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

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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-

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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.

2.2.1. Measurement of cell density

Optical density was measured at 600 nm using an Ultrospec 500 Pro spectrophotometer (Amersham Biosciences Ltd., Amersham, UK). Dry cell weight was measured by aliquoting 1 mL of culture into pre-dried and pre-weighed 2 mL Eppendorf tubes, centrifuging, removing supernatant and drying in an oven overnight at 100 °C.

2.2.2. Fab′ measurement

Fab′ concentration in the supernatant and total Fab′ concentration were analysed by HPLC (Agilent 1200, Agilent Technologies Inc., California, USA) using a 1 mL protein G column (HiTrap, GE Healthcare, Uppsala, Sweden). To measure total Fab′ concentration, sonication was carried out, followed by centrifugation. Bind and elute 20 mM phosphate buffers were used to process the samples, at pH 7.4 and pH 2.5 respectively. The concentration of eluted Fab′ was measured by absorbance at 220 nm. Purified Fab′ was kindly provided by UCB Pharma Ltd (Slough, UK).

2.2.3. DNA measurement

Quant-iT Picogreen assay (Life Technologies Ltd., Warrington, UK) was used to determine double stranded DNA concentration in the fermentation broth.

2.3. Preparation of material for rheological characterisation

2.3.1. Cell paste and supernatant

Cell paste was obtained for rheological measurements by taking 50 mL samples from the fermenter, and clarifying twice for 30 min each, at 10 °C and 1431 rcf (4000 rpm), using an Eppendorf Centrifuge 5810 R (Eppendorf AG, Germany), removing the supernatant after each run.

2.3.2. DNA and protein

Herring Sperm DNA (Promega, USA) was used as representative DNA for fabricating lysed cell suspensions. Bovine serum albumin (BSA) was used as a representative globular protein for fabricating lysed cell suspensions.

2.4. Rheological characterisation

Rheological experiments were carried out with a Kinexus Lab + rheometer (Malvern Instruments, Malvern, UK), using 50 mm parallel plates at 25 °C and a 300 μm gap size.

2.4.1. Viscosity measurements

Viscosity measurements were taken by controlling the applied shear rate over a range of 100–1000 s^{-1}, and the apparent viscosity was determined by recording the shear viscosity value at a shear rate of 100 s^{-1}.

2.4.2. Viscoelasticity measurements

Oscillatory testing was carried out using amplitude and frequency sweeps. Amplitude sweeps were taken over a shear strain range 0.05–10%, holding frequency constant at 1 Hz to determine the linear viscoelastic range (LVER), the critical strain limit at which a material starts to break down. Frequency sweeps were taken over the range 1–10 Hz, with shear strain held constant at 0.1%, within the critical strain region (LVER).

The loss tangent, $\tan\delta$, is a ratio of the loss modulus to storage modulus (G″/G′), and can be used to quantify the elasticity of a material. A loss tangent less than one indicates predominantly elastic or solid-like behaviour and a loss tangent greater than one indicates predominantly viscous or liquid-like behaviour.

2.4.3. Modelling cell lysis

An empirical model was created to determine the extent of cell lysis from viscosity measurements using the "polyfit" function and the fit was evaluated using the "polyval" function in Matlab. The model was created using a linear polyfit function (n = 1, where n is the degree of polynomial fit). Data was normalised to perform the modelling, and de-normalised to obtain final results.

3. Results & discussion

3.1. Understanding the effect of cell lysis on the rheological properties of fermentation broths

Fermentations were carried out as previously described [13]. Fig. 1(a) shows the growth profile of a typical fermentation run, with cells reaching a maximum dry cell weight of 48 g/L, which corresponded to an OD_{600} of 200. The exponential phase began around 24 h and heterologous protein expression (Fab′) was induced at 38 h with IPTG, when a dissolved oxygen spike was observed due to complete utilisation of the carbon source. This enabled rapid Fab′ production in stationary phase, reaching 1.6 g/L by the end of the fermentation. Glycerol was fed at a constant rate throughout the postinduction phase.

Fig. 1(b) shows the viscosity profile over the course of fermentation. The postinduction viscosity profile of *E. coli* fermentations has previously been shown to correlate well with cell lysis, loss of viability and product loss to extracellular space [13]. The storage modulus, G′, is the elastic component of the complex shear modulus, which describes the "elastic" or solid-like characteristics of a material, and therefore gives an indication of the strength of its internal structure. The average G′ of *E. coli* cell paste throughout the fermentation, shown in Fig. 1(b), suggests that cell strength rapidly decreases over the course of fermentation. This result aligns with a previous study [6] that used techniques such as adaptive focus acoustics (AFA) and ultra scale-down (USD) centrifugation performance to observe changes in cell strength over the course of a fermentation, for example a 2.6-fold increase in product release rate from the cell was observed and a 4-fold decrease in clarification performance was observed. In Fig. 1(b), a 9.35-fold reduction in cell strength, measured by the average storage modulus, was observed between induction point (38 h) and harvest point (71 h). Decreasing cell strength during fermentation leads to the degradation of the cells and release of intracellular content to the extracellular environment, which acts to increase the viscosity of the broth.

In order to gain a better understanding of the relative contributions of the major components of a cell broth to the overall viscosity increase in postinduction cultures, viscometry experiments were carried out on cell broth, cell paste and supernatant, as shown in Fig. 2. Fig. 2(a) plots the flow curves of the cell broth throughout fermentation, showing a 5-fold increase in viscosity from 0.0011 Pas at the start of the fermentation to 0.0053 Pas at the end of the fermentation, and an increase in shear thinning behaviour. The increase in shear thinning behaviour in Fig. 2(a) (flow behaviour index was greater than 0.95 for all samples) was due to the release of intracellular content to the fermentation broth. Fig. 2(b) shows the flow curves for cell paste during a fermentation; the viscosity of the cell paste displayed a decreasing trend as the fermentation progressed and a marked decrease was observed between 33 h postinduction (the critical point of product leakage and cell lysis for the fermentation presented in this article) and 57 h postinduction, showing rapid deterioration of the cells beyond 33 h postinduction. Additionally, a marked increase in shear thinning behaviour was observed in late stage fermentation (57 h postinduction), and a flow behaviour index of 0.505 was determined. Fig. 2(c) plots flow curves for the supernatant of the fermentation broth in mid exponential phase

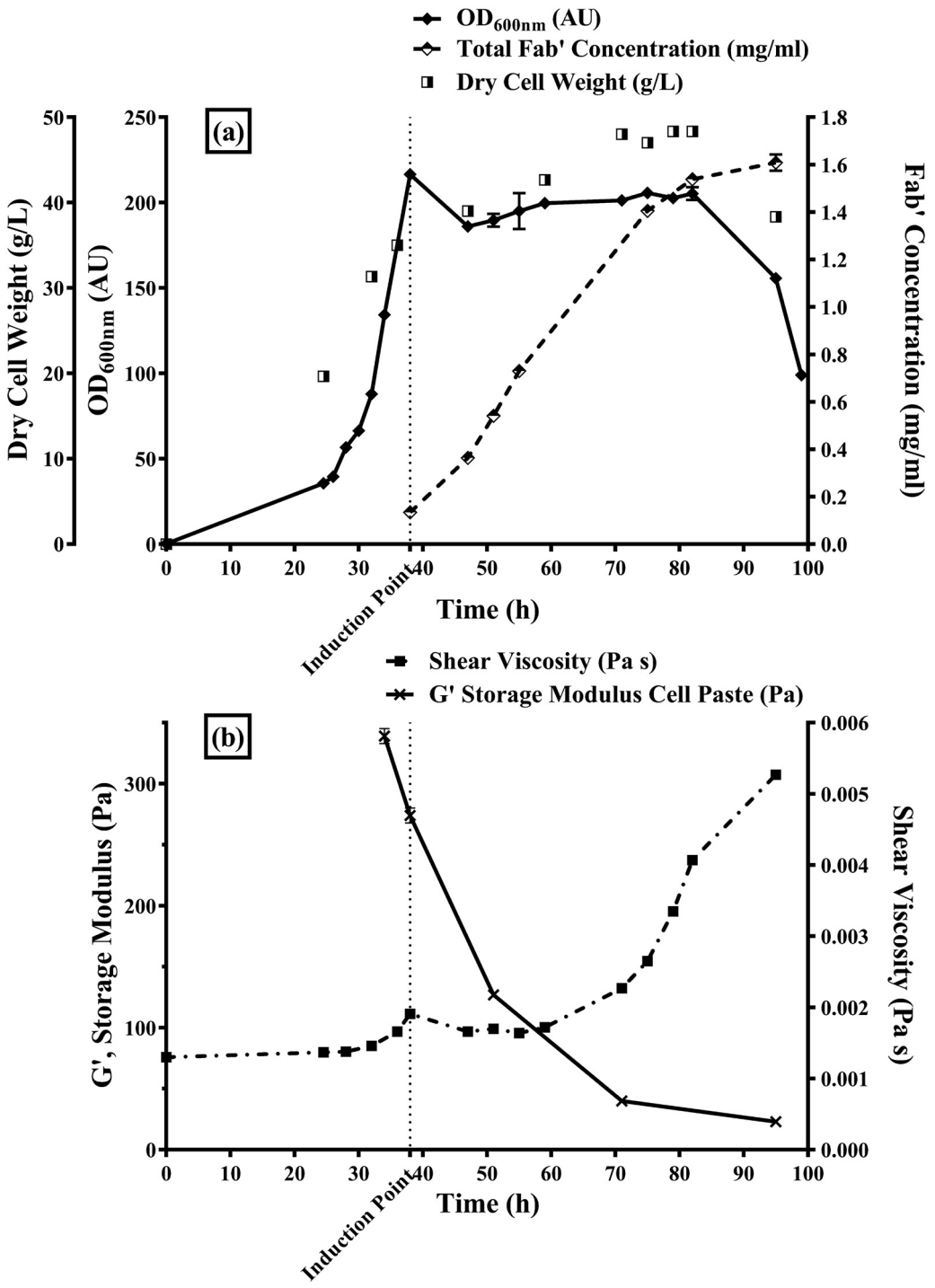

Fig. 1. Characterisation of an *E. coli* (Fab′) fermentation. Induced with IPTG at 38 h. (a) optical density at 600 nm (absorbance units (AU), in triplicate), dry cell weight (g/L, in triplicate) and total Fab′ concentration (mg/mL, in duplicate). (b) Shear viscosity (Pa s, single measurement, held at steady state for 10 s) and average storage modulus (G′, elastic component) of cell paste (Pa, measured in triplicate), calculated by averaging G′ over the linear viscoelastic range (LVER).

and late stage fermentation (57 h postinduction). A large increase in viscosity was seen for the supernatant. Although the viscosity of the cell paste decreased significantly during fermentation, we can see that overall broth viscosity increased, which is a result of the large increase in viscosity of the supernatant during cell lysis.

Oscillatory tests (amplitude and frequency sweeps) were carried out on the cell paste to give insight into the viscoelastic properties of the material. Viscoelasticity can be measured by both amplitude sweeps and frequency sweeps, however, the cell paste was determined to be a gel (independent of frequency), therefore frequency data are not presented in this article, as very little useful information was obtained from these experiments relevant to this immediate study. Oscillatory tests were carried out on cell paste only, as there was not enough structure present in the other materials studied. The linear viscoelastic range (LVER) can be defined as the limit at which the structure in a sample starts to break down and is where the rheological properties, i.e. shear modulus (G′ and G″) and phase angle, are independent of frequency. The strain limit of the LVER relates to the stability and robustness of a material, and G′ is a direct measure of the structure in a sample.

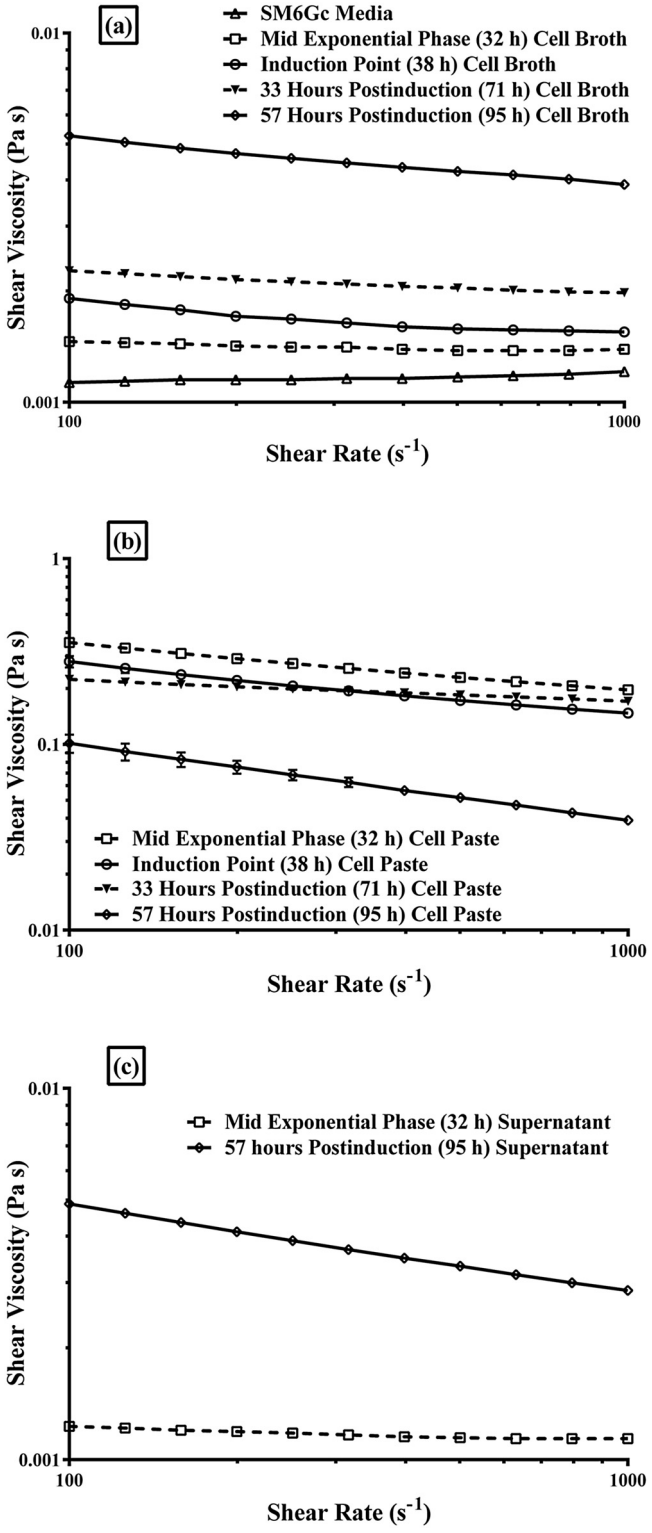

Fig. 2. Understanding the relative contributions of components of a cell broth to the viscosity increase in postinduction fermentation. Viscometry flow curves of *E. coli* (a) cell broth, (b) cell paste and (c) supernatant at various time points in the fermentation. For the cell broth, single measurements were recorded at each shear rate, held at steady state for 10 s, over a shear rate range 100–1000 s^{-1}. For cell paste and supernatant, measurements were carried out in the same way, in triplicate and over a shear rate range 100–1000 s^{-1}. Viscometry measurements were carried out at 25 °C using 50 mm parallel plates.

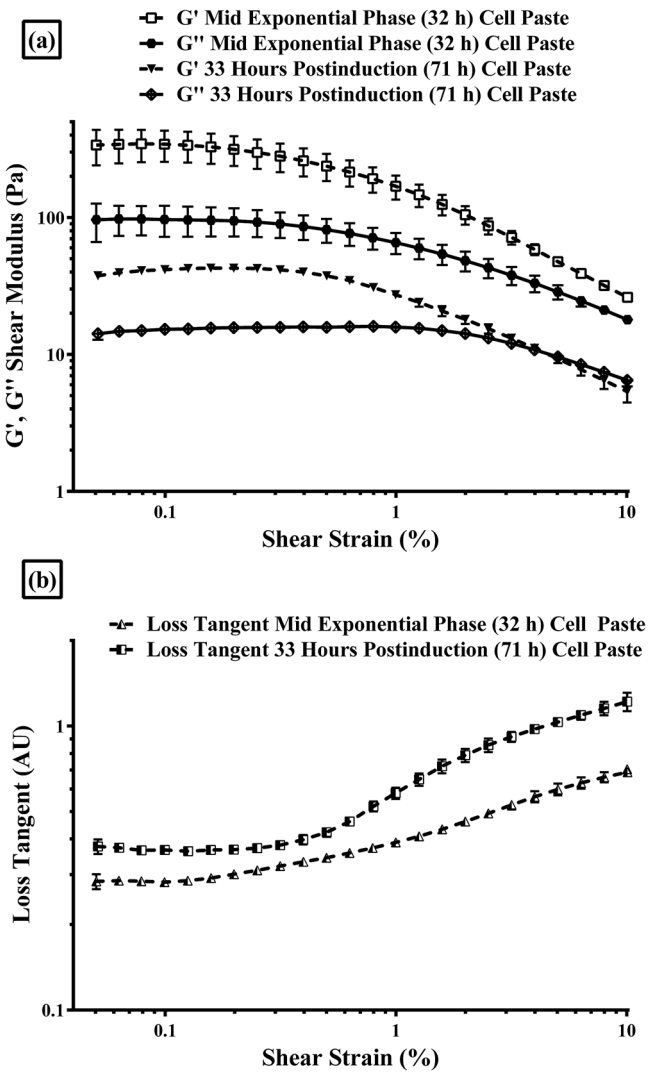

Fig. 3. Elucidating the rheological properties of an *E. coli* cell paste. Measurements carried out to compare the rheological properties (storage (G′) and loss (G″) moduli) of *E. coli* between two different growth stages; mid exponential phase and 33 h postinduction (typical harvest point). (a) Dynamic oscillation measurements were taken in triplicate using an amplitude sweep over a shear strain range 0.05–10%, holding frequency constant at 1 Hz. (b) The loss tangent, tanδ, is a ratio of the loss modulus to storage modulus (G″/G′), which gives a useful quantification of the elasticity of a material.

Fig. 3(a) shows the storage and loss moduli for two growth stages; cells in mid exponential phase and cells at 33 h postinduction (71 h fermentation time), to compare and characterise the rheological properties of *E. coli* cells. For both growth stages, the storage modulus, G′, was greater than the loss modulus, G″, which shows that both materials exhibited solid-like characteristics. Although the cells at 33 h postinduction may be leaking product and other intracellular content to the extracellular space due to porous membranes, this indicates that the cells haven't fragmented and broken down, i.e. the rod-like structure of the *E. coli* is still relatively intact. However, the magnitude of the storage modulus, G′, within the LVER is higher for mid exponential phase cell paste than 33 h postinduction cell paste, at 339 Pa and 40 Pa respectively, showing a considerable drop in the strength of the cells. The loss tangent, tan δ, is a ratio of the loss modulus to storage modulus (G″/G′), which provides a useful quantification of the elasticity of a material. In Fig. 3(b), the loss tangent is plotted for the cell paste at the two different growth stages; mid exponential and 33 h postinduction. The loss tangent was higher for the 33 h postinduction cell

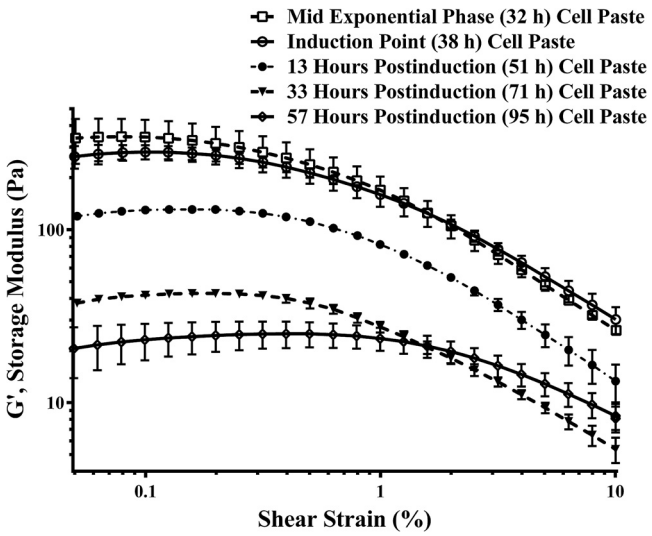

Fig. 4. Amplitude sweep of *E. coli* cell paste to determine the critical strain limit. Dynamic oscillation measurements were taken in triplicate to determine the linear viscoelastic range (LVER, the critical strain limit before a material starts to break down), using an amplitude sweep over a shear strain range 0.05–10%, holding frequency constant at 1 Hz. Measurements carried out at various time points throughout fermentation.

paste, which indicates a more fluid (i.e. weaker) system, if the shear viscosity increases (which we can see this happening from Fig. 1). This suggests that polymers in the cell paste are acting as fillers, and have a thickening effect (hence increased viscosity), which could be caused by the formation of a network due to interactions between cells and leaked intracellular content. This confirms the insight derived from Fig. 3(a), that the *E. coli* are still rod-like structures at 33 h postinduction (although leaking product), but the cells become weaker and lose rigidity as the fermentation progresses.

In Fig. 4, amplitude sweeps are presented for cell paste analysed over 5 time points throughout the fermentation, and two phenomena are evident. Firstly, we can see that the magnitude of the elastic component of the shear modulus (G′) decreases with fermentation time and the average G′ (within the LVER) was plotted in Fig. 1(b), showing a rapid drop in cell strength after induction with IPTG. Secondly, we can see that the length (or critical strain limit) of the LVER increases as the fermentation progresses, suggesting that although the strength of the cells decreases (G′), the critical strain limit increases due to interactions between cells and intracellular content such as DNA, which increases its "stickiness".

3.2. Modelling cell lysis in postinduction fermentation

The viscosity of fermentation broths is determined by two factors; cell concentration and solute concentration in the broth [22] (assuming other factors such as media composition, temperature, aeration and agitation are constant). Cells, representative protein and DNA were isolated and prepared at various concentrations, in order to gain an understanding of the contributions of these individual components to the overall viscosity of a fermentation broth. Fig. 5(a) plots flow curves for cell suspensions at various concentrations (dry cell weight), showing a slight increase in shear thinning behaviour as the concentration increases. In exponential phase and early stationary phase, viscosity is predominantly determined by cell concentration. Therefore, we can see that a linear relationship is evident between viscosity and cell concentration in the growth phase of *E. coli* fermentations. 100 s^{-1} was chosen as the characteristic parameter to obtain apparent viscosity values for all studies presented, because at lower shear rates, the measurement will pick up more of the polymer interactions (e.g., DNA) that are thought to cause an increase in viscosity during cell lysis. Taking the viscosity values from Fig. 5(a), Fig. 5(b) shows a linear relationship between cell concentration and viscosity, with an R^2 value of 0.98. The error bars for higher concentration cell suspensions are relatively large and it is thought that this may be caused by cell aggregation from centrifugation and resuspension in sample preparation steps. The relationship between cell concentration and viscosity is presented in Eq. (1), where C_{cells} is cell concentration (g.DCW/L) and η_{cells} is viscosity in Pas;

$$\eta_{cells} = 5.38 \times 10^{-5} C_{cells} + 0.00062 \quad (1)$$

This result confirms that Einstein's well-known viscosity equation, based on hydrodynamic theory for dilute suspensions of spherical particles [43], is appropriate for the growth stage of the *E. coli* fermentation system under study in this article. The Einstein equation is of a linear form and based on the volume fraction of spherical particles, i.e. cell concentration. At higher cell concentrations, this linearity may not be appropriate, however further work is being carried out in a separate study to determine the fit of the Einstein equation (and extensions to the Einstein equation) to non-spherical *E. coli* cells at higher cell concentrations.

Fig. 6 shows similar plots for flow curves of BSA protein at various concentrations. At all concentrations, protein solutions exhibited highly shear thinning behaviour, as seen in Fig. 6(a). However, plotting the relationship between protein concentration and viscosity in Fig. 6(b), there is a clear linear relationship between protein concentration and viscosity, with an R^2 value of 0.92. The relationship between protein and viscosity is presented in Eq. (2), where η_{prot} is the viscosity of protein in Pas and C_{prot} is the concentration of protein (g/L);

$$\eta_{prot} = 3.79 \times 10^{-5} C_{prot} + 0.0016 \quad (2)$$

E. coli contains a vast range of proteins and although they differ from BSA, we believe it is the total concentration of protein that determines the viscosity, and therefore we assume that the relationship between total protein in the fermentation broth and viscosity to be linear.

Fig. 7(a) plots the flow curves for DNA at various concentrations, displaying Newtonian behaviour; where viscosity is independent of shear rate. As seen in Fig. 7(b), a linear relationship between DNA and viscosity is also evident, with an R^2 value of 0.99. The relationship between DNA concentration and viscosity is shown in Eq. (3), where C_{DNA} is DNA concentration (g/L) and η_{DNA} is the viscosity of DNA in Pas;

$$\eta_{DNA} = 0.0005123 C_{DNA} + 0.001 \quad (3)$$

From Figs. 5–7, we can see that cell concentration, protein concentration and DNA concentration all have linear relationships with viscosity. It is generally believed that DNA causes the increase in viscosity in postinduction cell cultures, and gram for gram this is true; DNA has a much higher impact on viscosity. However, for the high cell density *E. coli* fermentation studied in this article, extracellular protein concentration reached up to 40 g/L and extracellular DNA typically reached around 3 g/L. Therefore, comparing Figs. 6 and 7, we can see that protein has an equal or greater contribution to viscosity, and a large impact on the shear thinning properties of fermentation broths.

The definition of cell lysis is that if cells are leaking DNA, cell lysis is occurring. Therefore, DNA leakage to the fermentation broth can be used to monitor cell lysis. From Fig. 7, we can see that a linear relationship between DNA and viscosity exists. In the growth phase of fermentation, viscosity is determined by cell concentration (i.e. the Einstein viscosity equation). However, in postinduction cell cultures (where cell concentration is relatively constant), there are other factors affecting broth viscosity, such as leakage of other

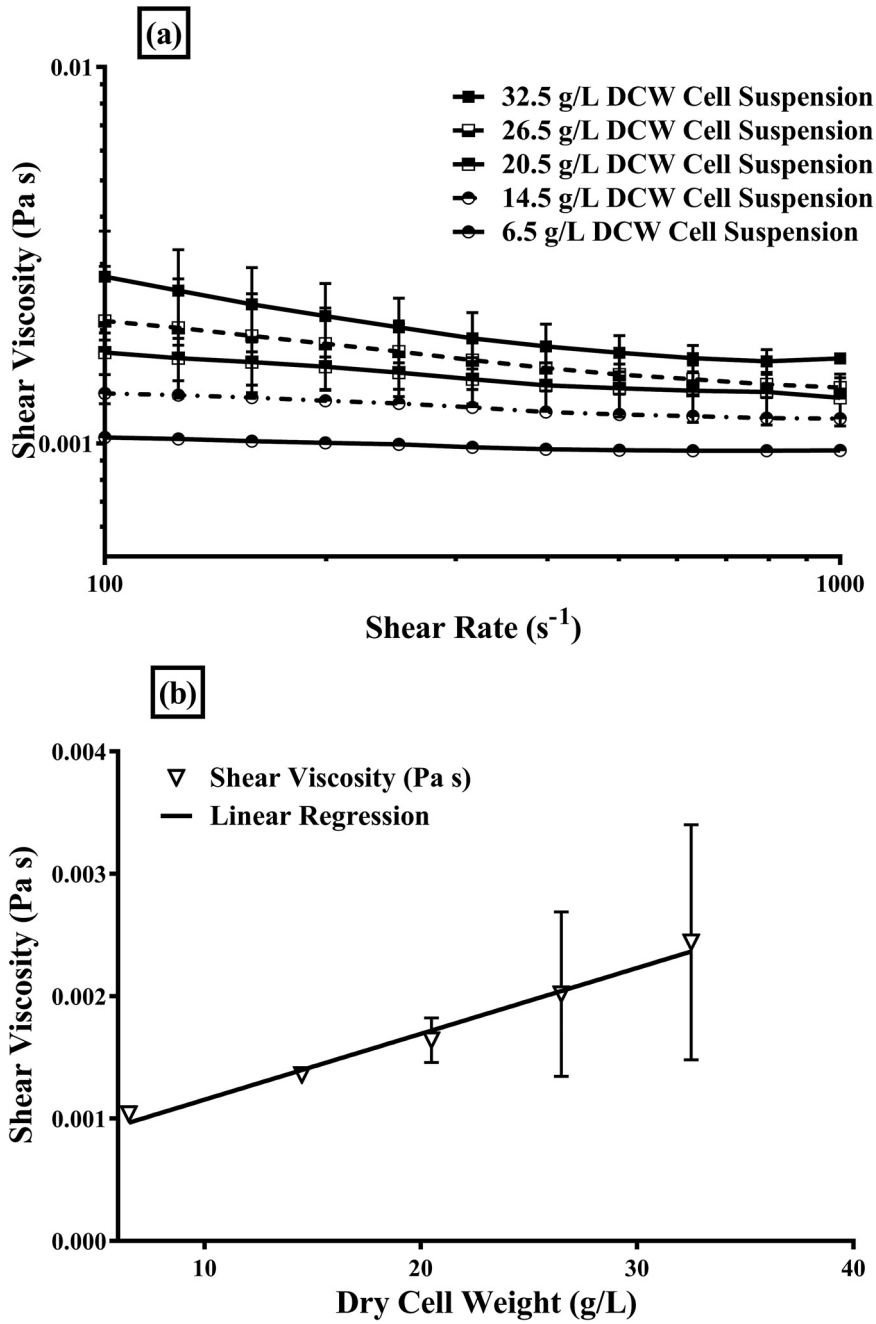

Fig. 5. Determining the relationship between cell concentration and viscosity. (a) Flow curves of cell suspensions (dry cell weight, g/L) measured over a shear rate 100–1000 s^{-1}, carried out in triplicate, using 50 mm parallel plates at 25 °C. (b) The linear relationship between cell concentration (dry cell weight, g/L) and viscosity at 100 s^{-1}, with an R^2 value of 0.98.

intracellular content. It has been shown previously [33] that a relatively linear relationship exists between DNA leakage and product loss. Therefore, assuming a proportional relationship between DNA and other intracellular content, by rapidly monitoring viscosity in fermentation (measurements in under 2 min), we can model DNA leakage to the broth, and infer cell lysis and product loss based on DNA release.

A linear model was created, shown in Fig. 8, using experimental data from 3 identical fermentation runs, with 23 total data points. The model was created using MATLAB, however this was done for convenience and to generate a logical methodology; we recommend that users could execute this model very easily in Microsoft Excel or other more accessible software programs. Having previously determined a linear relationship between viscosity and DNA, the model was created using a linear polyfit function (n = 1, where n is the degree of polynomial fit). The determined equation is presented in Eq. (4):

$$C_{DNA} = 905.8(\eta - \eta_0) + 0.1486 \quad (4)$$

where C_{DNA} is the concentration of DNA that has leaked to the fermentation broth, η is viscosity and η_0 is the viscosity at induction point in fermentation, taken as a zero point in order to negate differences in cell density between batches. By the time the cell density changes substantially, considerable cell lysis has already taken place (and a typical batch would have been terminated at a

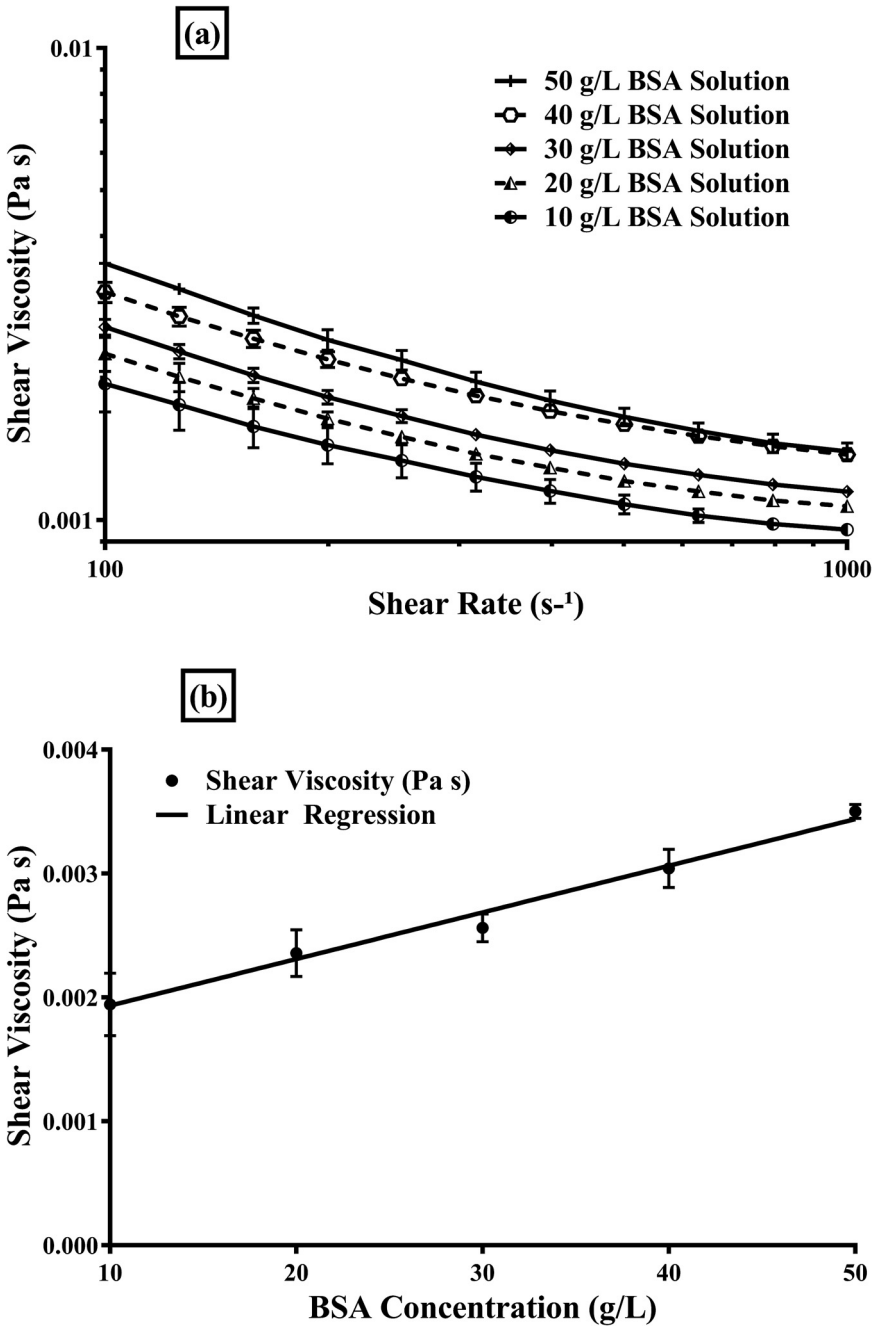

Fig. 6. Determining the relationship between protein concentration and viscosity. (a) Flow curves of protein (BSA) solutions measured over a shear rate 100–1000 s^{-1}, carried out in triplicate, using 50 mm parallel plates at 25 °C. (b) The linear relationship between protein concentration and viscosity at 100 s^{-1}, with an R^2 value of 0.92.

much earlier time point), therefore we assume that cell density is constant throughout the stationary phase.

An R^2 value of 0.91 was determined for the model, which shows a highly linear relationship between broth viscosity and DNA. Generally, the model performed very well for all three fermentation datasets, and the majority of data points fell within the 90% confidence bands, with the exception of three outliers seen in Fig. 8. A greater number of data points are at lower viscosities, and this is the most critical point to detect cell lysis and product leakage. In real fermentation runs the batch would be ended before reaching such high viscosities (typically when product loss is around 10%), which for this *E. coli* fermentation system corresponds to an increase in viscosity of up to 0.001 Pa s.

This model demonstrates that by taking rapid at-line or indeed online viscosity measurements, it is possible to instantly quantify DNA leakage to the fermentation broth, which enables the operator to make rapid decisions about cell harvesting, as DNA leakage has implications on many downstream processing unit operations. The potential application of this model for other fermentation systems would be particularly useful for host cells with intracellular protein products. However, generally, it is useful to know DNA leakage during fermentation, including for mammalian cell processes where the product is secreted to the extracellular space. This model also gives an indication of product loss, as product loss occurs simultaneously to DNA release [13,33].

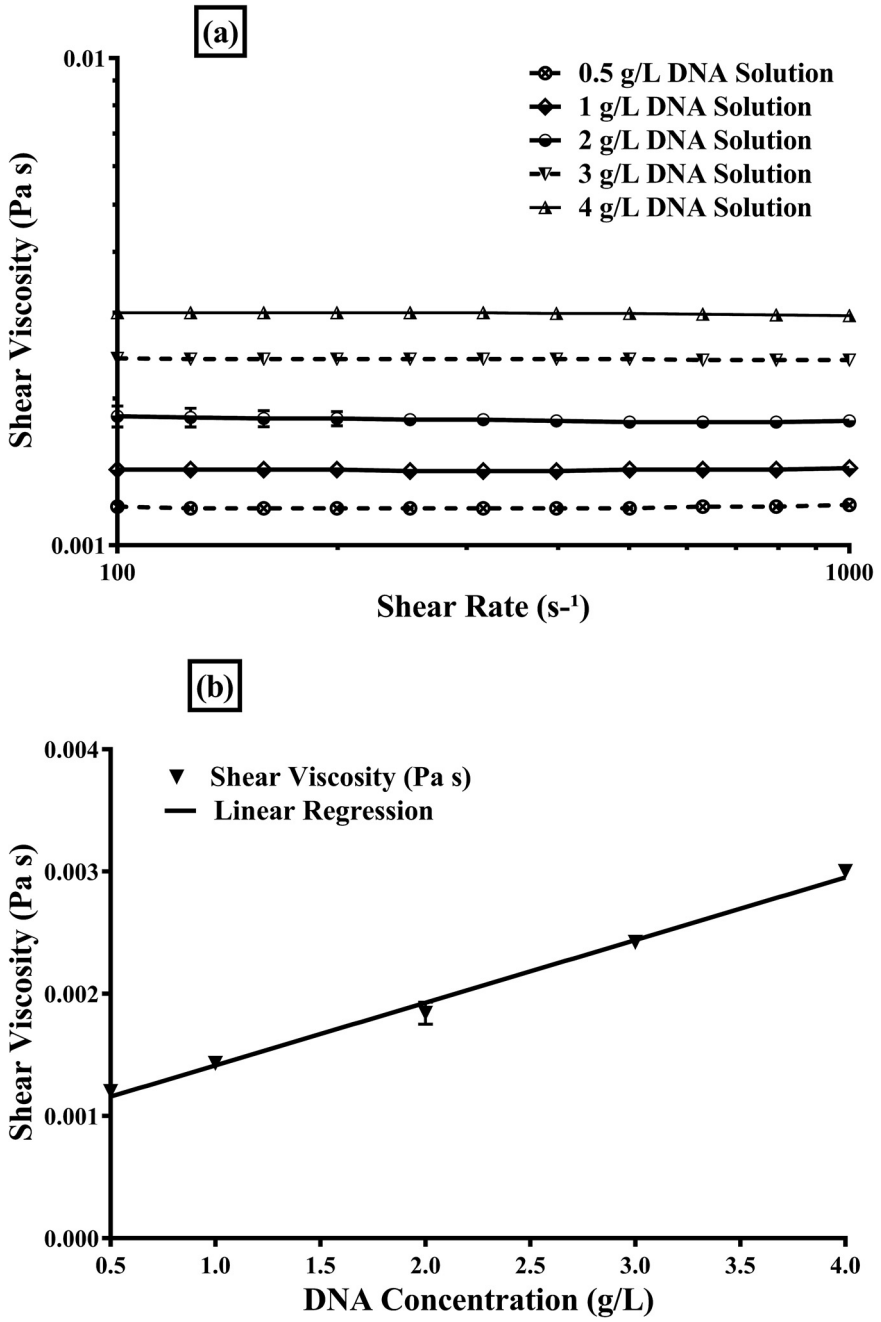

Fig. 7. Determining the relationship between DNA concentration and viscosity. (a) Flow curves of DNA solutions measured over a shear rate 100–1000 s^{-1}, carried out in triplicate, using 50 mm parallel plates at 25 °C. (b) The linear relationship between DNA concentration and viscosity at 100 s^{-1}, with an R^2 value of 0.99.

4. Conclusions

Newton et al. [13] have previously shown that rapid viscosity monitoring in fermentation can be a useful technique to detect cell lysis and product loss in postinduction fermentation. In this article, an industrially-relevant high cell density fermentation producing antibody fragments (Fab′) was carried out and analysed using rheology. A decrease in the shear modulus, G′, of cell paste and an increase in the LVER were observed, suggesting that although cell strength decreases over the fermentation, interactions are present between cells and leaked intracellular content such as DNA, which increases the "stickiness" of cell paste.

Linear relationships were determined between viscosity and cell concentration, protein concentration and DNA concentration. It was also observed that protein is shear thinning and has a significant contribution to viscosity in lysed cell broths, in addition to DNA, which has not previously been considered.

An empirical model was created where it is possible to accurately quantify cell lysis (by determining DNA concentration in the cell broth) from rapid viscosity measurements. This directly indicates product loss, as a proportional relationship exists between DNA leakage and product loss. We envisage that this model could be used for many industrial fermentation systems, simply by characterising the relationship between viscosity and DNA leakage, and implementing at-line viscosity monitoring. This article lays the groundwork for the development of an online, in-situ viscosity probe for fermentation monitoring, which can also measure volume fraction, allowing a cell concentration factor to be implemented

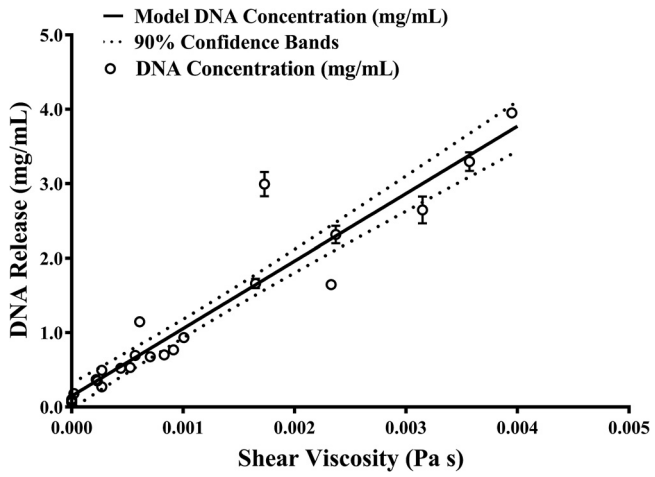

Fig. 8. Comparison of model prediction of cell lysis and experimental data. Results of an empirical model built with three fermentation datasets (23 data points), to determine DNA concentration in extracellular space, and hence cell lysis, from rapid viscosity measurements. The R^2 value was 0.91, and had 3 outliers in the dataset.

into the model to obtain greater accuracy at higher viscosities in late stage fermentation. Future work includes carrying out fermentations for different cell lines to widen the scope of the model.

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