can be grown to high densities using inexpensive media and glycosylation is not needed [2,3]. However, high cell density fermentations present additional complications with respect to poor mass and oxygen transfer, leading to problems with loss of viability and cell lysis. *E. coli* produce Fab′ fragments that can be targeted to the periplasmic space, however the capacity of the periplasm is limited; Fab′ fragments will leak when exceeding 6% of the volume of the periplasm [4]. Cell lysis due to factors such as poor mass and oxygen transfer in high cell density fermentation and over-expression of the recombinant protein product is a common challenge in modern fermentation processes. As cell lysis occurs in late stage fermentation, cells lose viability and leak the Fab′ product and other intracellular content to the fermentation broth. This has adverse consequences in the efficiency of subsequent downstream processing steps including homogenisation, microfiltration and centrifugation, and therefore a trade-off exists between harvest time and total product yield [5–12]. Monitoring cell lysis directly is challenging, and a detailed review has been carried out elsewhere [13], however common methods include optical density, HPLC, cytotoxicity assays and flow cytometry, each of which have their own difficulties.

Rheology is defined as the study of the deformation and flow of matter, which can be divided into two types of physical properties; viscosity and viscoelasticity. Viscosity relates to the internal friction of a fluid and is a measure of its resistance to flow. Viscoelasticity describes both the solid-like (elastic) and liquid-like (viscous) characteristics of a non-Newtonian material undergoing deformation (temporary or permanent). Essentially, viscoelasticity can help to quantify the properties of the internal structure and strength of a material. Rheology is frequently used to characterise materials in the processing industry, from oil and gas to cosmetics such as toothpaste and hand cream. In the biopharmaceutical industry, rheology is typically used in formulation for therapeutics.

In the 70's, 80's and early 90's, previous studies attempted to monitor viscosity in fermentation to determine biomass concen-

* Corresponding author.
E-mail addresses: joseph.matthew.newton@gmail.com (J.M. Newton), joanna@procellia.com (J. Vlahopoulou), y.zhou@ucl.ac.uk (Y. Zhou).

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tration, as cell concentration is directly related to viscosity [14–19], however this yielded relatively poor results and a detailed review of viscosity monitoring techniques and methods has been carried out elsewhere [13]. Of particular note, Badino et al. [20,21] have previously carried out studies using a custom online rheometer to develop empirical correlations between the rheological properties of the cell broth and biomass concentration, agitation rate and mycelial morphology in *Aspergillus awamori* fermentation. However, viscosity is not solely affected by biomass concentration [22], and it is well known that as cell lysis occurs, the viscosity of the fermentation broth increases. This has been shown by monitoring the change in viscosity of an *E. coli* broth during chemical (alkaline) lysis [23], and the co-expression of nuclease (to reduce plasmid DNA) has been shown to reduce the viscosity of the bioprocess feedstock [7]. Viscosity monitoring in fermentation was hindered in the early 1990s due to a lack of adequate measuring technology, however with much more powerful rheometers entering the marketplace recently, its use is starting to gather interest again, and rheology may be able to offer insight into the physical properties of bioprocesses. Newton et al. [13] have previously shown that viscosity and cell lysis are closely related. Viscosity monitoring may therefore be a useful tool to detect product loss, which can be done more rapidly than many common monitoring techniques such as flow cytometry, HPLC, DNA assays and cytotoxicity assays and can detect signs of cell lysis earlier than optical density and capacitance measurements [13,24–26]. Viscosity monitoring could also complement infrared or fluorescence-based monitoring, which have been widely researched in bioprocessing [27–32], to provide comprehensive information on both the physical (viscosity) and chemical (e.g. infrared) properties of the fermentation broth.

Using rheology to monitor the flow consistency index of the cell broth, which gives an indication of non-Newtonian behaviour, has also shown an increase during cell lysis in fermentation [33]. For bioprocesses that produce plasmid DNA as products, or that have high polysaccharide or protein concentrations, the viscosity and non-Newtonian behaviour of the broth can be significant [34]. This can have a major impact on mass transfer and bioreactor design, pumping and power consumption. Shear thinning behaviour is often observed in fermentation broths [13], after homogenisation [35], and during lysis [23] and is believed to be caused by complex interactions between cells and their excreted or leaked polymers in the composite system [11], and can have ramifications in process efficiency [34]. However, the exact cause of this behaviour is unclear, and using rheology to improve our understanding of cell lysis from a bioprocessing perspective may have useful applications in process design, monitoring and optimisation, including upstream in synthetic biology, such as with the co-expression of nuclease to reduce the viscosity of the broth [7].

Viscoelastic measurements have also been used in bioprocess research and development. Vlahopoulou [36,37] previously reported the use of dynamic oscillatory testing to investigate the effect of cell biomass and *exo*-polymers produced by *Streptococcus theromphilus* and *Lactobacillus bulgaricus* strains during yoghurt fermentation. Oscillatory testing has been carried out on flocculated gels of *E. coli* lysate after undergoing chemical lysis [38], demonstrating the sensitivity of the elastic modulus of the gel-matrix to shear strain, and results from the rheological studies were used to inform research strategies for process synthesis. Viscoelasticity has also been used to study mixing and fluid flow in simulated xanthan fermentation broths [39], to study the effects of starter cultures on the properties of yoghurt gels [40] and to analyse the viscoelastic nature of filamentous fermentation broths [41]. Essentially, viscoelasticity has been shown to be a very useful method to analyse the material properties of fermentation broths.

Newton et al. [13] have previously shown that rapid offline viscosity monitoring (results in less than two minutes) can be a useful tool to detect cell lysis and product loss in fermentation. A correlation has been developed showing that 10% product leakage corresponded to a 25% increase in broth viscosity in postinduction cell cultures [13]. This article aims to add to this work, by characterising the fundamental rheological properties of an *E. coli* cell broth and its individual components (such as cell paste and supernatant), to understand the physiological changes in *E. coli* cells over the course of fermentation. Monitoring viscoelasticity may also provide novel insight into the physical properties of cells, such as changes in the strength, stability and robustness of the cell population during fermentation. From this information, it may be possible to infer conclusions to aid decision-making in cell culture processes.

A second aim of this article is to investigate the relationship between fundamental rheological properties such as viscosity and viscoelasticity, and individual biomass components such as cells, DNA and protein concentrations. This will provide a novel understanding of the relative contributions of these components to the overall viscosity, and facilitate the determination of the cause of the increase in viscosity and non-Newtonian behaviour in postinduction cell cultures. By improving our understanding of the rheology of cell broths, this article aims to demonstrate the utility of using rheology monitoring as a tool in process development.

A third aim of this article is to use the insight gained from the rheological characterisation of an *E. coli* cell broth and its components, to develop a model to quantify cell lysis in late stage fermentation, by using rapid viscosity measurements. The ability to rapidly monitor cell lysis in fermentation is central to the improvement of biopharmaceutical manufacturing [6] and will aid the implementation of quality by design (QbD) initiatives in process development. This article therefore aims to lay the groundwork for the development of an online, in-situ viscosity probe for fermentation monitoring.

2. Materials and methods

2.1. Strain

An *E. coli* w3110 strain (ATCC 27325) containing the plasmid pTTOD A33 IGS2, was kindly donated by UCB Pharma Ltd. (Slough, UK), coding for a 46 kDa antibody fragment (Fab′) utilising a *tac* promoter. All chemicals were provided by Sigma-Aldrich (Dorset, UK) unless otherwise stated and used as supplied.

2.2. Fermentation

High-cell density fed-batch fermentations were carried out using an autoclavable 7 L Applikon vessel (Applikon Biotechnology B.V., Schiedam, Holland), with a 5 L working volume. Cells were grown initially using complex LB broth, before being transferred to SM6Gc media, using a method previously described by Garcia-Arrazola et al. [42] and Li et al. [35]. Agitation was controlled between 300–1200 rpm using a cascade control system and dissolved oxygen tension was controlled at 30%. Temperature was initially controlled at 30 °C and dropped to 25 °C thereafter upon reaching an optical density (OD_{600}) of 38. At an OD_{600} of around 200 (38 h postinoculation), a dissolved oxygen spike and pH spike indicated that the culture had utilised all of the glycerol carbon source in the media. At this point, isopropyl β-D-1-thiogalactopyranoside (IPTG) (Generon Ltd., Maidenhead, UK) was added to a target bioreactor concentration of 0.03 g L^{-1} in order to induce Fab′ expression, and 80% w/w glycerol solution was fed at a rate of 6.4 mL h^{-1}. To control foaming, 1 mL of 100% polypropylene glycol (PPG) was added to the fermenter prior to inoculation, and as necessary thereafter up to a maximum of 2 mL total PPG. The fermentation was typically continued up to 60 h postinduction.