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On-line near infrared bioreactor monitoring of cell density and concentrations of glucose and lactate during insect cell cultivation

Jiang Qiu[a,*], Mark A. Arnold[b], David W. Murhammer[a]

[a] Department of Chemical and Biochemical Engineering, Optical Science and Technology Center, University of Iowa, Iowa City, IA 52242, United States
[b] Department of Chemistry, Optical Science and Technology Center, University of Iowa, Iowa City, IA 52242, United States

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ABSTRACT

Near infrared spectroscopy is demonstrated as a suitable method for monitoring real time cell density and concentrations of glucose and lactate during insect cell cultivation. The utility of this approach is illustrated during the cultivation of *Trichoplusia ni* BTI-Tn-5B1-4 insect cells in a stirred-tank bioreactor. *On-line* near infrared measurements are made by passing unaltered culture medium through an autoclavable near infrared flow-through sample cell during the cultivation process. Single-beam near infrared spectra were collected over the combination spectral range (5000–4000 cm^{-1}) through a 1.5 mm path length sample. Cell density calibration model was established by uni-variable linear regressions with measured mean absorbance values of on-line spectra collected during a cultivation run. Calibration models are generated for glucose and lactate by regression analysis of both *off line* and *on line* spectra collected during a series of pre-measurement cultivation runs. Analyte-specific calibration models are generated by using a combination of spectra from both natural, unaltered samples and samples spiked with known levels of glucose and lactate. Spiked samples are used to destroy concentration correlations between solutes, thereby enhancing the selectivity of the calibration models. Absorbance spectra are used to build partial least squares calibration models for glucose and lactate. The calibration model for cell density corresponds to a univariate linear regression calibration model based on the mean absorbance between 4750 and 4250 cm^{-1}. The standard errors of prediction are 1.54 mM, 0.83 mM, and 0.38 × 10^6 cells/mL for the glucose, lactate, and cell density models, respectively.

1. Introduction

Insect cell cultures, e.g., *Trichoplusia ni* BTI-Tn-5B1-4 (Tn-5B1-4) cell line, is used to produce recombinant proteins with the baculovirus expression vector system (BEVS). The BEVS has the potential for high protein expression levels and the capability to perform most posttranslational modifications (Chico and Jäger, 2000; Donaldson et al., 1999; Saarinen and Murhammer, 2003; Saarinen et al., 1999). System productivity can potentially be improved through the control of nutrient concentrations and minimization of by-product accumulation. Therefore, monitoring of cell density and nutrient and by-product concentrations is important to accomplish optimization and control of these processes.

Infrared spectroscopy (near and mid-infrared) offers a non-invasive method for rapidly measuring multiple components within a sample. This approach has been used in the biotechnology field to quantifying multiple species in complicated matrixes, such as samples corresponding to microbial fermentations (Crowley et al., 2005; Egidio et al., 2010; Hall et al., 1996; Petersen et al., 2010; Qiu et al., 2001; Vaccari et al., 1994; Vaidyanathan et al., 2001), cell cultures (Cervera et al., 2009; Lewis et al., 2000; McShane and Coté, 1998; Rhiel et al., 2002; Riley et al., 1998, 1997; Sandor et al., 2013; Yano et al., 1997; Yano and Harata, 1994), and downstream processing (Rodrigues et al., 2008; Yeung et al., 1999).

NIR spectra correspond to the overtone and combination vibration transitions associated with C—H, N—H, and O—H molecular groups. The corresponding absorption features are broad and weak compared to fundamental vibration absorptions, which creates strongly overlapping absorbance spectra from complex sample matrixes. Multivariate calibration methods, such as partial least squares (PLS) regression, are generally required to extract selective analytical information from such spectra. Multivariate calibration methods are prone, however, to systematic errors on the basis of concentration correlations. A natural concentration correlation is generated between glucose and lactate within cultivation samples (Riley et al., 1998). Such correlations must be eliminated within the data set in order to realize selective measurements (ASTM, 1995). The adaptive procedure described elsewhere was used in this study to minimize the significance of the

* Corresponding author at: Ricerca Biosciences, 7528 Auburn Road, Concord, OH 44077, United States. Tel.: +1 319 594 6844.
E-mail address: jiangqiu@hotmail.com (J. Qiu).

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concentration correlation between glucose and lactate (Riley et al., 1998).

Cell density is a critical parameter in monitoring the status of a cultivation run. Traditional methods of measuring cell density, such as dry cell mass and microscopic cell count, are tedious and difficult to perform *on line*. For this reason, a multitude of innovative methods are reported for *on line* biomass measurements. Techniques include both direct and indirect methods, such as optical density (Akhnoukh et al., 1996; Bédard et al., 1994), dielectric spectroscopy (Hoffmann et al., 2000; Zeiser et al., 1999, 2000), and glucose metabolism rate (Ducommun et al., 2001). NIR measurements are also proposed for both *off line* and *on line* cell density measurements (Arnold et al., 2002; Finn et al., 2006; Ge et al., 1994; Hall et al., 1996; Macaloney et al., 1994; Vaidyanathan et al., 2001; Yamane et al., 1996).

The primary objective of the experiments reported here is to demonstrate the ability to extract both cell density and concentrations of substrate (glucose) and by-product (lactate) during insect cell cultures from one NIR spectrum collected over the combination spectral region. In this study, cultivation medium is circulated through a closed-loop system where raw culture medium is taken from a stirred tank bioreactor, passed through a sterile NIR sample cell, and returned to the bioreactor. Samples are not filtered before the optical measurement and significant light scattering is created by the suspended insect cells. Previous work in our group illustrates the ability to quantify proteins dissolved in buffer solutions that contain different levels of scattering material (Green et al., 2002). This previous work shows that polystyrene microspheres significantly alter the signal-to-noise ratio of the NIR spectra by affecting the amount of transmitted light, and hence the radiant power at the detector. Nevertheless, accurate analytical measurements were possible in spite of the scattering particles as long as the scattering bodies were appropriately represented within the calibration data (Green et al., 2002). These findings suggest that cell density and solute concentrations can be extracted simultaneously from NIR spectra obtained from highly scattering samples. The functional utility of this approach is tested by using samples collected *off line* and *on line* during previous cultivation runs to build calibration models that correctly predict concentrations and cell density during subsequent *on line* monitoring.

2. Materials and methods

2.1. Cell line and cultivation conditions

The Tn-5B1-4 insect cell line was purchased from Invitrogen (San Diego, CA) and adapted to grow as a suspension in serum-free medium by a previously described procedure (Saarinen et al., 1999). Express Five SFM (Gibco BRL) serum-free medium was used throughout.

The Tn-5B1-4 cells (seeded at $\sim 0.5 \times 10^6$ cells/mL) were cultured in 250-mL Erlenmeyer flasks using 40 mL of medium at 27 °C and 125 rpm rotational speed. Larger flasks were used to grow sufficient cells for seeding the bioreactor for the corresponding experiments. A 3 L water-jacketed bioreactor (Applikon, Foster City, CA) equipped with oxygen electrodes (Mettler-Toledo, Wilmington, MA) was used with 1.6 L working volume. The dissolved oxygen concentration was monitored with the electrode and controlled at 50% air saturation via sparging with pure oxygen using LabView software (National Instruments, Austin, TX). Temperature was maintained at 27 °C and agitation at 200 rpm. The bioreactor headspace was flushed with air at a flow rate of 200 mL/min to avoid carbon dioxide accumulation (Mitchell-Logean and Murhammer, 1997).

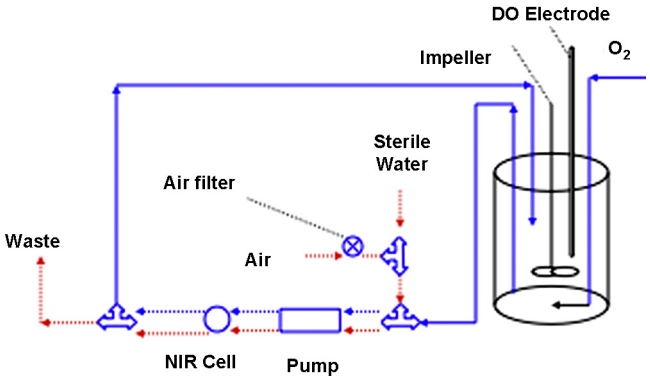

Fig. 1. Flow path diagram for coupling the NIR sample cell with a stirred-tank bioreactor.

2.2. Off line and on line spectra

All NIR spectra were collected at 128 co-added, double-sided interferograms with a Nexus 670 Fourier transform spectrometer (Thermo-Nicolet, Madison, WI). Fourier processing was accomplished with the Omnic software by using a triangular apodization and Mertz phase correction. This process resulted in single beam spectra with 4 cm^{-1} resolution. The spectrometer was equipped with a tungsten halogen lamp source, calcium fluoride beam splitter, and indium antimonide (InSb) cryogenically cooled detector. A multi-layer interference K-band filter (Barr and Associates, Westford, MA) was placed before the sample flow-though cell to confine the spectral range to 5000–4000 cm^{-1}. Generally, spectra were collected in triplicate after thermal equilibration. Background spectra of sterilized water were collected before each sample collection.

The sample cell was an autoclavable aluminum jacketed flow-through cell (Wilmad Glass, Buena, NJ) with sapphire windows (Meller Optics, Providence, RI). The sample thickness was maintained at 1.5 mm with a set of Teflon spacers between the sapphire windows. Temperature of the sample was monitored by placing a thermocouple probe within the jacket of the flow-through cell. Sample temperature was controlled at 27.0 ± 0.1 °C by circulating thermostated water though the outer jacket. The disturbance of NIR sample cell attached and entrapped with air bubbles, cells, and proteins can be easily detected from the background spectra. The NIR sample cell can also be easily cleaned and dried by purging sterilized water and air. The flow path schematic diagram for coupling the NIR sample cell with a stirred-tank bioreactor is shown in Fig. 1.

Samples collected *off line* were continuously circulated through the thermostated sample cell with a peristaltic pump at a flow rate of 8 mL/min. After the solution temperature stabilized at 27 °C, spectra were collected in triplicate.

On line spectra were collected by using the flow path presented schematically in Fig. 1. In this configuration, the autoclavable flow-through cell described above was connected to the stirred-tank bioreactor with autoclavable silicon tubing (Masterflex Tygon extended life silicon tubing, 96440-14). Solution flow was controlled by a series of three-way valves in conjunction with a peristaltic pump. Samples were drawn directly from the stirred-tank bioreactor at a flow rate of 8 mL/min. The cell-containing medium was continuously passed through the sample cell while spectra were collected. After at least three or four spectra were obtained, a sample was collected for *off line* reference analysis (see below).

For both *off line* and *on line* samples, the flow cell was rinsed with sterile water and dried with the sterile filtered air between samples. During this cleansing step, the sample chamber was flushed