

IN SITU RAMAN SPECTROSCOPY SYSTEMS AND METHODS FOR CONTROLLING PROCESS VARIABLES IN CELL CULTURES

5 CROSS REFERENCE TO RELATED APPLICATIONS

This application claims benefit of and priority to US Provisional Patent Applications 62/572,828 filed on October 16, 2018, and 62/662,322 filed on April 25, 2018, all of which are incorporated by reference in their entireties where permissible.

10 FIELD OF THE INVENTION

The invention is generally directed to bioreactor systems and methods including *in situ* Raman spectroscopy methods and systems for monitoring and controlling one or more process variables in a bioreactor cell culture.

15 BACKGROUND OF THE INVENTION

The Process Analytical Technology (PAT) framework of the Food and Drug Administration (FDA) encourages the voluntary development and implementation of innovative solutions for process development, process analysis, and process control to better understand processes and control the quality of products. Process parameters are monitored and controlled during the manufacturing process. For example, the feeding of nutrients to a cell culture in a bioreactor during the manufacturing of bioproducts is an important process parameter. Current bioproduct manufacturing involves a feed strategy of daily bolus feeds. Under current methods, daily bolus feeds increase the nutrient concentration in the cell cultures by at least five times each day. To ensure that the culture is not depleted of nutrients in between feedings, the daily bolus feeds maintain nutrients at high concentration levels. Indeed, each feed is designed to have all of the nutrients that the culture requires to sustain it until the next feed. However, the large amount of nutrients in each daily bolus feed can cause substantial swings in nutrient levels in the bioreactor leading to inconsistencies in the product quality output of the production culture.

30 In addition, the high concentration of nutrients in each daily bolus feed contributes to an increase in post-translational modifications in the resulting bioproduct. For example, high concentrations of glucose in the cell culture can lead to an increase in glycation in the final bioproduct. Glycation is the nonenzymatic addition of a reducing sugar to an amino acid residue of the protein, typically occurring at the N-terminal amine of proteins and the

positively charged amine group. The resulting products of glycation can have yellow or brown optical properties, which can result in colored drug product (Hodge JE (1953) J Agric Food Chem. 1:928-943). Glycation can also result in charge variants within a single production batch of a therapeutic monoclonal antibody (mAb) and result in binding inhibition (Haberger M et al. (2014) MAbs. 6:327-339).

Accordingly, in an effort to further the PAT initiative, there remains a need for a method or system that is able to optimize nutrient concentrations within the cell culture leading to higher quality products.

SUMMARY OF THE INVENTION

In situ Raman spectroscopy methods and systems for monitoring and controlling one or more process variables in a bioreactor cell culture are disclosed herein.

One embodiment of the present invention includes a method for controlling cell culture medium conditions including quantifying one or more analytes in the cell culture medium using *in situ* Raman spectroscopy; and adjusting the one or more analyte concentrations in the cell culture medium to match predetermined analyte concentrations that maintain post-translational modifications of proteins in the cell culture medium to 1.0 to 30 percent. In some embodiments, the post-translational modification includes glycation. In other embodiments, proteins in the cell culture include an antibody, antigen-binding fragment thereof, or a fusion protein. In still other embodiments, the cell culture medium includes mammalian cells, for example, Chinese Hamster Ovary cells.

In some embodiments, the analyte is glucose. In this aspect, the predetermined glucose concentration is 0.5 to 8.0 g/L. In another embodiment, the predetermined glucose concentration is 1.0 g/L to 3.0 g/L. In still another embodiment, the glucose concentration is 2.0 g/L or 1.0 g/L. In other embodiments, the predetermined analyte concentrations maintain post-translational modifications of proteins in the cell culture medium to 1.0 to 20 percent or 5.0 to 10 percent. In still other embodiments, the quantifying of analytes is performed continuously, intermittently, or in intervals. For example, the quantifying of analytes is performed in 5 minute intervals, 10 minute intervals, or 15 minute intervals. In yet other embodiments, the quantifying of analytes is performed hourly or at least daily. In some embodiments, the adjusting of analyte concentrations is performed automatically. In still other embodiments, at least two or at least three or at least four different analytes are quantified.

Another embodiment of the present invention includes a method for reducing post-translation modifications of a secreted protein including culturing cells secreting the protein in a cell culture medium including 0.5 to 8.0 g/L glucose; incrementally determining the concentration of glucose in the cell culture medium during culturing of the cells using *in situ* Raman spectroscopy; and adjusting the glucose concentration to maintain the concentration of glucose to 0.5 to 8.0 g/L by automatically delivering multiple doses of glucose per hour to maintain post-translational modifications of the secreted protein to 1.0 to 30.0 percent. In one embodiment, the concentration of glucose is 1.0 to 3.0 g/L.

Still another embodiment of the present invention includes a system for controlling cell culture medium conditions including one or more processors in communication with a computer readable medium storing software code for execution by the one or more processors in order to cause the system to receive data including a concentration of one or more analytes in the cell culture medium from an *in situ* Raman spectrometer; and adjust the one or more analyte concentrations in the cell culture medium to match predetermined analyte concentrations that maintain post-translational modifications of proteins in the cell culture medium to 1.0 to 30 percent. In one embodiment, the software code is further configured to cause the system to perform chemometric analysis, for example, Partial Least Squares regression modeling, on the data. In other embodiments, the software code is further configured to cause the system to perform one or more signal processing techniques, for example, a noise reduction technique, on the data.

Another embodiment of the present invention includes a system for reducing post-translation modifications of a secreted protein including one or more processors in communication with a computer readable medium storing software code for execution by the one or more processors in order to cause the system to incrementally receive spectral data including a concentration of glucose in a cell culture medium during culturing of cells secreting the protein from an *in situ* Raman analyzer; and adjust the glucose concentration to maintain the concentration of glucose to 0.5 to 8.0 g/L, for example, to 1.0 to 3.0 g/L, by automatically delivering multiple doses of glucose per hour to maintain post-translational modifications of the secreted protein to 1.0 to 30.0 percent. In one embodiment, the software code is further configured to cause the system to correlate peaks within the spectral data to glucose concentrations. In another embodiment, the software code is further configured to perform Partial Least Squares regression modeling on the spectral data. In still another embodiment, the software code is further configured to perform a noise reduction technique

on the spectral data. In yet other embodiments, the adjustment of the glucose concentration is performed by automated feedback control software.

BRIEF DESCRIPTION OF THE DRAWINGS

5 Further features and advantages of the invention can be ascertained from the following detailed description that is provided in connection with the drawings described below:

FIG. 1 is a flow chart of a method for controlling process variables in a cell culture according to one embodiment of the present invention.

10 FIG. 2 is a schematic diagram of a system for controlling process variables in a cell culture associated with FIG 1 in accordance with the present invention.

FIG. 3 is a graph showing predicted nutrient process values confirmed by offline nutrient samples.

15 FIG. 4 is a graph showing filtered final nutrient process values after a signal processing technique according to the present invention.

FIG. 5 is a graph showing the predicted nutrient process values and the filtered final nutrient process values after a shift in the predefined set point of nutrient concentration.

20 FIG. 6 is a line graph showing the effects of glucose concentration on post-translational modifications for a feedback controlled continuous nutrient feed in accordance with the present invention and for a bolus nutrient feed.

FIG. 7 is a graph showing the *in situ* Raman predicted glucose concentration values for a feedback controlled continuous nutrient feed in accordance with the present invention and for a bolus nutrient feed.

25 FIG. 8 is a line graph showing the antibody titer for a feedback controlled continuous nutrient feed in accordance with the present invention and for a bolus nutrient feed.

FIG. 9 is a bar graph showing shows the normalized percentage of post-translational modifications as a result of glucose concentration.

30 FIG. 10 is a graph showing the glucose concentrations for a feedback controlled continuous nutrient feed in accordance with the present invention and for a bolus nutrient feed.

FIG. 11 is a graph showing that feedback control cell culture can reduce the PTMs by as much as 50% compared to bolus fed strategy cell culture.

DETAILED DESCRIPTION

I. Definitions

As used herein, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise.

- 5 Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein.

- 10 Use of the term “about” is intended to describe values either above or below the stated value in a range of approx. +/- 10%; in other embodiments, the values may range in value either above or below the stated value in a range of approx. +/- 5%; in other embodiments, the values may range in value either above or below the stated value in a range of approx. +/- 2%; in other embodiments, the values may range in value either above or below the stated value in a range of approx. +/- 1%. The preceding ranges are intended to be made clear by
15 context, and no further limitation is implied. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (*e.g.*, “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should
20 be construed as indicating any non-claimed element as essential to the practice of the invention.

 The term “bioproduct” refers to any antibody, antibody fragment, modified antibody, protein, glycoprotein, or fusion protein as well as final drug substances manufactured in a bioreactor process.

- 25 The terms “control” and “controlling” refer to adjusting an amount or concentration level of a process variable in a cell culture to a predefined set point.

 The terms “monitor” and “monitoring” refer to regularly checking an amount or concentration level of a process variable in a cell culture or a process condition in the cell culture.

- 30 The term “steady state” refers to maintaining the concentration of nutrients, process parameters, or the quality attributes in the cell culture at an unchanging, constant, or stable level. It is understood that an unchanging, constant, or stable level refers to a level within predetermined set points. Set points, and therefore steady state levels, may be shifted during the time period of a production cell culture by the operator.

II. Methods for Producing Bioproducts

One embodiment provides methods for monitoring and controlling one or more process variables in a bioreactor cell culture in order to improve product quality and consistency. Process variables include but are not limited to concentrations of glucose, amino acids, vitamins, growth factors, proteins, viable cell count, oxygen, nitrogen, pH, dead cell count, cytokines, lactate, glutamine, other sugars such as fructose and galactose, ammonium, osmolality, and combinations thereof. The disclosed methods and systems utilize *in situ* Raman spectroscopy and chemometric modeling techniques for real-time assessments of cell cultures, combined with signal processing techniques, for precise continuous feedback and model predictive control of cell culture process variables. *In situ* Raman spectroscopy of the bioreactor contents allows the analysis of one or more process variables in the bioreactor without having to physically remove a sample of the bioreactor contents for testing. Through the use of real-time data from Raman spectroscopy, the process variables within the cell culture may be continuously or intermittently monitored and automated feedback controllers maintain the process variables at predetermined set points or maintain a specific feeding protocol that delivers variable amounts of agents to the bioreactor to maximize bioproduct quality.

The disclosed methods and systems control one or more process variables in a cell culture process. The terms, "cell culture" and "cell culture media," may be used interchangeably and include any solid, liquid or semi-solid designed to support the growth and maintenance of microorganisms, cells, or cell lines. Components such as polypeptides, sugars, salts, nucleic acids, cellular debris, acids, bases, pH buffers, oxygen, nitrogen, agents for modulating viscosity, amino acids, growth factors, cytokines, vitamins, cofactors, and nutrients may be present within the cell culture medium. One embodiment provides a mammalian cell culture process and include mammalian cells or cell lines. For example, a mammalian cell culture process may utilize a Chinese Hamster Ovary (CHO) cell line grown in a chemically defined basal medium.

The cell culture process may be performed in a bioreactor. The bioreactors include seed train, fed-batch, and continuous bioreactors. The bioreactors may range in volume from about 2 L to about 10,000 L. In one embodiment, the bioreactor may be a 60 L stainless steel bioreactor. In another embodiment, the bioreactor may be a 250 L bioreactor. Each bioreactor should also maintain a cell count in the range of about 5×10^6 cells/mL to about 100×10^6 cells/mL. For example, the bioreactor should maintain a cell count of about 20×10^6 cells/mL to about 80 cells/mL.

The disclosed methods and system can monitor and control any analyte that is present in the cell culture and has a detectable Raman spectrum. For example, the methods of the present invention may be used to monitor and control any component of the cell culture media including components added to the cell culture, substances secreted from the cell, and cellular components present upon cell death. Components of the cell culture media that may be monitored and/or controlled by the disclosed systems and methods include, but are not limited to, nutrients, such as amino acids and vitamins, lactate, co-factors, growth factors, cell growth rate, pH, oxygen, nitrogen, viable cell count, acids, bases, cytokines, antibodies, and metabolites.

One embodiment provides the methods for monitoring and controlling nutrient concentrations in a cell culture. As used herein, the term “nutrient” may refer to any compound or substance that provides nourishment essential for growth and survival. Examples of nutrients include, but are not limited to, simple sugars such as glucose, galactose, lactose, fructose, or maltose; amino acids; and vitamins, such as vitamin A, B vitamins, and vitamin E. In another embodiment, the methods of the present invention may include monitoring and controlling glucose concentrations in a cell culture. By controlling the nutrient concentrations, for example, glucose concentrations, in a cell culture, it has been discovered that bioproducts, such as proteins, can be produced in a lower concentration range than was previously possible using a daily bolus nutrient feeding strategy.

Moreover, by controlling nutrient concentrations and other process variables in the cell culture, the methods of the present invention further provide for modulating one or more post-translational modifications of a protein. Without being bound by any particular theory, it is believed that, by providing lower nutrient concentrations within the cell culture, post-translational modifications in proteins and antibodies may be decreased. Examples of post-translational modifications that may be modulated by the present invention include, but are not limited to, glycation, glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, and modification by non-naturally occurring amino acids. Another embodiment provides methods and systems for modulating the glycation of a protein. For instance, by providing lower concentration ranges of glucose in cell culture media, levels of glycation in secreted protein or antibody can be decreased in the final bioproduct.

FIG. 1 is a flow chart of an exemplary method for controlling one or more process variables, for example, nutrient concentration, in a bioreactor cell culture. Predetermined set points for each of the process variables to be monitored and controlled can be programed into

the system. The predefined set points represent the amount of process variable in the cell culture that is to be maintained or adjusted throughout the process. Glucose concentration is one example of a nutrient that can be monitored and modulated. As briefly discussed above, it has been discovered that bioproducts (for example, proteins, antibodies, fusion proteins, and drug substances) can be produced by cells in a culture medium that contains low levels of glucose compared to glucose concentrations in media using a daily bolus nutrient feeding strategy. In one embodiment, the predefined set point for nutrient concentration is the lowest concentration of a nutrient necessary to grow and propagate a cell line. The disclosed methods and systems can deliver multiple small doses of nutrients to the culture medium over a period of time or can provide a steady stream of nutrient to the culture medium. In some embodiments, the predefined set point may be increased or decreased during the process depending on the conditions within the cell culture media. For example, if the predefined amount of nutrient concentration results in cell death or sub-optimal growth conditions within the cell culture media, the predefined set point may be increased. However, the nutrient concentration should be maintained at a predefined set point of about 0.5 g/L to about 10 g/L. In another embodiment, the nutrient concentration should be maintained at a predefined set point of about 0.5 g/L to about 8 g/L. In still another embodiment, the nutrient concentration should be maintained at a predefined set point of about 1 g/L to about 3 g/L. In yet another embodiment, the nutrient concentration should be maintained at a predefined set point of about 2 g/L. These predefined set points essentially provide a baseline level at which the nutrient concentration should be maintained throughout the process.

In one embodiment, the monitoring of the one or more process variables, for example, the nutrient concentration, in a cell culture is performed by Raman spectroscopy (step 101). Raman spectroscopy is a form of vibrational spectroscopy that provides information about molecular vibrations that can be used for sample identification and quantitation. In some embodiments, the monitoring of the process variables is performed using *in situ* Raman spectroscopy. *In situ* Raman analysis is a method of analyzing a sample in its original location without having to extract a portion of the sample for analysis in a Raman spectrometer. *In situ* Raman analysis is advantageous in that the Raman spectroscopy analyzers are noninvasive, which reduces the risk of contamination, and nondestructive with no impact to cell culture viability or protein quality.

The *in situ* Raman analysis can provide real-time assessments of one or more process variables in cell cultures. For example, the raw spectral data provided by *in situ* Raman spectroscopy can be used to obtain and monitor the current amount of nutrient concentration

in a cell culture. In this aspect, to ensure that the raw spectral data is continuously up to date, the spectral data from the Raman spectroscopy should be acquired about every 10 minutes to 2 hours. In another embodiment, the spectral data should be acquired about every 15 minutes to 1 hour. In still another embodiment, the spectral data should be acquired about every 20
5 minutes to 30 minutes.

In this aspect, the monitoring of the one or more process variables in the cell culture can be analyzed by any commercially available Raman spectroscopy analyzer that allows for *in situ* Raman analysis. The *in situ* Raman analyzer should be capable of obtaining raw spectral data within the cell culture (for example, the Raman analyzer should be equipped
10 with a probe that may be inserted into the bioreactor). Suitable Raman analyzers include, but are not limited to, RamanRXN2 and RamanRXN4 analyzers (Kaiser Optical Systems, Inc. Ann Arbor, MI).

In step 102, the raw spectral data obtained by *in situ* Raman spectroscopy may be compared to offline measurements of the particular process variable to be monitored or
15 controlled (for example, offline nutrient concentration measurements) in order to correlate the peaks within the spectral data to the process variable. For instance, if the process variable to be monitored or controlled is glucose concentration, offline glucose concentration measurements may be used to determine which spectral regions exhibit the glucose signal. The offline measurement data may be collected through any appropriate analytical method.
20 Additionally, any type of multivariate software package, for example, SIMCA 13 (MKS Data Analytic Solutions, Umea, Sweden), may be used to correlate the peaks within the raw spectral data to offline measurements of the particular process variable to be monitored or controlled. However, in some embodiments, it may be necessary to pretreat the raw spectral data with spectral filters to remove any varying baselines. For example, the raw spectral data
25 may be pretreated with any type of point smoothing technique or normalization technique. Normalization may be needed to correct for any laser power variation and exposure time by the Raman analyzer. In one embodiment, the raw spectral data may be treated with point smoothing, such as 1st derivative with 21 cm⁻¹ point smoothing, and normalization, such as Standard Normal Variate (SNV) normalization.

30 Chemometric modeling may also be performed on the obtained spectral data. In this aspect, one or more multivariate methods including, but not limited to, Partial Least Squares (PLS), Principal Component Analysis (PCA), Orthogonal Partial least squares (OPLS), Multivariate Regression, Canonical Correlation, Factor Analysis, Cluster Analysis, Graphical Procedures, and the like, can be used on the spectral data. In one embodiment, the obtained

spectral data is used to create a PLS regression model. A PLS regression model may be created by projecting predicted variables and observed variables to a new space. In this aspect, a PLS regression model may be created using the measurement values obtained from the Raman analysis and the offline measurement values. The PLS regression model provides
5 predicted process values, for example, predicted nutrient concentration values.

After chemometric modeling, a signal processing technique may be applied to the predicted process values (for example, the predicted nutrient concentration values) (step 103). In one embodiment, the signal processing technique includes a noise reduction technique. In this aspect, one or more noise reduction techniques may be applied to the predicted process
10 values. Any noise reduction technique known to those skilled in the art may be utilized. For example, the noise reduction technique may include data smoothing and/or signal rejection. Smoothing is achieved through a series of smoothing algorithms and filters while signal rejection uses signal characteristics to identify data that should not be included in the analyzed spectral data. In one embodiment, the predicted process values are noise mitigated
15 by a noise reduction filter. The noise reduction filter provides final filtered process values (for example, final filtered nutrient concentration values). In this aspect, the noise reduction technique combines raw measurements with a model-based estimate for what the measurement should yield according to the model. In one embodiment, the noise reduction technique combines a current predicted process value with its uncertainties. Uncertainties
20 can be determined by the repeatability of the predicted process values and the current process conditions. Once the next predicted process value is observed, the estimate of the predicted process value (for example, predicted nutrient concentration value) is updated using a weighted average where more weight is given to the estimates with higher certainty. Using an iterative approach, the final process values may be updated based on the previous
25 measurement and the current process conditions. In this aspect, the algorithm should be recursive and able to run in real time so as to utilize the current predicted process value, the previous value, and experimentally determined constants. The noise reduction technique improves the robustness of the measurements received from the Raman analysis and the PLS predictions by reducing noise upon which the automated feedback controller will act.

30 Upon obtaining the final filtered process values (for example, the final filtered nutrient concentration values), the final values may be sent to an automated feedback controller (step 104). The automated feedback controller may be used to control and maintain the process variable (for example, the nutrient concentration) at the predefined set point. The automated feedback controller may include any type of controller that is able to

calculate an error value as the difference between a desired set point (*e.g.*, the predefined set point) and a measured process variable and automatically apply an accurate and responsive correction. The automated feedback controller should also have controls that are capable of being changed in real time from a platform interface. For instance, the automated feedback controller should have a user interface that allows for the adjustment of a predefined set point. The automated feedback controller should be capable of responding to a change in the predefined set point.

In one embodiment, the automated feedback controller may be a proportional-integral-derivative (PID) controller. In this aspect, the PID controller is operable to calculate the difference between the predefined set point and the measured process variable (for example, the measured nutrient concentration) and automatically apply an accurate correction. For example, when a nutrient concentration of a cell culture is to be controlled, the PID controller may be operable to calculate a difference between a filtered nutrient value and a predefined set point and provide a correction in nutrient amount. In this aspect, the PID controller may be operatively connected to a nutrient pump on the bioreactor so that the corrective nutrient amount may be pumped into the bioreactor (step 105).

Through the use of Raman real time analysis and feedback control, the methods of the present invention are able to provide continuous and reduced concentrations of nutrients to the cell culture. That is, the method of the present invention is able to provide steady-state nutrient addition to the cell culture. In one embodiment, in order to maintain the predefined nutrient concentration, the nutrients may be pumped to the cell culture, via the nutrient pump, continuously over a period of time. In another embodiment, the nutrients may be added to the cell culture, via the nutrient pump, in a duty cycle. For instance, in this aspect, the addition of the nutrients may be staggered or occur intermittently over a period of time.

The disclosed methods and systems also allow for the production of bioproducts in culture media that contains lower nutrient concentration range, for example, glucose concentration range, than nutrient concentrations in culture media using a daily bolus nutrient feeding strategy. In one embodiment, the nutrient concentrations, for example, glucose concentrations, are at least 3 g/L lower than bolus nutrient feedings. In another embodiment, the nutrient concentrations, for example, glucose concentrations, are at least 5 g/L lower than nutrient concentrations in culture media obtained using bolus nutrient feedings. In still another embodiment, the nutrient concentrations, for example, glucose concentrations, are at least 6 g/L lower than nutrient concentrations obtained using bolus nutrient feedings.

Moreover, the lower nutrient concentrations in culture media and steady-state addition achieved by the disclosed systems and methods allow for a decrease in post-translational modification in proteins and monoclonal antibodies. In one embodiment, the disclosed methods and systems deliver nutrients near or at the rate the nutrients are taken up or
5 consumed by cells in the culture. The steady-state addition of small doses of nutrients over time allows for the production of bioproducts having lower levels of post-translational modifications, for example, lower levels of glycation, in comparison to standard bolus feed addition. Importantly, the steady-state addition of the reduced concentrations of nutrients does not affect antibody production. In one embodiment, the reduced nutrient concentrations
10 provide for a decrease in post-translation modification by as much as 30% when compared to the post-translation modifications observed in standard bolus feed addition. In another embodiment, the reduced nutrient concentrations provide for a decrease in post-translation modification by as much as 40% when compared to the post-translation modifications observed in standard bolus feed addition. In still another embodiment, the reduced nutrient
15 concentrations provide for a decrease in post-translation modification by as much as 50% when compared to the post-translation modifications observed in standard bolus feed addition.

III. Bioreactor Systems

Another embodiment provides systems for monitoring and controlling one or more
20 process variables in a bioreactor cell culture. Multiple components are integrated into a single system with a single user interface. Referring to FIG. 2, Raman analyzer 200 may be operatively connected to bioreactor 300. In this aspect, a Raman probe may be inserted into the bioreactor 300 to obtain raw spectral data of one or more process variables, for example, nutrient concentration, within the cell culture. The Raman analyzer 200 may also be
25 operatively connected to computer system 500 so that the obtained raw spectral data may be received and processed.

Computer system 500 may typically be implemented using one or more programmed general-purpose computer systems, such as embedded processors, systems on a chip, personal computers, workstations, server systems, and minicomputers or mainframe computers, or in
30 distributed, networked computing environments. Computer system 500 may include one or more processors (CPUs) 502A-502N, input/output circuitry 504, network adapter 506, and memory 508. CPUs 502A-502N execute program instructions in order to carry out the

functions of the present systems and methods. Typically, CPUs 502A-502N are one or more microprocessors, such as an INTEL CORE® processor.

Input/output circuitry 504 provides the capability to input data to, or output data from, computer system 500. For example, input/output circuitry may include input devices, such as keyboards, mice, touchpads, trackballs, scanners, analog to digital converters, etc., output devices, such as video adapters, monitors, printers, etc., and input/output devices, such as, modems, etc. Network adapter 506 interfaces device 500 with a network 510. Network 510 may be any public or proprietary LAN or WAN, including, but not limited to the Internet.

Memory 508 stores program instructions that are executed by, and data that are used and processed by, CPU 502 to perform the functions of computer system 500. Memory 508 may include, for example, electronic memory devices, such as random-access memory (RAM), read-only memory (ROM), programmable read-only memory (PROM), electrically erasable programmable read-only memory (EEPROM), flash memory, etc., and electro-mechanical memory, such as magnetic disk drives, tape drives, optical disk drives, etc., which may use an integrated drive electronics (IDE) interface, or a variation or enhancement thereof, such as enhanced IDE (EIDE) or ultra-direct memory access (UDMA), or a small computer system interface (SCSI) based interface, or a variation or enhancement thereof, such as fast-SCSI, wide-SCSI, fast and wide-SCSI, etc., or Serial Advanced Technology Attachment (SATA), or a variation or enhancement thereof, or a fiber channel-arbitrated loop (FC-AL) interface.

Memory 508 may include controller routines 512, controller data 514, and operating system 520. Controller routines 512 may include software routines to perform processing to implement one or more controllers. Controller data 514 may include data needed by controller routines 512 to perform processing. In one embodiment, controller routines 512 may include multivariate software for performing multivariate analysis, such as PLS regression modeling. In this aspect, controller routines 512 may include SIMCA-QPp (MKS Data Analytic Solutions, Umea, Sweden) for performing chemometric PLS modeling. In another embodiment, controller routines 512 may also include software for performing noise reduction on a data set. In this aspect, the controller routines 512 may include MATLAB Runtime (The Mathworks Inc., Natick, MA) for performing noise reduction filter models. Moreover, controller routines 512 may include software, such as MATLAB Runtime, for operating the automated feedback controller, for example, the PID controller. The software for operating the automated feedback controller should be able to calculate the difference between the predefined set point and the

measured process variable (for example, the measured nutrient concentration) and automatically apply an accurate correction. Accordingly, the computer system 500 may also be operatively connected to nutrient pump 400 so that the corrective nutrient amount may be pumped into the bioreactor 300.

- 5 The disclosed systems may control and monitor process variables in a single bioreactor or a plurality of bioreactors. In one embodiment, the system may control and monitor process variables in at least two bioreactors. In another embodiment, the system may control and monitor process variables in at least three bioreactors or at least four bioreactors. For example, the system can monitor up to four bioreactors in an hour.

10

Examples

The following non-limiting examples demonstrate methods for controlling one or more process variables in a bioreactor cell culture in accordance with the present invention. The examples are merely illustrative of the preferred embodiments of the present invention, and are not to be construed as limiting the invention, the scope of which is defined by the appended claims.

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Example 1

Materials and Methods

- 20 The mammalian cell culture process utilized a Chinese Hamster Ovary (CHO) cell line grown in a chemically defined basal medium. The production was performed in a 60L pilot scale stainless steel bioreactor controlled by RSLogix 5000 software (Rockwell Automation, Inc. Milwaukee, WI).

- The data collection for the model included spectral data from both Kaiser
25 RamanRXN2 and RamanRXN4 analyzers (Kaiser Optical Systems, Inc. Ann Arbor, MI) utilizing BIO-PRO optic (Kaiser Optical Systems, Inc. Ann Arbor, MI). The RamanRXN2 and RamanRXN4 analyzers operating parameters were set to a 10 second scan time for 75 accumulations. An OPC Reader/Writer to RSLinx OPC Server was used for data flow.

- SIMCA 13 (MKS Data Analytic Solutions, Umea, Sweden) was used to correlate
30 peaks within the spectral data to offline glucose measurements. The following spectral filtering was performed on the raw spectral data: 1st derivative with 21cm^{-1} point smoothing to remove varying baselines and Standard Normal Variate (SNV) normalization to correct for laser power variation and exposure time.

A Partial Least Squares regression model was created with corresponding offline measurements taken on the Nova Bioprofile Flex (Nova Biomedical, Waltham, MA). Table 1A below shows the details of the nutrient chemometric Partial Least Squares regression model.

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TABLE 1A: NUTRIENT CHEMOMETRIC PARTIAL LEAST SQUARES REGRESSION MODEL DETAILS

Nutrient PLS Model Variable	Value
Observations	223
Wavelength Range (cm ⁻¹)	350-3100
Nutrient Concentration Range (g/L)	0.65-8.63
RMSEE	0.430
RSMECV	0.662
R ² X	0.982
Q ²	0.869

Signal processing techniques, specifically, noise reduction filtering, were also performed. The noise reduction technique combined the raw measurement with a model-based estimate for what the measurement should yield according to the model. Using an iterative approach, it allows for the filtered measurement to be updated based on the previous measurement and the current process conditions.

A reverse-acting proportional-integral-derivative (PID) Control having an algorithm programmed separately in MATLAB Runtime (The Mathworks Inc., Natick, MA) was utilized. All variables of the PID controller, such as tuning constants, have the ability to be changed in real time from the platform interface.

Results

FIG. 3 shows the predicted nutrient process values confirmed by offline nutrient samples. As can be seen from FIG. 3, the Raman analyzer and the chemometric model predicted nutrient concentration values within the offline analytical method's variability.

- 5 This demonstrates that *in situ* Raman spectroscopy and chemometric modeling according to the methods of the present invention provide accurate measurements of nutrient concentration values.

- FIG. 4 shows the filtered final nutrient process values after the signal processing technique. As can be seen from FIG. 4, the signal processing technique reduces noise of raw
10 predicted nutrient process values. The noise reduction filtering of the predicted nutrient values increases the robustness of the overall feedback control system.

- FIG. 5 shows the predicted nutrient process values and the filtered final nutrient process values after a shift in the predefined set point of nutrient concentration in a feedback controlled continuous nutrient feed batch. As can be seen by the adjustment in filtered
15 nutrient process values, a successful response from the feedback controller is observed when a shift in nutrient concentration set point occurs. Indeed, the PID controller was able to quickly respond to a set point change operating off the noise filtered nutrient process value.

- Based on the results shown in FIGS. 3-5, the methods of the present invention provide real time data that enables automated feedback control for continuous and steady nutrient
20 addition.

Example 2

Materials and Methods

- The production was performed in 250L single use bioreactors. A Partial Least Squares regression model was created. Table 1B below shows the details of the nutrient
25 chemometric Partial Least Squares regression model.

TABLE 1B: NUTRIENT CHEMOMETRIC PARTIAL LEAST SQUARES REGRESSION MODEL DETAILS

Nutrient PLS Model Variable	Value
Observations	147
Wavelength Range (cm ⁻¹)	350-3100
Nutrient Concentration Range (g/L)	0.6-3.61
RMSEE	0.352
RSMECV	0.520
R ² _X	0.769
Q ²	0.617

Noise filtering techniques were not used in this example.

Results

- 5 FIG. 6 shows the effects of glucose concentration on post-translational modifications. As can be seen from FIG. 6, the greater the glucose concentration, the higher the percentage of PTM. The data points in FIG. 6 for normalized % of post-translational modification (PTM) and glucose concentration over the batch day are shown in Table 2 below.

TABLE 2: NORMALIZED % PTM AND GLUCOSE CONCENTRATION DATA POINTS FOR FIG. 6

Time (hours)	% PTM	Glucose Concentration	Normalized % PTM	Glucose Concentration (g/L)
192	18.7	4.83	0.623333333	4.83
192	20.4	9.75	0.68	9.75
195	20.6	8.4	0.686666667	8.4
198	20.2	8.3	0.673333333	8.3
200	16.2	7.68	0.54	7.68
214	16.6	3.96	0.553333333	3.96
214	17.7	9.34	0.59	9.34
220	17.4	9.09	0.58	9.09
223	17.5	8.03	0.583333333	8.03
225	20.9	7.68	0.696666667	7.68
238	21.5	4.56	0.716666667	4.56
238	22.3	8.22	0.743333333	8.22
243	21.8	7.78	0.726666667	7.78
246	23.1	7.19	0.77	7.19
248	18.6	7.08	0.62	7.08
267	17	4.11	0.566666667	4.11
291	19.1	3.3	0.636666667	3.3
310	19.4	4.62	0.646666667	4.62
315	19	4.55	0.633333333	4.55
318	24	4.23	0.8	4.23
320	24.7	4	0.823333333	4
334	26	2.53	0.866666667	2.53
340	25.3	2.15	0.843333333	2.15
343	25.9	1.86	0.863333333	1.86
345	20.7	1.67	0.69	1.67
357	19.7	0.59	0.656666667	0.59
358	20.2	11.18	0.673333333	11.18
362	20.6	10.34	0.686666667	10.34
366	20.5	10.31	0.683333333	10.31
381	25.9	7.74	0.863333333	7.74

FIG. 7 shows the *in situ* Raman predicted glucose concentration values for a feedback controlled continuous nutrient feed in accordance with the present invention and for a bolus nutrient feed. The bolded black line in FIG. 7 represents the pre-defined set point. The pre-defined set point (SP1) was initially set at 3 g/L (SP1) and was increased to 5 g/L (SP2). As can be seen from FIG. 7, the Raman predicted glucose concentrations accurately adjusted during a shift in pre-defined set points. The data points in FIG. 7 for the Raman predicted glucose concentration values over the batch day are shown in Table 3 below.

TABLE 3: RAMAN PREDICTED GLUCOSE CONCENTRATION DATA POINTS FOR FIG. 7

Time (Glucose Feedback Control) (Elapsed Days)	Raman Glucose Feedback Concentration (g/L)	Time (Glucose Bolus Feed) (Elapsed Days)	Raman Bolus Feed Concentration (g/L)
2	5.27449	2	#N/A
2.023263889	6.057528	2.023263889	#N/A
2.044097222	6.093102	2.044097222	#N/A
2.064930556	6.030814	2.064930556	#N/A
2.085763889	5.928053	2.085763889	#N/A
2.106597222	6.112341	2.106597222	#N/A
2.127430556	5.877689	2.127430556	#N/A
2.148263889	5.881066	2.148263889	#N/A
2.169097222	5.929256	2.169097222	#N/A
2.189930556	5.928593	2.189930556	#N/A
2.210763889	5.929407	2.210763889	#N/A
2.231597222	5.672209	2.231597222	#N/A
2.252430556	5.796999	2.252430556	#N/A
2.273263889	5.572541	2.273263889	#N/A
2.294097222	5.771776	2.294097222	#N/A
2.31494213	5.521614	2.31494213	#N/A
2.335775463	5.630873	2.335775463	#N/A
2.356608796	5.53435	2.356608796	#N/A
2.37744213	5.628556	2.37744213	#N/A
2.398275463	5.575116	2.398275463	#N/A
2.419108796	5.675688	2.419108796	#N/A
2.43994213	5.356216	2.43994213	#N/A
2.460775463	5.019809	2.460775463	#N/A
2.481608796	5.571718	2.481608796	#N/A
2.50244213	5.424471	2.50244213	#N/A
2.523275463	4.974746	2.523275463	#N/A
2.544108796	5.105621	2.544108796	#N/A
2.56494213	4.882367	2.56494213	#N/A
2.585775463	5.156937	2.585775463	#N/A
2.606608796	4.882068	2.606608796	#N/A
2.62744213	5.054303	2.62744213	#N/A
2.648275463	5.034556	2.648275463	6.109157
2.669108796	4.835382	2.669108796	5.83853
2.689953704	5.057273	2.689953704	6.071649
2.710787037	4.504433	2.710787037	6.257731

Time (Glucose Feedback Control) (Elapsed Days)	Raman Glucose Feedback Concentration (g/L)	Time (Glucose Bolus Feed) (Elapsed Days)	Raman Bolus Feed Concentration (g/L)
2.73162037	4.725886	2.73162037	5.978051
2.752453704	4.707865	2.752453704	5.687498
2.773275463	4.474821	2.773275463	5.510823
2.794108796	4.595435	2.794108796	5.745687
2.814953704	4.846455	2.814953704	5.493782
2.835787037	4.349487	2.835787037	5.420269
2.85662037	4.623514	2.85662037	5.677184
2.877453704	4.35981	2.877453704	5.499728
2.898287037	4.580013	2.898287037	5.273839
2.91912037	4.233418	2.91912037	5.523314
2.939953704	4.033472	2.939953704	5.601781
2.960787037	3.875247	2.960787037	5.556786
3.0009375	4.083802	3.0009375	5.661055
3.023287037	3.564172	3.023287037	5.20255
3.04412037	3.788096	3.04412037	5.251106
3.064953704	3.721753	3.064953704	5.24757
3.12525463	3.615655	3.12525463	5.073968
3.166898148	3.759606	3.166898148	5.125836
3.208564815	3.402011	3.208564815	5.700113
3.250231481	3.312303	3.250231481	5.346854
3.291898148	3.384652	3.291898148	5.366998
3.333553241	2.754262	3.333553241	5.469024
3.416898148	2.657981	3.416898148	4.906005
3.458564815	2.661131	3.458564815	4.953602
3.500231481	2.683549	3.500231481	5.018805
3.541909722	2.315241	3.541909722	5.040889
3.583564815	2.470533	3.583564815	4.669607
3.625243056	2.895316	3.625243056	4.677879
3.666909722	3.167133	3.666909722	4.748203
3.708564815	2.959319	3.708564815	4.306628
3.750243056	3.334286	3.750243056	4.003834
3.791898148	3.10766	3.791898148	4.363513
3.833587963	3.058263	3.833587963	4.014596
3.875243056	2.723771	3.875243056	4.028898
3.916909722	2.612081	3.916909722	4.080404
3.958576389	2.666911	3.958576389	3.442322

Time (Glucose Feedback Control) (Elapsed Days)	Raman Glucose Feedback Concentration (g/L)	Time (Glucose Bolus Feed) (Elapsed Days)	Raman Bolus Feed Concentration (g/L)
4.00025463	2.121485	4.00025463	3.755342
4.040208333	2.498356	4.040208333	3.691836
4.063460648	2.796938	4.063460648	3.801793
4.084293981	3.222628	4.084293981	3.397573
4.105127315	3.059871	4.105127315	3.198539
4.125960648	3.144483	4.125960648	6.444279
4.146793981	2.912629	4.146793981	6.634366
4.167627315	2.798553	4.167627315	6.147713
4.188460648	2.657885	4.188460648	6.247666
4.209305556	2.724152	4.209305556	6.187882
4.230127315	2.72257	4.230127315	6.114422
4.250960648	2.797554	4.250960648	5.93613
4.271793981	3.035758	4.271793981	5.516821
4.332094907	2.726879	4.332094907	5.486897
4.373726852	2.984358	4.373726852	5.457622
4.415405093	2.487146	4.415405093	5.381355
4.457060185	2.364557	4.457060185	5.195489
4.498738426	2.894607	4.498738426	4.731695
4.540393519	3.171245	4.540393519	4.725901
4.623738426	3.579278	4.623738426	4.398326
4.665405093	3.227408	4.665405093	4.601714
4.707071759	2.769516	4.707071759	3.739007
4.74875	3.303736	4.74875	4.125107
4.810706019	2.604359	4.810706019	3.918031
4.833958333	2.666446	4.833958333	3.87917
4.854791667	2.436089	4.854791667	3.812785
4.875625	2.365274	4.875625	#N/A
4.896458333	3.052339	4.896458333	#N/A
4.917291667	3.356655	4.917291667	#N/A
4.938125	3.536857	4.938125	#N/A
4.958958333	3.254377	4.958958333	8.184118
4.979803241	2.647855	4.979803241	7.679708
5.000625	2.479576	5.000625	7.4381
5.021458333	3.108576	5.021458333	6.956085
5.042291667	2.733165	5.042291667	6.785896
5.063136574	2.161332	5.063136574	6.765765

Time (Glucose Feedback Control) (Elapsed Days)	Raman Glucose Feedback Concentration (g/L)	Time (Glucose Bolus Feed) (Elapsed Days)	Raman Bolus Feed Concentration (g/L)
5.083958333	2.115124	5.083958333	6.793903
5.104803241	2.617033	5.104803241	6.765692
5.125636574	2.554023	5.125636574	6.222265
5.146458333	2.480167	5.146458333	6.749342
5.167291667	2.715101	5.167291667	5.725123
5.188136574	2.735876	5.188136574	5.549073
5.208969907	2.725627	5.208969907	5.06423
5.229803241	2.575811	5.229803241	5.338056
5.250636574	2.212894	5.250636574	5.471513
5.271458333	2.233998	5.271458333	5.151946
5.292303241	2.213399	5.292303241	5.546629
5.313136574	2.766555	5.313136574	5.259173
5.333969907	2.52938	5.333969907	4.601235
5.354803241	2.933614	5.354803241	4.772757
5.375636574	3.028033	5.375636574	4.52338
5.396469907	3.41555	5.396469907	4.513873
5.417303241	3.193063	5.417303241	4.173473
5.438136574	3.138092	5.438136574	3.831865
5.458981481	2.893515	5.458981481	3.9247
5.479814815	3.43812	5.479814815	3.336164
5.500636574	3.013834	5.500636574	3.628655
5.521469907	3.132246	5.521469907	3.92468
5.542314815	3.046817	5.542314815	7.176596
5.563148148	3.078321	5.563148148	6.633468
5.583981481	2.615919	5.583981481	6.08785
5.604803241	2.751108	5.604803241	6.244726
5.625636574	2.824868	5.625636574	5.927638
5.646469907	2.517154	5.646469907	7.42588
5.667314815	1.988747	5.667314815	6.687646
5.688148148	2.344756	5.688148148	7.307424
5.708969907	3.218347	5.708969907	6.437283
5.729814815	2.85646	5.729814815	5.960429
5.750648148	2.43488	5.750648148	6.032461
5.771493056	2.792278	5.771493056	6.137525
5.811608796	2.982295	5.811608796	6.469258
5.833981481	2.991141	5.833981481	6.484286

Time (Glucose Feedback Control) (Elapsed Days)	Raman Glucose Feedback Concentration (g/L)	Time (Glucose Bolus Feed) (Elapsed Days)	Raman Bolus Feed Concentration (g/L)
5.854814815	3.201134	5.854814815	5.838443
5.875659722	2.563264	5.875659722	5.693282
5.896481481	2.42295	5.896481481	6.134384
5.917314815	2.673206	5.917314815	5.663696
5.938148148	2.654685	5.938148148	5.459308
5.958981481	2.747516	5.958981481	5.10138
5.979814815	2.548837	5.979814815	5.754516
6.019282407	2.525679	6.019282407	4.844961
6.060914352	2.808173	6.060914352	5.415936
6.102592593	2.547346	6.102592593	5.179432
6.144259259	2.485466	6.144259259	4.849273
6.185925926	2.707999	6.185925926	4.904904
6.227592593	3.150225	6.227592593	4.450798
6.269259259	2.60164	6.269259259	4.495592
6.310925926	2.741736	6.310925926	3.395906
6.352592593	2.407971	6.352592593	4.206471
6.394259259	1.757518	6.394259259	3.473652
6.435925926	2.549188	6.435925926	3.669552
6.477604167	3.543268	6.477604167	8.226236
6.519270833	3.739929	6.519270833	8.798409
6.5609375	3.384398	6.5609375	8.077047
6.602604167	3.33986	6.602604167	7.873461
6.644270833	2.969001	6.644270833	7.76911
6.6859375	2.726888	6.6859375	7.415218
6.727604167	2.846601	6.727604167	6.526413
6.769270833	2.275316	6.769270833	6.82022
6.8109375	2.198233	6.8109375	6.822738
6.852615741	3.320418	6.852615741	6.629892
6.894282407	3.746778	6.894282407	6.207532
6.935949074	3.943445	6.935949074	6.731417
6.977615741	3.363937	6.977615741	5.485258
7.019282407	2.890475	7.019282407	6.309702
7.060949074	3.262214	7.060949074	5.860365
7.102615741	2.954454	7.102615741	5.880978
7.144282407	2.153391	7.144282407	5.84526
7.185960648	2.378666	7.185960648	5.735903

Time (Glucose Feedback Control) (Elapsed Days)	Raman Glucose Feedback Concentration (g/L)	Time (Glucose Bolus Feed) (Elapsed Days)	Raman Bolus Feed Concentration (g/L)
7.227662037	2.9512	7.227662037	5.541218
7.269293981	3.551366	7.269293981	5.192567
7.310960648	3.218829	7.310960648	9.177272
7.352627315	3.12968	7.352627315	8.703374
7.394293981	2.593928	7.394293981	8.983128
7.435960648	2.394028	7.435960648	8.965026
7.477627315	2.21824	7.477627315	8.120359
7.519293981	3.134434	7.519293981	8.137175
7.560960648	2.766007	7.560960648	8.314145
7.602627315	2.512249	7.602627315	8.698809
7.644305556	2.630357	7.644305556	8.641541
7.685972222	2.416168	7.685972222	8.071362
7.727638889	2.661644	7.727638889	8.489848
7.769305556	2.79807	7.769305556	8.062885
7.810960648	2.972875	7.810960648	7.448528
7.852638889	2.41065	7.852638889	8.106278
7.894305556	2.495323	7.894305556	7.770178
7.935972222	2.934737	7.935972222	8.291804
7.977638889	2.847816	7.977638889	7.42387
8.01931713	3.15902	8.01931713	8.205845
8.060983796	3.667069	8.060983796	7.910364
8.102638889	3.282952	8.102638889	7.724277
8.14431713	2.793275	8.14431713	7.616001
8.185983796	2.452958	8.185983796	7.379514
8.227650463	2.630365	8.227650463	7.477386
8.26931713	2.729709	8.26931713	6.807137
8.310983796	2.807003	8.310983796	6.842168
8.352650463	2.620657	8.352650463	9.308379
8.39431713	3.13093	8.39431713	8.968605
8.436030093	2.627208	8.436030093	9.14572
8.477662037	2.251114	8.477662037	8.747909
8.51931713	2.646687	8.51931713	8.726134
8.56099537	3.079137	8.56099537	8.391006
8.602662037	2.563705	8.602662037	8.450653
8.644328704	3.087527	8.644328704	7.990832
8.68599537	2.590317	8.68599537	8.18066

Time (Glucose Feedback Control) (Elapsed Days)	Raman Glucose Feedback Concentration (g/L)	Time (Glucose Bolus Feed) (Elapsed Days)	Raman Bolus Feed Concentration (g/L)
8.727662037	2.968817	8.727662037	7.942457
8.769340278	3.12238	8.769340278	7.713663
8.811006944	3.547524	8.811006944	8.415674
8.852673611	4.297379	8.852673611	7.626019
8.894340278	4.161104	8.894340278	8.069413
8.936018519	5.030762	8.936018519	8.045293
8.977673611	5.637126	8.977673611	8.527124
9.019351852	5.298599	9.019351852	7.610373
9.061006944	4.932112	9.061006944	7.099549
9.102685185	5.059932	9.102685185	7.573514
9.144351852	4.555223	9.144351852	7.538042
9.186018519	4.263374	9.186018519	7.441958
9.227685185	4.428963	9.227685185	7.639114
9.269351852	4.978399	9.269351852	6.761559
9.311018519	5.80515	9.311018519	7.284119
9.352685185	5.421699	9.352685185	7.794689
9.394351852	5.041867	9.394351852	9.245949
9.436018519	4.245652	9.436018519	10.85137
9.477685185	4.627719	9.477685185	10.59078
9.519363426	5.043918	9.519363426	10.01031
9.561030093	5.134606	9.561030093	9.805758
9.602696759	4.84806	9.602696759	10.12079
9.644398148	3.838338	9.644398148	10.16871
9.686030093	4.53542	9.686030093	9.679668
9.727696759	4.92595	9.727696759	9.62599
9.769351852	4.769973	9.769351852	9.378336
9.811030093	5.17225	9.811030093	10.05829
9.852696759	4.80986	9.852696759	8.640112
9.894363426	5.148977	9.894363426	9.457369
9.936030093	4.672589	9.936030093	9.403243
9.977708333	4.188494	9.977708333	9.422581
10.019375	4.707168	10.019375	9.496971
10.06104167	4.721385	10.06104167	8.947212
10.10269676	4.783384	10.10269676	8.878696
10.144375	4.512029	10.144375	9.005632
10.18604167	4.258463	10.18604167	8.788143

Time (Glucose Feedback Control) (Elapsed Days)	Raman Glucose Feedback Concentration (g/L)	Time (Glucose Bolus Feed) (Elapsed Days)	Raman Bolus Feed Concentration (g/L)
10.22770833	4.029292	10.22770833	8.814812
10.269375	4.322887	10.269375	8.966389
10.31104167	4.08165	10.31104167	8.892519
10.35265046	4.958148	10.35265046	9.361223
10.394375	5.847916	10.394375	8.628824
10.43604167	6.32333	10.43604167	8.199861
10.47770833	6.265306	10.47770833	7.797361
10.51938657	5.801625	10.51938657	8.34846
10.56104167	5.735916	10.56104167	9.992476
10.60271991	5.45328	10.60271991	10.55201
10.64438657	5.33565	10.64438657	10.78163
10.68605324	5.542859	10.68605324	10.40103
10.72771991	5.033404	10.72771991	9.900923
10.76938657	4.913043	10.76938657	9.858058
10.81105324	5.076824	10.81105324	10.93733
10.85275463	4.666098	10.85275463	10.56453
10.89439815	4.554989	10.89439815	10.63292
10.93605324	4.729548	10.93605324	10.1317
10.97771991	4.089445	10.97771991	10.15173
11.01938657	3.973743	11.01938657	10.03745
11.06105324	4.564354	11.06105324	9.908442
11.10273148	4.511001	11.10273148	9.87036
11.14439815	5.108614	11.14439815	10.1959
11.18606481	4.441917	11.18606481	9.519185
11.22773148	4.69673	11.22773148	9.621466
11.26939815	4.755281	11.26939815	10.03958
11.31106481	4.227083	11.31106481	8.765776
11.35273148	4.190309		
11.39439815	4.416976		
11.43606481	4.467027		
11.47773148	5.739811		
11.51939815	5.667678		
11.56107639	5.399963		
11.60273148	5.114323		
11.64440972	5.493369		
11.68607639	4.566129		

Time (Glucose Feedback Control) (Elapsed Days)	Raman Glucose Feedback Concentration (g/L)	Time (Glucose Bolus Feed) (Elapsed Days)	Raman Bolus Feed Concentration (g/L)
11.72774306	4.238223		
11.76940972	4.256388		
11.81107639	3.624721		
11.85274306	4.105767		
11.89440972	5.08095		
11.93607639	5.102737		
11.97775463	5.012239		

FIG. 8 shows the antibody titer for a feedback controlled continuous nutrient feed and for a bolus nutrient feed. As can be seen in FIG. 8, antibody production is unaffected by either method. Tables 4 and 5 below show the bolus fed antibody titer and feedback control antibody titer data points, respectively, for FIG. 8.

TABLE 4: BOLUS FED ANTIBODY TITER DATA POINTS FOR FIG. 8

Bolus Feed Time (Elapsed Days)	Bolus feed Ab Titer (mg/L)	Bolus Fed Normalized Ab Titer
0	0.866	0.000721667
0.831180556	2.362	0.001968333
1.668321759	#N/A	#N/A
2.614583333	32.606	0.027171667
3.625787037	89.425	0.074520833
4.531863426	148.02	0.12335
5.726122685	301.873	0.251560833
6.67775463	421.186	0.350988333
7.65849537	519.165	0.4326375
8.641284722	670.959	0.5591325
9.714537037	#N/A	#N/A
10.66090278	#N/A	#N/A
11.64418981	#N/A	#N/A
12.62819444	1158.82	0.965683333

TABLE 5: FEEDBACK CONTROL ANTIBODY TITER DATA POINTS FOR FIG. 8

Feedback Control Time	Feedback Control Ab Titer	Feedback Control Normalized Ab Titer
0	#N/A	#N/A
0.753171296	2.556	0.00213
1.749884259	15.36	0.0128
2.757048611	48.048	0.04004
3.710439815	105.017	0.087514167
4.757465278	205.669	0.171390833
5.814016204	#N/A	#N/A
6.735243056	423.018	0.352515
7.729918981	543.108	0.45259
8.767893519	683.645	0.569704167
9.742418981	795.66	0.66305
10.70917824	913.834	0.761528333
11.73123843	1034.809	0.862340833
12.79594907	1134.383	0.945319167

FIG. 9 shows the normalized percentage of PTM as a result of glucose concentration. As can be seen from FIG. 9, there is a decrease in PTM as the glucose concentration decreases from about 6 g/L – 8 g/L (set point for bolus-fed harvest) to 5 g/L (set point 2) to 3 g/L (set point 1). In other words, lower exposure to nutrients results in a decrease in PTM. The data points in FIG. 9 for the normalized percentage of PTM are shown in Table 6 below.

TABLE 6: NORMALIZED % PTM DATA POINTS FOR FIG. 9

Condition	% Post Translational Modification	Normalized % Post Translational Modification
Day -1 of SP Increase	12.03	0.401
Day 0 of SP Increase	11.79	0.393
Day 1 of SP Increase	14.88	0.496
Day 2 of SP Increase	16.48	0.549333333
Day 3 of SP Increase	17.58	0.586
Day 4 of SP Increase	20.63	0.687666667
Bolus-Fed Harvest	27.2	0.906666667

FIG. 10 shows the glucose concentrations for a feedback controlled continuous nutrient feed in accordance with the present invention and for a bolus nutrient feed. As can be seen by FIG. 10, the methods of the present invention are able to provide reduced, steady

concentrations of glucose. The data points in FIG. 10 for the glucose concentrations are shown in Table 7 below.

TABLE 7: GLUCOSE CONCENTRATION DATA POINTS FOR FIG. 10

Time (hrs) Feedback Control	Glucose Concentration Feedback Control (g/L)	Time (hrs) Bolus Fed	Glucose Concentration Bolus Fed (g/L)
0	5.29985	0	3.9606
0.443888889	3.95717	0.443888889	3.92564
0.888055556	3.87786	0.888055556	3.82241
1.331944444	3.94245	1.554444444	3.84826
1.554444444	3.88536	1.998333333	3.78432
1.998333333	3.88327	2.442222222	3.81402
2.442222222	3.84436	4.382222222	3.83029
2.886111111	3.7485	5.334444444	3.75084
3.589444444	6.98909	6.226666667	3.80185
4.157777778	3.83584	7.119166667	3.72134
5.11	3.78798	8.011388889	3.68723
6.0025	3.7856	8.900833333	3.71741
6.894722222	3.73533	20.45444444	3.40678
7.787222222	3.68673	30.1175	4.74804
8.679444444	3.66978	31.01027778	4.80446
20.23388889	3.40307	31.90277778	4.76064
21.12222222	3.40884	32.79527778	4.69968
21.56944444	3.37754	33.68777778	4.7881
29.72194444	3.11293	43.51138889	4.50823
30.78583333	3.15921	44.40333333	4.44888
31.67833333	3.08833	45.295	4.56108
32.57111111	2.95089	46.18777778	4.44496
33.46333333	3.04687	47.07722222	4.43893
34.35305556	2.90941	56.46	4.27974
43.2875	2.92864	57.35194444	4.30659
44.17888889	2.81226	58.24444444	4.29294
45.07138889	2.85354	59.13638889	4.18843
45.96333333	2.83553	60.02611111	4.13743
46.85583333	2.79272	69.4075	4.95997
56.23555556	2.67934	71.02194444	4.9194
57.12805556	2.67136	71.46583333	4.41552
58.02	2.57063	71.90972222	4.38365
58.91194444	2.54624	72.35361111	4.42239
59.80472222	2.50303	73.02027778	4.31899

Time (hrs) Feedback Control	Glucose Concentration Feedback Control (g/L)	Time (hrs) Bolus Fed	Glucose Concentration Bolus Fed (g/L)
69.18361111	2.97555	73.46416667	4.37885
70.07583333	3.77294	73.90805556	4.3449
70.80111111	4.34847	74.35194444	4.23448
71.46583333	4.08935	75.01861111	4.24824
71.90972222	4.00212	75.4625	4.14202
72.35361111	3.99123	75.90638889	4.14761
72.7975	4.01331	76.35027778	4.07654
73.02027778	3.99191	77.01694444	4.04303
73.46416667	3.91424	77.46083333	4.10848
73.90805556	3.85688	77.90472222	4.02519
74.35194444	3.84475	78.34861111	3.97673
74.79583333	3.67941	79.01527778	3.97045
75.01861111	3.64752	79.45916667	3.99019
75.4625	3.66484	79.90305556	3.90772
75.90638889	3.6525	80.34694444	4.13212
76.35027778	3.55085	81.01361111	3.94071
76.79416667	3.45215	81.4575	3.93964
77.01694444	3.42771	81.90138889	3.93305
77.46083333	3.5292	82.34527778	3.90002
77.90472222	3.47243	83.01194444	3.78135
78.34861111	3.48275	83.45583333	3.80974
78.7925	3.44748	83.89972222	3.72092
79.01527778	3.51503	84.34361111	3.54584
79.45916667	3.40908	85.01055556	3.79766
79.90305556	3.4091	85.45472222	3.73607
80.34694444	3.40949	85.89861111	3.6327
80.79083333	3.37424	86.34277778	3.60241
81.01361111	3.66927	87.01	3.64506
81.4575	3.40708	87.45416667	3.4821
81.90138889	3.29053	87.89805556	3.49399
82.34527778	3.33054	88.34194444	3.50496
82.78916667	3.3244	89.00888889	3.53164
83.01194444	3.2331	89.45305556	3.31505
83.45583333	3.24332	89.89722222	3.27601
83.89972222	3.39759	90.34111111	3.33213
84.34361111	3.15861	91.00805556	3.43951
84.78777778	3.22317	91.45222222	3.38503

Time (hrs) Feedback Control	Glucose Concentration Feedback Control (g/L)	Time (hrs) Bolus Fed	Glucose Concentration Bolus Fed (g/L)
85.01055556	3.24632	91.89611111	3.1468
85.45472222	3.31019	92.34027778	3.4265
85.89861111	3.17534	93.00694444	3.24971
86.34277778	3.14291	93.45083333	3.19635
86.78694444	3.11793	93.895	3.27543
87.01	3.16349	94.33888889	3.09075
87.45388889	3.0751	95.24694444	2.49991
87.89805556	2.9869	95.69111111	2.57693
88.34194444	3.00619	96.135	2.5465
88.78583333	2.95103	96.57916667	4.02104
89.00888889	3.05399	97.02305556	3.98664
89.45305556	2.81784	97.46722222	3.95544
89.89694444	2.94564	97.91138889	3.86852
90.34111111	2.82913	98.35527778	3.66631
90.785	2.83378	99.0225	3.62051
91.00805556	2.91134	99.46638889	3.76868
91.45222222	3.09505	99.91027778	3.69577
91.89611111	2.86231	100.3544444	3.74638
92.34027778	2.95479	101.0216667	3.61072
92.78416667	2.84231	101.4655556	3.65232
93.00694444	2.81938	101.9094444	3.65673
93.45083333	2.79815	102.3536111	3.50981
93.895	2.83839	103.0205556	3.59905
94.33888889	2.93334	103.4647222	3.50056
95.02611111	2.94485	103.9086111	3.58028
95.69083333	3.01962	104.3525	3.51239
96.135	3.08518	105.0194444	3.35906
96.57888889	2.90996	105.4636111	3.46452
97.02305556	2.822	105.9077778	3.4217
97.46722222	2.60949	106.3516667	3.52777
97.91111111	2.98458	107.0186111	3.37968
98.35527778	2.99921	107.4627778	3.24786
98.79944444	2.89195	107.9066667	3.17432
99.02222222	2.88476	108.3508333	3.26832
99.46638889	2.80296	109.0180556	3.09402
99.91027778	2.81875	109.4619444	3.19621
100.3544444	2.88799	109.9061111	3.15208

Time (hrs) Feedback Control	Glucose Concentration Feedback Control (g/L)	Time (hrs) Bolus Fed	Glucose Concentration Bolus Fed (g/L)
100.7986111	2.7446	110.3502778	3.08408
101.0213889	2.71513	111.0169444	3.12704
101.4655556	2.62124	111.4611111	3.09169
101.9094444	2.7469	111.905	3.13017
102.3536111	2.6358	112.3488889	3.10825
102.7977778	2.64662	113.0161111	3.05118
103.0205556	2.64383	113.4602778	2.96148
103.4644444	2.48012	113.9041667	3.13752
103.9086111	2.56149	114.3483333	3.07076
104.3525	2.61773	115.0152778	2.97416
104.7966667	2.58291	115.4594444	3.11854
105.0194444	2.49816	115.9033333	3.01764
105.4636111	2.46984	117.2877778	6.00949
105.9075	2.5008	117.7316667	5.96736
106.3516667	2.47808	118.1758333	5.92612
106.7955556	2.24744	118.6194444	5.64293
107.0186111	2.57076	119.2863889	5.49402
107.4625	2.47027	119.7302778	5.43498
107.9066667	2.43396	120.1741667	5.47254
108.3508333	2.43259	120.6180556	5.28723
108.7947222	2.4977	121.2847222	5.26741
109.0177778	2.38829	121.7286111	5.17114
109.4619444	2.34725	122.1725	5.22748
109.9058333	2.22657	122.6163889	5.18455
110.35	2.27469	123.2830556	5.05853
110.7941667	2.3519	123.7269444	5.09368
111.0169444	2.28667	124.1708333	5.06618
111.4608333	2.29553	124.6147222	4.92785
111.905	2.30401	125.2813889	4.95126
112.3488889	2.1131	125.7252778	5.12272
112.7930556	2.05542	126.1694444	5.04657
113.0158333	2.15201	126.6133333	4.89878
113.46	2.15773	127.28	4.89227
113.9041667	2.1462	127.7236111	4.83168
114.3480556	2.0095	128.1675	4.73809
114.7922222	2.00685	128.6113889	4.62723
115.015	2.08611	129.2783333	4.56662

Time (hrs) Feedback Control	Glucose Concentration Feedback Control (g/L)	Time (hrs) Bolus Fed	Glucose Concentration Bolus Fed (g/L)
115.4591667	2.23016	129.7222222	4.5413
115.9033333	1.89489	130.1661111	4.39996
116.3475	2.03546	130.61	4.36069
117.0672222	2.11907	131.2766667	4.47573
117.7316667	2.10383	131.7205556	4.19303
118.1755556	1.91726	132.1644444	4.17655
118.6194444	1.93228	132.6083333	4.24852
119.0636111	1.78201	133.275	4.07631
119.2863889	1.90199	133.7188889	4.01898
119.7302778	1.76972	134.1627778	3.97811
120.1741667	1.81882	134.6066667	3.7236
120.6180556	1.90338	135.2736111	3.78111
121.0619444	1.86254	135.7177778	3.82847
121.2847222	1.89595	136.1613889	3.56015
121.7286111	1.95022	136.6052778	3.56488
122.1725	2.03028	137.2722222	3.59907
122.6163889	2.02368	137.7158333	3.53736
123.0602778	1.80358	138.1597222	3.51143
123.2830556	1.86305	138.6036111	3.48144
123.7269444	1.68852	139.2705556	3.69714
124.1708333	2.16485	139.7144444	3.53598
124.6147222	2.68219	140.1583333	3.56975
125.0586111	3.84445	140.6022222	3.46682
125.2813889	3.75849	141.5097222	3.27107
125.7252778	3.05046	141.9536111	3.37317
126.1691667	1.60889	142.3975	3.19992
126.6133333	1.55251	142.8413889	3.29018
127.0569444	1.49635	143.285	5.29681
127.28	1.4625	143.7288889	5.42912
127.7238889	1.5599	144.1730556	5.31815
128.1675	1.411	144.6169444	5.49514
128.6113889	1.59737	145.2836111	5.31922
129.0555556	1.49927	145.7275	5.50698
129.2783333	1.55528	146.1713889	5.40168
129.7222222	1.68831	146.6152778	5.21572
130.1661111	1.65586	147.2819444	5.22277
130.61	1.69803	147.7258333	5.32597

Time (hrs) Feedback Control	Glucose Concentration Feedback Control (g/L)	Time (hrs) Bolus Fed	Glucose Concentration Bolus Fed (g/L)
131.0538889	1.51503	148.1697222	5.25509
131.2766667	1.62337	148.6133333	5.18307
131.7205556	1.56305	149.0683333	5.08164
132.1644444	1.53581	149.3183333	4.88397
132.6083333	1.39492	149.5683333	5.06794
133.0522222	1.35263	149.8183333	5.01549
133.275	1.2922	150.0686111	4.91031
133.7188889	1.21502	150.3186111	4.92284
134.1627778	1.38027	150.5686111	4.88071
134.6066667	1.30947	150.8186111	4.90576
135.0505556	1.3538	151.0686111	4.7337
135.2736111	1.36581	151.3186111	4.98071
135.7175	1.19768	151.5686111	4.66753
136.1613889	1.41395	151.8186111	4.73602
136.6052778	1.08014	152.2625	4.67663
137.0494444	1.32496	152.7066667	4.66436
137.2719444	1.34268	153.1505556	4.79716
137.7158333	1.45098	153.5947222	4.70976
138.16	1.3088	154.0388889	4.68658
138.6038889	1.39873	154.4827778	4.45627
139.0475	1.36488	154.9269444	4.69575
139.2705556	1.19001	155.3711111	4.61841
139.7144444	1.40293	156.0380556	4.58039
140.1583333	1.41103	156.4822222	4.6775
140.6022222	1.5462	156.9263889	4.4771
141.2888889	2.01927	157.3702778	4.35384
141.9536111	2.42777	158.0372222	4.4401
142.3975	2.63074	158.4811111	4.56737
142.8413889	2.83209	158.9252778	4.42704
143.285	2.72224	159.3691667	4.07445
143.7288889	2.63608	160.0361111	4.36575
144.1730556	2.69195	160.4802778	4.13995
144.6169444	2.71345	160.9241667	4.22379
145.0608333	2.50984	161.3680556	4.17469
145.2836111	2.6369	162.035	4.28975
145.7275	2.60541	162.4788889	4.13539
146.1713889	2.67274	162.9230556	3.87281

Time (hrs) Feedback Control	Glucose Concentration Feedback Control (g/L)	Time (hrs) Bolus Fed	Glucose Concentration Bolus Fed (g/L)
146.6152778	2.69351	163.3672222	4.87836
147.0591667	2.50699	164.0338889	5.2242
147.2819444	2.68272	164.4780556	5.24807
147.7258333	2.80848	165.165	5.03418
148.1697222	2.71963	165.8297222	4.81739
148.6133333	3.27574	166.2736111	4.73886
152.2625	1.84522	166.7177778	4.87246
152.7066667	2.02054	167.1616667	4.77461
153.1505556	1.89572	167.6058333	4.68469
153.5947222	1.7493	168.2725	4.5802
154.0386111	1.82994	168.7166667	4.5102
154.4827778	2.03299	169.1608333	4.70917
154.9269444	1.84201	169.6047222	4.54906
155.3708333	2.33961	170.2716667	4.58545
155.815	2.17287	170.7155556	4.46504
156.0380556	2.09251	171.1597222	4.47254
156.4822222	2.00326	171.6036111	4.42642
156.9263889	2.00972	172.2705556	4.48492
157.3702778	1.95632	172.7147222	4.27087
157.8144444	1.85693	173.1586111	4.16092
158.0372222	1.87511	173.6027778	4.23464
158.4811111	2.25587	174.2697222	4.18793
158.9252778	2.41394	174.7138889	4.17626
159.3691667	2.27275	175.1580556	4.12183
159.8133333	2.33431	175.6022222	4.31591
160.0361111	2.11631	176.2691667	3.96654
160.48	2.15315	176.7130556	3.86951
160.9241667	2.21482	177.1572222	4.05681
161.3680556	2.10691	177.6013889	3.80757
161.8119444	1.9879	178.2683333	3.88444
162.0347222	2.07513	178.7122222	3.7184
162.4788889	2.09918	179.1563889	3.76801
162.9230556	2.045	179.6002778	3.65193
163.3669444	2.0579	180.2672222	3.8665
163.8111111	1.9786	180.7113889	3.60753
164.0338889	2.04415	181.1552778	3.56228
164.4780556	2.11519	181.5994444	3.51562

Time (hrs) Feedback Control	Glucose Concentration Feedback Control (g/L)	Time (hrs) Bolus Fed	Glucose Concentration Bolus Fed (g/L)
164.9219444	2.04256	182.2663889	3.53538
165.8297222	1.92716	182.7105556	3.58554
166.2736111	1.74054	183.1544444	3.52299
166.7177778	2.17775	183.5986111	3.50055
167.1616667	2.21902	184.2655556	3.35449
167.6055556	2.23581	184.7097222	3.15678
168.0497222	2.1295	185.1536111	3.49221
168.2725	2.06408	185.5977778	3.31856
168.7166667	1.95822	186.2644444	3.1794
169.1608333	1.87785	186.7086111	3.261
169.6047222	2.38464	187.1525	3.26585
170.0486111	2.52549	187.5963889	3.11678
170.2716667	2.48755	188.0405556	3.29677
170.7155556	2.39386	188.4847222	3.13789
171.1594444	2.26082	188.9288889	3.04174
171.6036111	2.10124	189.8586111	2.84437
172.0477778	2.04631	190.7886111	2.97215
172.2705556	1.96783	191.7183333	2.74657
172.7144444	2.03789	192.6480556	2.85061
173.1586111	1.96485	193.5780556	2.71859
173.6025	1.75977	194.5077778	2.64369
174.0466667	2.13635	195.4377778	2.23807
174.2697222	2.35361	196.3675	2.16861
174.7138889	2.19967	197.2975	2.18502
175.1577778	2.2276	198.2275	2.02487
175.6019444	2.26713	199.1572222	2.00279
176.0461111	2.27076	200.0861111	2.05927
176.2688889	2.08234	201.0158333	1.77877
176.7130556	2.05613	201.9455556	3.21063
177.1569444	1.98094	202.8752778	5.70505
177.6011111	2.09971	203.8047222	5.55309
178.0452778	2.13739	204.7341667	5.62934
178.2683333	1.81014	205.6636111	5.40796
178.7122222	2.33795	206.5933333	5.26706
179.1561111	2.27909	207.5230556	5.24844
179.6002778	2.13411	208.4522222	5.04861
180.0441667	2.28842	208.8961111	4.9106

Time (hrs) Feedback Control	Glucose Concentration Feedback Control (g/L)	Time (hrs) Bolus Fed	Glucose Concentration Bolus Fed (g/L)
180.2672222	2.3228	209.3402778	4.83827
180.7113889	2.20826	209.7844444	5.05838
181.1552778	2.1662	210.2286111	4.83412
181.5991667	1.97546	210.6725	4.76257
182.0433333	2.11621	211.1166667	4.64707
182.2661111	2.07917	211.7836111	4.80408
182.7102778	1.95	212.2275	4.53231
183.1544444	2.00555	212.6716667	4.68255
183.5983333	2.1972	213.1155556	4.661
184.0425	1.99805	213.7827778	4.53894
184.2652778	1.90735	214.2266667	4.38914
184.7094444	2.07147	214.6705556	4.51892
185.1536111	2.30457	215.1147222	4.35161
185.5975	1.94533	215.7816667	4.2933
186.0416667	2.04383	216.2255556	4.2022
186.2644444	2.02201	216.6697222	4.14232
186.7086111	2.00486	217.1136111	4.19824
187.1525	1.87491	217.7808333	3.98641
187.5963889	1.71041	218.225	4.17967
188.0405556	2.27353	218.6688889	4.12755
188.4844444	2.27361	219.1130556	3.98162
188.9286111	2.21939	219.7797222	4.18885
189.6158333	2.32112	220.2238889	3.99614
190.5455556	2.23684	220.6680556	3.88445
191.4752778	2.00438	221.1122222	4.00875
192.4052778	2.08773	221.7794444	4.02466
193.335	1.98721	222.2233333	4.92433
194.2647222	2.34499	222.6675	5.31792
195.1947222	2.07045	223.1116667	5.10258
196.1247222	1.87379	223.7786111	5.18651
197.9844444	2.44455	224.2227778	5.33129
198.9144444	1.43529	224.6669444	5.31647
199.8438889	2.10835	225.1111111	5.22186
200.7730556	2.16165	225.7780556	5.09756
201.7027778	2.03911	226.2219444	5.0919
202.6325	2.02224	226.6661111	5.09598
203.5619444	2.04709	227.1102778	5.20148

Time (hrs) Feedback Control	Glucose Concentration Feedback Control (g/L)	Time (hrs) Bolus Fed	Glucose Concentration Bolus Fed (g/L)
204.4916667	1.74866	227.7775	5.27139
205.4205556	2.42807	228.2213889	5.17647
206.3502778	2.3646	228.6655556	4.97104
207.28	2.29919	229.1097222	4.95102
208.2313889	2.37703	229.7766667	5.02617
208.8961111	2.39499	230.2208333	4.89217
209.3402778	1.97051	230.6647222	5.06075
209.7841667	2.24512	231.1088889	4.91127
210.2283333	2.25347	231.7758333	4.75924
210.6725	2.08371	232.6836111	4.86344
211.1166667	2.15365	233.1275	4.66869
211.5605556	2.29691	233.5713889	4.77352
211.7833333	2.03092	234.0155556	4.63601
212.2275	1.97129	234.4597222	4.71014
212.6716667	1.9721	234.9038889	4.69685
213.1155556	2.07924	235.3477778	4.83778
213.5597222	1.93054	235.7919444	4.73268
213.7825	2.09871	236.2358333	4.72232
214.2266667	2.01653	236.6797222	4.70191
214.6705556	1.97157	237.1238889	4.61924
215.1147222	2.08205	237.7908333	5.82279
215.5586111	2.20945	238.2347222	5.95289
215.7816667	1.90401	238.6788889	5.7376
216.2255556	2.25764	239.1227778	5.39835
216.6697222	2.20062	239.7897222	5.55047
217.1136111	2.38191	240.2336111	5.45566
217.5577778	2.30704	240.6777778	5.56575
217.7808333	2.3666	241.1219444	5.37954
218.225	2.21814	241.7888889	5.28663
218.6688889	2.22546	242.2330556	5.22091
219.1130556	2.29399	242.6769444	5.31419
219.5569444	2.35247	243.1211111	5.269
219.7797222	2.36244	243.7880556	5.33359
220.2238889	2.42202	244.2322222	5.20919
220.6677778	3.90842	244.6763889	5.16646
221.1119444	3.226	245.1202778	4.87647
221.5561111	3.24104	245.7872222	5.19865

Time (hrs) Feedback Control	Glucose Concentration Feedback Control (g/L)	Time (hrs) Bolus Fed	Glucose Concentration Bolus Fed (g/L)
221.7791667	3.44121	246.2313889	5.26332
222.2233333	2.10021	246.6755556	5.27455
222.6675	1.65588	247.1194444	4.9051
223.1116667	2.00054	247.7863889	4.96193
223.5555556	2.2584	248.2302778	4.95473
223.7786111	2.18337	248.6744444	4.87265
224.2227778	2.22002	249.1186111	4.88933
224.6666667	2.02996	249.7855556	4.95339
225.1108333	2.11005	250.2297222	4.91535
225.555	1.98403	250.6738889	4.8415
225.7780556	1.97535	251.1180556	4.73406
226.2219444	2.1047	251.785	4.75863
226.6661111	2.14528	252.2291667	4.83177
227.1102778	2.11167	252.6733333	4.73776
227.5541667	1.96546	253.1175	4.80804
227.7772222	2.1583	253.7841667	4.47607
228.2213889	2.09114	254.2283333	4.44379
228.6655556	2.03119	254.6722222	4.61578
229.1097222	2.03169	255.1163889	4.35294
229.5536111	1.805	255.7833333	4.35565
229.7766667	1.75306	256.4702778	4.63822
230.2208333	2.03753	257.4	4.1795
230.6647222	1.98862	258.3291667	4.3277
231.1088889	1.85836	259.2588889	4.10085
231.5530556	1.81241	260.1886111	4.15495
231.7758333	1.83977	261.1183333	3.90911
232.4627778	1.76471	262.0477778	3.81073
233.1275	1.63967	262.9772222	3.87842
233.5713889	1.79819	263.9061111	5.04643
234.0155556	1.74429	264.8347222	4.97527
234.4594444	1.77757	265.7641667	4.93942
234.9036111	1.82093	266.6936111	4.81825
235.3477778	1.75825	267.6225	4.80283
235.7919444	1.71644	268.2875	4.75164
236.2358333	1.64919	268.7313889	4.93642
236.6797222	1.65067	269.1755556	4.75401
237.1238889	1.59211	269.6197222	4.51092

Time (hrs) Feedback Control	Glucose Concentration Feedback Control (g/L)	Time (hrs) Bolus Fed	Glucose Concentration Bolus Fed (g/L)
237.5677778	2.09602	270.2866667	4.5984
237.7905556	2.0281	270.7308333	4.54899
238.2347222	2.0728	271.1747222	4.70999
238.6786111	1.96003	271.6188889	4.4307
239.1227778	2.13435	272.2858333	4.3134
239.5666667	2.14529	272.7297222	4.46242
239.7897222	2.12039	273.1738889	4.44403
240.2336111	2.1226	273.6177778	4.31874
240.6777778	2.17822	274.2847222	4.43482
241.1216667	2.09458	274.7286111	4.22428
241.5658333	1.93963	275.1727778	4.50794
241.7888889	1.78058	275.6166667	4.37905
242.2327778	1.92457	276.2836111	4.28183
242.6769444	2.54728	276.7277778	4.36293
243.1208333	2.7696	277.1716667	4.06209
243.565	2.96879	277.6155556	4.27271
243.7880556	3.0983	278.2825	4.05231
244.2322222	2.44977	278.7266667	4.19835
244.6761111	3.0513	279.1705556	4.10201
245.1202778	4.46037	279.6147222	4.0479
245.5641667	3.64992	280.2816667	4.14879
245.7872222	2.63717	280.7258333	4.01384
246.2311111	2.23246	281.1697222	3.94503
246.6752778	1.96177	281.6138889	3.82963
247.1194444	1.9733	282.2808333	4.01967
247.5633333	1.92291	282.725	4.08182
247.7863889	1.9421	283.1688889	3.83589
248.2302778	2.29655	283.6130556	3.8807
248.6744444	2.15675	284.28	3.60671
249.1186111	2.06017	284.7238889	3.74206
249.5625	1.83718	285.1680556	3.61191
249.7855556	2.26354	285.6119444	3.64284
250.2297222	2.15135	286.2788889	3.49373
250.6736111	2.13613	286.7227778	3.75384
251.1177778	2.01012	287.1666667	5.50193
251.5619444	1.91997	287.6108333	5.36619
251.785	2.04497	288.2777778	5.44722

Time (hrs) Feedback Control	Glucose Concentration Feedback Control (g/L)	Time (hrs) Bolus Fed	Glucose Concentration Bolus Fed (g/L)
252.2288889	1.76215	288.7219444	5.23718
252.6730556	1.91976	289.1661111	5.48611
253.1172222	2.17963	289.61	5.29237
253.5613889	2.49015	290.2769444	5.09807
253.7841667	2.31233	290.7211111	5.27902
254.2280556	2.25077	291.165	5.21127
254.6722222	2.41304	291.8522222	4.93468
255.1161111	2.32947	292.5169444	5.3445
255.5602778	2.27885	292.9611111	4.9385
255.7833333	1.94173	293.4052778	5.0282
256.2272222	2.39855	293.8491667	4.92491
257.1572222	1.97358	294.2933333	4.94234
258.0863889	2.13599	294.7375	5.14301
259.0161111	2.20439	295.1813889	5.09006
259.9458333	2.07312	295.6252778	4.97926
260.8752778	2.35689	296.2922222	4.79825
261.8052778	2.16814	296.7363889	4.98856
262.7347222	2.00509	297.1802778	4.64638
263.6638889	2.06753	297.6241667	4.83557
264.5925	1.85036	298.2913889	4.66544
265.5213889	2.46909	298.7352778	4.46933
266.4508333	2.44871	299.1794444	4.42644
267.3797222	2.44656	299.6233333	4.40905
268.2872222	2.48505	300.2902778	4.48562
268.7313889	2.63435	300.7344444	4.29635
269.1755556	3.24711	301.1786111	4.21742
269.6194444	2.23888	301.6227778	4.5645
270.0636111	2.07904	302.2894444	4.37116
270.2863889	2.21563	302.7333333	4.30076
270.7305556	1.91896	303.1775	4.357
271.1747222	2.09629	303.6216667	4.19275
271.6188889	2.04491	304.2888889	4.3476
272.0627778	1.96894	304.7327778	4.12702
272.2858333	2.10447	305.1766667	4.22847
272.7297222	1.98481	305.6208333	4.14018
273.1736111	1.88517	306.2877778	3.91622
273.6177778	2.02339	306.7319444	4.0101

Time (hrs) Feedback Control	Glucose Concentration Feedback Control (g/L)	Time (hrs) Bolus Fed	Glucose Concentration Bolus Fed (g/L)
274.0616667	2.1536	307.1758333	4.08905
274.2844444	1.94488	307.62	3.69736
274.7286111	2.09537	308.2866667	3.89877
275.1725	1.94546	308.7308333	3.91969
275.6166667	1.97124	309.1747222	3.95032
276.0605556	2.10351	309.6188889	3.83547
276.2833333	2.15169	310.2858333	5.15943
276.7275	2.06851	310.73	4.85061
277.1713889	1.95511	311.1738889	4.90129
277.6155556	2.17411	311.6177778	4.69627
278.0594444	1.91116	312.2847222	4.98669
278.2825	1.89503	312.7286111	4.99629
278.7263889	2.13133	313.1727778	4.92192
279.1705556	2.23375	313.6166667	4.91592
279.6147222	2.07922	314.2838889	4.79179
280.0588889	2.15941	314.7277778	4.82191
280.2816667	2.10306	315.1719444	4.62895
280.7258333	2.09977	315.6158333	4.68028
281.1697222	1.90922	316.5236111	4.39498
281.6138889	1.97935	316.9675	4.51145
282.0577778	1.98323	317.4116667	4.64399
282.2808333	2.13178	317.8558333	4.35246
282.725	2.05535	318.5230556	4.39085
283.1688889	2.17687	318.9669444	4.51255
283.6130556	2.10914	319.4111111	4.26767
284.0569444	1.88863	319.855	4.41338
284.28	1.90439	320.5225	4.04934
284.7238889	2.27687	320.9663889	4.54584
285.1680556	2.27819	321.4105556	4.21321
285.6119444	1.97363	321.8547222	4.26114
286.0561111	2.474	322.5219444	4.16462
286.2788889	2.08995	322.9658333	4.03369
286.7227778	2.25392	323.41	4.07753
287.1666667	2.16887	323.8541667	4.06638
287.6108333	2.53164	324.5213889	4.03094
288.0547222	2.19634	324.9652778	4.01644
288.2777778	2.18478	325.4094444	4.21972

Time (hrs) Feedback Control	Glucose Concentration Feedback Control (g/L)	Time (hrs) Bolus Fed	Glucose Concentration Bolus Fed (g/L)
288.7219444	2.05544	325.8536111	4.08692
289.1661111	2.28481	326.5205556	3.84686
289.61	2.18665	326.9644444	4.04213
290.0538889	2.44092	327.4086111	3.77223
290.2769444	2.30768	327.8527778	3.9225
290.7208333	2.09997	328.52	3.99757
291.165	2.13653	328.9638889	3.76221
291.6091667	2.29461	329.4080556	3.68814
292.5166667	1.89174	329.8522222	3.89506
293.405	3.35168	330.5194444	3.79475
293.8491667	3.81949	330.9636111	3.69956
294.2933333	1.83112	331.4075	3.64703
294.7372222	1.8642	331.8516667	3.57235
295.1813889	2.16381		
295.6252778	2.17022		
296.0691667	1.98928		
296.2919444	1.90433		
296.7361111	2.24558		
297.1802778	2.07294		
297.6241667	2.00742		
298.0680556	2.04407		
298.2911111	1.82856		
298.7352778	2.18444		
299.1791667	2.38328		
299.6233333	1.94764		
300.0675	2.35273		
300.2902778	2.10771		
300.7344444	2.18582		
301.1783333	2.28062		
301.6225	2.18726		
302.0666667	2.01366		
302.2894444	2.08052		
302.7333333	2.115		
303.1775	2.1862		
303.6213889	2.23513		
304.0655556	1.88516		
304.2886111	2.01393		

Time (hrs) Feedback Control	Glucose Concentration Feedback Control (g/L)	Time (hrs) Bolus Fed	Glucose Concentration Bolus Fed (g/L)
304.7327778	2.13416		
305.1766667	1.95372		
305.6205556	2.34303		
306.0647222	2.20315		
306.2877778	2.26925		
306.7316667	2.10713		
307.1758333	2.12814		
307.62	2.36701		
308.0636111	2.16943		
308.2866667	1.82948		
308.7308333	2.26683		
309.1747222	2.1141		
309.6188889	2.49		
310.0630556	2.27842		
310.2858333	2.20096		
310.73	2.17509		
311.1738889	2.15439		
311.6177778	2.33172		
312.0616667	2.19789		
312.2847222	2.15463		
312.7286111	2.27852		
313.1725	1.99785		
313.6166667	1.96589		
314.0608333	2.49224		
314.2836111	2.40053		
314.7277778	2.37773		
315.1716667	2.46324		
315.6158333	2.55963		
316.3027778	2.42319		
317.4113889	3.20763		
317.8558333	4.52655		
318.2997222	2.16813		
318.5227778	1.901		
318.9669444	1.79215		
319.4111111	2.46673		
319.855	2.19232		
320.2991667	2.22674		

Time (hrs) Feedback Control	Glucose Concentration Feedback Control (g/L)	Time (hrs) Bolus Fed	Glucose Concentration Bolus Fed (g/L)
320.522222	2.16041		
320.9663889	2.30146		
321.4102778	2.35759		
321.8544444	2.06147		
322.2986111	2.2465		
322.5216667	1.90065		
322.9658333	2.42279		
323.41	2.29138		
323.8541667	2.21841		
324.2980556	2.42145		
324.5211111	2.35336		
324.9652778	2.25286		
325.4094444	2.25769		
325.8536111	2.31652		
326.2975	2.24343		
326.5205556	2.28121		
326.9644444	2.32713		
327.4086111	2.38217		
327.8527778	2.14074		
328.2966667	2.30334		
328.5197222	2.2444		
328.9638889	2.10546		
329.4080556	2.16617		
329.8522222	2.30982		
330.2961111	2.12672		
330.5191667	2.19646		
330.9633333	1.81375		
331.4075	2.20783		

THE CLAIMS

What is claimed is:

1. A method for controlling cell culture medium conditions comprising:
quantifying one or more analytes in the cell culture medium using in situ Raman spectroscopy; and
adjusting the one or more analyte concentrations in the cell culture medium to match predetermined analyte concentrations that maintain post-translational modifications of proteins in the cell culture medium to 1.0 to 30 percent.
2. The method of claim 1, wherein the post-translational modification comprises glycation.
3. The method of claim 1, wherein proteins in the cell culture comprise an antibody or antigen-binding fragment thereof.
4. The method of claim 1, wherein proteins in the cell culture comprise a fusion protein.
5. The method of claim 1, wherein the cell culture medium comprises mammalian cells.
6. The method of claim 5, wherein the mammalian cells comprise Chinese Hamster Ovary cells.
7. The method of claim 1, wherein the analyte is glucose.
8. The method of claim 7, wherein the predetermined glucose concentration is 0.5 to 8.0 g/L.
9. The method of claim 7, wherein the glucose concentration is 1.0 g/L to 3.0 g/L.
10. The method of claim 7, wherein the glucose concentration is 2.0 g/L.
11. The method of claim 7, wherein the glucose concentration is 1.0 g/L.
12. The method of claim 1, wherein the predetermined analyte concentrations maintain post-translation modifications of proteins in the cell culture medium to 1.0 to 20 percent.
13. The method of claim 1, wherein the predetermined analyte concentrations maintain post-translation modifications of proteins in the cell culture medium to 5.0 to 10 percent.
14. The method of claim 1, wherein the quantifying of analytes is performed continuously.
15. The method of claim 1, wherein the quantifying of analytes is performed intermittently.
16. The method of claim 1, wherein the quantifying of analytes is performed in intervals.
17. The method of claim 1, wherein the quantifying of analytes is performed in 5 minute intervals.

18. The method of claim 1, wherein the quantifying of analytes is performed in 10 minute intervals.
19. The method of claim 1, wherein the quantifying of analytes is performed in 15 minute intervals.
20. The method of claim 1, wherein the quantifying of analytes is performed hourly.
21. The method of claim 1, wherein the quantifying of analytes is performed at least daily.
22. The method of claim 1, wherein the adjusting of analyte concentrations is performed automatically.
23. The method of claim 1, wherein at least two different analytes are quantified.
24. The method of claim 1, wherein at least three different analytes are quantified.
25. The method of claim 1, wherein at least four different analytes are quantified.
26. A method for reducing post-translation modifications of a secreted protein comprising:
 - culturing cells secreting the protein in a cell culture medium comprising 0.5 to 8.0 g/L glucose;
 - incrementally determining the concentration of glucose in the cell culture medium during culturing of the cells using *in situ* Raman spectroscopy;
 - adjusting the glucose concentration to maintain the concentration of glucose to 0.5 to 8.0 g/L by automatically delivering multiple doses of glucose per hour to maintain post-translational modifications of the secreted protein to 1.0 to 30.0 percent.
27. The method of claim 26, wherein the concentration of glucose is 1.0 to 3.0 g/L.
28. A system for controlling cell culture medium conditions comprising:
 - one or more processors in communication with a computer readable medium storing software code for execution by the one or more processors in order to cause the system to
 - receive data comprising a concentration of one or more analytes in the cell culture medium from an *in situ* Raman spectrometer; and
 - adjust the one or more analyte concentrations in the cell culture medium to match predetermined analyte concentrations that maintain post-translational modifications of proteins in the cell culture medium to 1.0 to 30 percent.
29. The system of claim 28, wherein the software code is further configured to cause the system to perform chemometric analysis on the data.
30. The system of claim 29, wherein the chemometric analysis comprises Partial Least Squares regression modeling.

31. The system of claim 28, wherein the software code is further configured to cause the system to perform one or more signal processing techniques on the data.
32. The system of claim 31, wherein the signal processing technique comprises a noise reduction technique.
33. A system for reducing post-translation modifications of a secreted protein comprising:
one or more processors in communication with a computer readable medium storing software code for execution by the one or more processors in order to cause the system to
incrementally receive spectral data comprising a concentration of glucose in a cell culture medium during culturing of cells secreting the protein from an *in situ* Raman analyzer; and
adjust the glucose concentration to maintain the concentration of glucose to 0.5 to 8.0 g/L by automatically delivering multiple doses of glucose per hour to maintain post-translational modifications of the secreted protein to 1.0 to 30.0 percent.
34. The system of claim 33, wherein the software code is further configured to cause the system to correlate peaks within the spectral data to glucose concentrations.
35. The system of claim 33, wherein the software code is further configured to perform Partial Least Squares regression modeling on the spectral data.
36. The system of claim 33, wherein the software code is further configured to perform a noise reduction technique on the spectral data.
37. The system of claim 33, wherein the adjustment of the glucose concentration is performed by automated feedback control software.
38. The system of claim 33, wherein the concentration of glucose is 1.0 to 3.0 g/L.