

Thermal profiling for parallel on-line monitoring of biomass growth in miniature stirred bioreactors

N. K. Gill · M. Appleton · G. J. Lye

Received: 13 March 2008 / Accepted: 17 March 2008
© Springer Science+Business Media B.V. 2008

Abstract Recently we have described the design and operation of a miniature bioreactor system in which 4–16 fermentations can be performed (Gill et al., *Biochem Eng J* 39:164–176, 2008). Here we report on the use of thermal profiling techniques for parallel on-line monitoring of cell growth in these bioreactors based on the natural heat generated by microbial culture. Results show that the integrated heat profile during *E. coli* TOP10 pQR239 fermentations followed the same pattern as off-line optical density (OD) measurements. The maximum specific growth rates calculated from off-line OD and on-line thermal profiling data were in good agreement, at 0.66 ± 0.04 and $0.69 \pm 0.05 \text{ h}^{-1}$ respectively. The combination of a parallel miniature bioreactor system with a non-invasive on-line technique for estimation of culture kinetic parameters provides a valuable approach for the rapid optimisation of microbial fermentation processes.

Keywords Fermentation heat measurements · On-line monitoring · Miniature bioreactor · Thermal profiling

N. K. Gill · G. J. Lye (✉)
The Advanced Centre for Biochemical Engineering,
Department of Biochemical Engineering,
University College London, Torrington Place,
London WC1E 7JE, UK
e-mail: g.lye@ucl.ac.uk

M. Appleton
Bioxplore, Unit 9–10, Capital Business Park, Manor Way,
Borehamwood, Hertfordshire WD6 1GW, UK

Nomenclature

P_{eh} Power output of the heater (W)
 V_{ch} Voltage supplied to the calibration heater (V)
 R_{ch} Resistance of the calibration heater (Ω)

Introduction

The advent of miniature bioreactor systems and high throughput methodologies is now proving to be an effective tool for bioprocess development. Systems that enable parallel and automated operation of several fermentations simultaneously have the potential to increase the rate at which the necessary experiments are performed allowing reduced development times (Fernandes and Cabral 2006; Lye et al. 2003). They also provide significant reductions in labour intensity, media costs and space requirements compared to conventional bioreactors (Betts and Baganz 2006; Lye et al. 2003). For the majority of miniature bioreactors reported to date, temperature, pH and dissolved oxygen are the most widely measured and controlled in situ parameters. These measurements are typically achieved using miniaturised probes or fluorescence based optical sensors. When working at these scales (7–100 ml) taking large numbers of samples for off-line analysis of biomass growth becomes unrealistic and alternative methods for continuous on-line monitoring are required. In some cases, this has been addressed by integrating commercially available optical density probes for continuous monitoring of biomass

growth (Marose et al. 1999; Sarrafzadeh et al. 2005). However these occupy a significant portion of the vessel volume altering the flow characteristics at the scale.

An alternative non-invasive method for on-line monitoring is calorimetry. Given that heat generation is a natural occurrence in the majority of bioprocesses (von Stockar and Marison 1989), a wealth of information can be inferred from simple measurements of heat production by microbes during culture. These can be valuable for assessing the state of the culture and for process control (Voisard et al. 2002). An added advantage is the absence of probes inserted into the vessel and associated electrical or fibre optic cables. There have been several publications on the application of calorimetric techniques for monitoring and controlling various bioprocesses. The majority have used modified calorimeters such as the RC1 (e.g. Marison et al. 1998) developed by Mettler-Toledo and the BSC-81 (e.g. Meier-Schneiders et al. 1995) developed by Ciba-Geigy initially designed for calorimetric investigation of chemical processes. More recently Hüttl et al. (2008) have reported on a small-scale (25 ml working volume) calorimetric vessel modified to operate as bioreactor. This vessel facilitates accurate calorimetric measurements but the complexity of the design would appear to limit parallel operation. In some cases conventional bioreactors have been modified to perform as calorimeters (e.g. Meier-Schneiders and Schafer 1996). Data collection in these cases is based on conventional calorimetric techniques, i.e. a complete heat balance, and yields the precise quantity of heat generated during a fermentation time course.

Although data collected in this way is useful for deriving enthalpic parameters, it is necessary to accurately determine all heat flows that result from stirring, aeration, loss to the environment, from acid and base feeds and carbon dioxide vaporisation (Voisard et al. 2002). In most cases however, particularly those applications for which miniature bioreactors have been designed, a general indication of the rate at which heat is released may provide sufficiently useful information on the kinetics of cell growth. In the case of parallel miniature bioreactors this approach may allow estimation of cell growth rates and yields without the need for multiple samples. Here we demonstrate how valuable information on culture heat generation can readily be extracted from the temperature control system of a recently commercialised miniature stirred bioreactor (Gill et al. 2008) to estimate kinetic parameters from

parallel cultures. Given the absence of a detailed heat balance this approach is referred to here as 'thermal profiling' rather than calorimetry.

Materials and methods

In-situ thermal profiling of miniature (100 ml) bioreactor fermentations

The design characteristics of the parallel miniature bioreactors used here have been described in detail elsewhere (Gill et al. 2008). Each bioreactor stand holds up to four bioreactors capable of independent operation. Recombinant *E. coli* TOP10 pQR239 expressing cyclohexanone monooxygenase (CHMO) under the control of an L-arabinose promoter (Doig et al. 2001) was used in this study. Batch fermentations were carried as previously described (Gill et al. 2008) with 100 ml culture media and 2 ml inoculum at 37°C. pH was automatically maintained at 7 by the addition of 3 M NaOH or 3 M H₃PO₄. The dissolved O₂ was monitored using a miniature polarographic O₂ electrode. The agitation and aeration rates for all fermentations were controlled at 1,000 rpm and 1 vvm respectively. Each miniature bioreactor was lagged with glass fibre insulating wool (HEL Ltd., UK) to minimise general heat losses throughout the culture.

Analytical techniques

Biomass concentration was determined by off-line OD₆₀₀ measurements as described by Gill et al. (2008). All biomass concentrations reported here are dry cell weight (DCW) concentrations and were determined from calibration curves of known DCW and the corresponding optical density. All calculations of heater energy requirements were carried out with the aid of IQ software (HEL Ltd., UK).

Results and discussion

In-situ culture heat generation measurements

Thermal profiling of cell growth

The temperature control system of each miniature bioreactor system employs an external electrical disc

heater (50 W) positioned directly beneath the vessel driven by pulse-width modulation (PWM). The PWM drive regime was derived from a continuously variable parameter termed the 'request power' (expressed as a percentage) that was determined by a PID control loop. The heater was then turned on and off at a fixed level to maintain the set-point temperature. Previous experiments have demonstrated that the requested power of the heater is linearly related to the magnitude of the input power from the calibration heater (data not shown).

To determine the actual power output during fermentation a calibration procedure was undertaken, involving lagging the entire vessel containing media to maintain the vessel at 37°C. A calibration heater was then placed into the bioreactor and once the requested power had stabilised, the calibration heater was activated at a known power and the magnitude of the control system response recorded. The calibration heater used was a stainless steel-cased 100 Ω resistor which was driven at 2.5 V, giving an output of 0.0625 W as calculated from:

$$P_{eh} = \frac{V_{ch}^2}{R_{ch}} \quad (1)$$

where P_{eh} is the power output of the heater (W), V_{ch} is the voltage supplied to the calibration heater (V) and R_{ch} is the resistance of the calibration heater (Ω).

Figure 1 shows that when the calibration heater was activated the miniature bioreactor temperature control system backed off by 0.4% giving a calibration ratio of approximately 0.16 W percent of the requested heater power. Thus under typical fermentation conditions, as microbial growth progresses and heat is generated, the reduction in requested heater power will be assumed to be proportional to the amount of heat generated by the fermentation.

Analysis of thermal profiling results from repeated E. coli TOP10 pQR239 fermentations

In order to test this simple thermal profiling approach a total of ten identical *E. coli* TOP10 pQR239 fermentations were carried out. Considering a single typical fermentation, Fig. 2a shows the power delivered from the electrical heater to maintain the bioreactor at 37°C. It can be seen that the power required from the electrical heater gradually decreased from 0.023 to 0.0003 W due to the increasing heat evolved from the microbial culture.

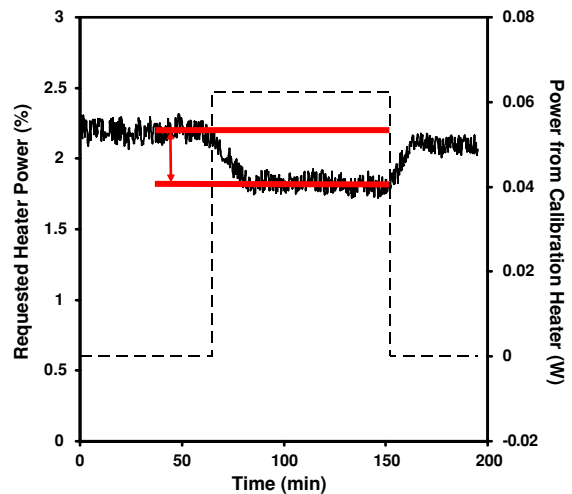


Fig. 1 Calibration of the requested power of the electrical heater (—) used for temperature control of the miniature bioreactor. A known power was introduced to the vessel using a calibration heater (---). The average decrease in requested power when the calibration heater was activated, as indicated by the arrow, was 0.4%. Calibration was performed using a well-lagged vessel containing fermentation medium

At approximately 350 min the heater power reduces to a minimum value of 1.85×10^{-5} W which coincided with the time at which the fermentation was in the late log phase, as indicated in Fig. 2b. Once the microbial culture entered stationary phase, there was a corresponding increase in the power supplied by the electrical heater in the absence of any cell growth. From Fig. 2b it can be seen that the actual rate of heat evolution increased with biomass concentration, achieving a maximum value of 0.12 W l^{-1} . After 370 min a series of small peaks in the heat signal were recorded although no measurable increase in biomass was detected. This pattern is typical of fermentations on complex media, since various substrates that are still present can be slowly oxidised (Voisard et al. 2002).

The integrated heat curve based on the data in Fig. 2b is shown in Fig. 3. This is seen to follow the same profile as the off-line biomass concentration curve, achieving a maximum heat evolution of 2.4 kJ l^{-1} . During exponential growth of the culture heat evolution also increased proportionally. The calculated rate constant for heat generation was similar to that of biomass production, 0.68 and 0.65 h^{-1} (μ_{\max}) respectively. The data collected from the ten identical *E. coli* fermentations provide good agreement between the maximum specific growth

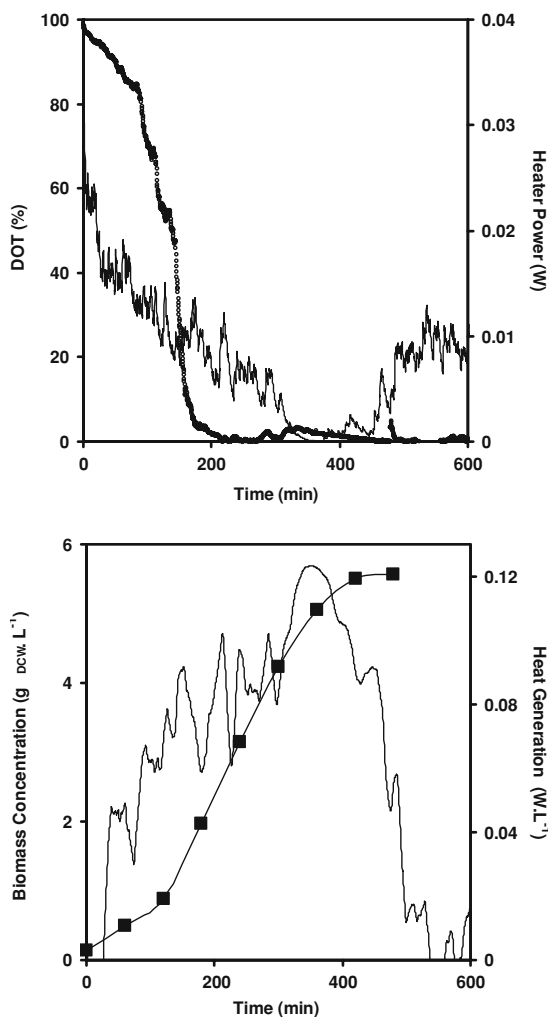


Fig. 2 On-line heat measurements during a typical *E. coli* TOP10 pQR239 fermentation. (a) Profiles of dissolved oxygen tension and power supplied by the heater to maintain the fermentation at 37°C: (○) DOT; (–) heater power. (b) Comparison of biomass growth kinetics from off-line OD₆₀₀ measurements and calculated heat generation: (■) OD₆₀₀; (–) heat generation

rates predicted from the off-line biomass measurements and the integrated heat curves of, 0.66 ± 0.04 and $0.69 \pm 0.05 \text{ h}^{-1}$ respectively.

Thermal profiling as a tool for in-situ on-line monitoring

For the majority of miniature bioreactor system reported to date, on-line biomass measurements are most commonly achieved using optical density probes or optical sensors. While such probes provide

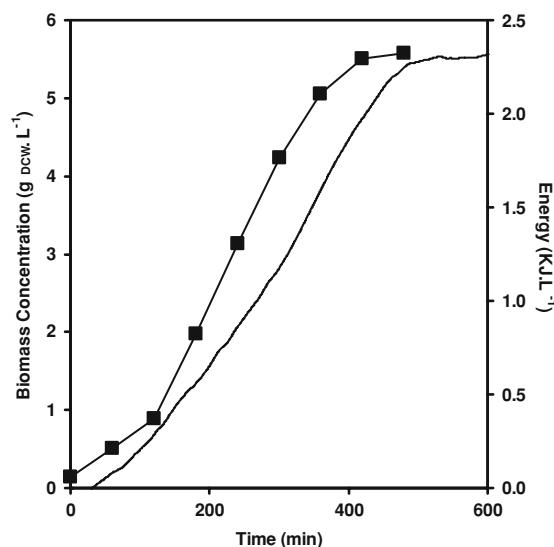


Fig. 3 Comparison of biomass growth kinetics from a typical parallel *E. coli* TOP10 pQR239 fermentation based on off-line OD₆₀₀ measurements and the integrated heat generation curves: (■) OD₆₀₀; (–) integrated heat curve

valuable information about cell growth, they rely solely on turbidity changes of the fermentation broth to estimate this. Although some of the changes in the appearance of the fermentation broth can be attributed to growth and increase in cell number, this measurement is not able to differentiate between active cells, dead cells, cell fragments or any other particulates that may have accumulated during the fermentation (Kaiser et al. 2007). As shown here simple quantification of the changes in the natural heat evolved (Fig. 2) potentially provides a valuable indicator of the growth of a microbial culture and is specific to actively growing cells. In this sense it has some parallels with more accurate biocalorimetry methods (e.g. Bou-Diab et al. 2001; Jassen et al. 2005; Voisard et al. 2002) and techniques such as flow cytometry (Hewitt et al. 2000) which can also distinguish viable cells from dead ones.

In the specific case of *parallel* miniature bioreactors monitoring a characteristic that is already naturally occurring (the generation of heat) during cell cultivation, provides a valuable real-time, non-invasive way of estimating cell growth kinetics in potentially 10³'s of parallel fermentations. This enables operators to focus on fermentation process design and optimisation rather than extensive analysis of off-line samples.

Conclusions

The thermal profiling results presented here demonstrated that a wealth of information can be gained simply from monitoring the natural heat generated by a microbial culture during fermentation. It has been shown that quantitative information regarding biomass growth kinetics can be easily obtained from the standard temperature control system of the miniature bioreactors, without any additional modifications that are necessary with conventional calorimetry methods (e.g. Bou-Diab et al. 2001; Jassen et al. 2005; Voisard et al. 2002). Calculated maximum specific growth rates estimated from the integrated heat curve and off-line biomass concentration curve are seen to be comparable. The thermal profiling technique thus appears to be a valuable and competitive monitoring technique for parallel miniature bioreactors. The technique is in principle applicable to any microbial growth process with significant heat generation.

Acknowledgements The authors would like to thank the UK Joint Infrastructure Fund (JIF), the Science Research Investment Fund (SRIF) and the Gatsby Charitable Foundation for funds to establish the UCL Centre for Micro Biochemical Engineering. Financial support from the UK Engineering and Physical Sciences Research Council (EPSRC) and Bioxplore, in the form of an Engineering Doctorate (EngD) studentship for NKG, is also acknowledged.

References

- Betts JI, Baganz F (2006) Miniature bioreactors: current practices and future opportunities. *Microb Cell Fact* 5:1–14
- Bou-Diab A, Schenker B, Marison I, Ampuero S, von Stockar U (2001) Improvements of continuous calibration based on temperature oscillation and application to biochemical reaction calorimetry. *Chem Eng* 81:113–127
- Doig SD, O'Sullivan LM, Patel S, Ward JM, Woodley JM (2001) Large scale production of cyclohexanone monooxygenase from *Escherichia coli* TOP10 pQR239. *Enzyme Microb Technol* 28:265–274
- Fernandes P, Cabral JMS (2006) Review: microlitre/millilitre shaken bioreactors in fermentative and biotransformation process. *Biocatal Biotransformation* 24:237–252
- Gill NK, Appleton M, Baganz F, Lye GJ (2008) Design and characterisation of a miniature stirred bioreactor system for parallel microbial fermentations. *Biochem Eng J* 39:164–176
- Hewitt CJ, Caron G, Nienow AW, McFarlane CM (2000) Use of multi-staining flow cytometry to characterise the physiological state of *Escherichia coli* W3110 in high cell density fed-batch cultures. *Biotechnol Bioeng* 63:705–711
- Hüttl R, Harmel J, Lißner A, Wolf G, Klare P, Vonau W, Berthold F, Herrmann S (2008) A small-scale calorimetric reactor system combined with several chemical sensors for the investigation of microbial growth processes. *Eng Life Sci* 8:56–61
- Jassen M, Patino R, von Stockar U (2005) Application of bench-scale biocalorimetry to photoautotrophic cultures. *Thermochim Acta* 435:18–27
- Kaiser C, Carvell JP, Luttmann R (2007) A sensitive, compact, in-situ biomass measurement system: controlling and monitoring microbial fermentations using radio-frequency impedance. *Bioprocess Int* 5:52–56
- Lye GJ, Ayazi-Shamlou P, Baganz F, Dalby PA, Woodley JM (2003) Accelerated design of bioconversion processes using automated microscale processing techniques. *Trends Biotechnol* 21:29–37
- Marison I, Liu JS, Ampuero S, von Stockar U, Schenker B (1998) Biological reaction calorimetry: development of high sensitivity bio-calorimeters. *Thermochim Acta* 309:157–173
- Marose S, Lindemann C, Ulber R, Scheper T (1999) Optical sensor systems for bioprocess monitoring. *Trends Biotechnol* 17:30–34
- Meier-Schneiders M, Schafer F (1996) Quantification of small enthalpic differences in anaerobic microbial metabolism—a calorimetry supported approach. *Thermochim Acta* 275:1–16
- Meier-Schneiders M, Gosshans U, Busch C, Eigenberger G (1998) Biocalorimetry-supported analysis of fermentation processes. *Appl Microbiol Biotechnol* 43:431–439
- Sarrafzadeh MH, Belloy L, Estaban G, Navarro JM, Ghommidh C (2005) Dielectric monitoring of growth and sporulation of *Bacillus thuringiensis*. *Biotechnol Lett* 27:511–517
- Voisard D, Pugeaud P, Kumar AR, Jenny K, Jayaraman K, Marison IW, von Stockar U (2002) Quantitative calorimetric investigation of fed-batch cultures of *Bacillus sphaericus* 1593M. *Thermochim Acta* 394:99–111
- von Stockar U, Marison IW (1989) The use of calorimetry in biotechnology. *Adv Biochem Eng Biotechnol* 40:93–136