

METHOD FOR MODULATING GLYCOSYLATION OF A BIOLOGICAL PRODUCT

FIELD

The present disclosure is directed to a process for the control of glycosylation of a biological product having a pre-determined target glycosylation profile.

BACKGROUND

Protein products, such as antibodies, undergo post-translational modifications during their expression from a cell, including the attachment of sugar moieties. One such modification is N-linked glycosylation of immunoglobulin G (IgG) that occurs at position Asn 297 of the CH₂ domain of mammalian IgG heavy chains. N-linked glycosylation is achieved by the initial addition of a pre-formed oligosaccharide which is then subject to subsequent enzymatic modification to remove or add sugars, including removal of mannose and glucose residues, and addition of N-acetylglucosamine (GlcNAc), fucose, galactose or sialic acid species. The final oligosaccharide may include or exclude higher mannose content, fucose, galactose, or sialic acid species, depending on what enzymatic reactions occurred in the N-glycosylation pathway.

Glycosylation can have strong effects on the biological activity of the protein. In particular, antibody-dependent cellular cytotoxicity (ADCC), which is an important mechanism of action of many therapeutic antibodies, is dependent on the type of glycosylation found on the antibody. Thus, production of antibody products in which glycosylation can be modulated is advantageous for some therapeutic approaches, especially in oncology.

Further, since the type and extent of glycosylation can affect biological activity, the glycan profile of a therapeutic antibody is an important critical quality attribute (CQA) that must be reported to regulatory authorities and consistently reproduced. However, when the manufacture of a therapeutic antibody is transferred from one process to another (or even between manufacturing sites), variations can occur to CQAs such as glycosylation that may result in the need to tune the transferred process to achieve the profile of glycosylation in the previously obtained range for that product.

There is a need, therefore, to be able to design a system to improve upon existing methods of glycosylation control.

SUMMARY

In embodiments, provided herein is a process for the control of glycosylation of a biological product having a pre-determined target glycosylation profile, comprising:

providing a predictive model for glycosylation of said biological product, said predictive model including data selected from concentration of a hexose sugar, a metal ion cofactor, an amino monosaccharide, and an alpha mannosidase inhibitor in a nutrient media, said predictive model configured to predetermine a target glycosylation profile of said biological product;

growing cells in said nutrient media for producing said biological product;

measuring a first measured glycosylation profile of said biological product;

inputting said first measured glycosylation profile into said predictive model, said predictive model calculating concentrations of hexose sugar, the metal ion cofactor, the amino monosaccharide, and the alpha mannosidase inhibitor required in said nutrient media, in order to modulate said first measured glycosylation profile to said pre-determined target glycosylation profile;

creating a media supplement based on the concentration of a hexose sugar, a metal ion cofactor, an amino monosaccharide, and the alpha mannosidase inhibitor calculated by said predictive model; and

adding said media supplement to said nutrient media.

Other features and aspects of the present disclosure are discussed in greater detail below.

BRIEF DESCRIPTION OF THE DRAWINGS

A full and enabling disclosure of the present disclosure is set forth more particularly in the remainder of the specification, including reference to the accompanying figures, in which:

FIG. 1 is a schematic view of one embodiment of a bioreactor system in accordance with the present disclosure.

DETAILED DESCRIPTION

It is to be understood by one of ordinary skill in the art that the present discussion is a description of exemplary embodiments only, and is not intended as limiting the broader aspects of the present disclosure.

In embodiments, provided herein is a method for modulating glycosylation of an antibody product, which is typically intended to (i) modify the biological activity and/or half-life of the antibody e.g. increase antibody-dependent cellular cytotoxicity (ADCC) or (ii) fine-tune the glycosylation in a process to match the levels obtained previously.

In some embodiments, three main approaches to controlling glycosylation are provided: cell-line engineering, process parameter changes, and cell culturing media supplementation. All approaches can impact the activity of the enzymes active in the glycosylation process. Cell-line engineering approaches are limited by the amount of time required to develop and select new cell lines and could have unintended effects on productivity and culture health. Process parameter changes have been shown to work, such as with the use of decreased culturing temperatures to affect glycosylation. Changes to pH and osmolality have also been shown to affect glycosylation. The key drawbacks of process changes are unintended effects on metabolic activity, lack of knowledge on the mechanisms behind the effects, and the need to stay within predefined operating limits in cGMP production processes.

In embodiments, the media supplementation approach described herein explores a wide variety of nutrients.

In some embodiments, the disclosure utilizes multiple supplements for glycosylation control. “Glycosylation,” a type of post-translational modification, is the process of adding sugar units to a molecule, including proteins such as antibodies (also called “antibody products” herein). There are four types of glycosylation: fucosylation, galactosylation, mannosylation, and sialylation. This disclosure focuses on the modulation of fucosylation, galactosylation, and mannosylation.

“Fucosylation,” a type of glycosylation, is the process of adding fucose sugar units to a molecule, including proteins such as antibody products. “Afucosylation,” as used herein, refers to the absence of fucose sugar units on a particular molecule, such as a particular antibody product. In a preparation of antibody product, the level of afucosylation is the percentage of antibody molecules that lack a fucose sugar unit.

“Galactosylation,” a type of glycosylation, is the process of adding galactose sugar units to a molecule, including proteins such as antibody products. “Galactosylation,” as used herein, refers to the presence of galactose sugar units on a particular molecule, such as a particular antibody product. In a preparation of antibody product, the level of galactosylation is the percentage of antibody molecules that possess a galactose sugar unit.

“Mannosylation,” a type of glycosylation, is the process of adding mannose sugar units to a molecule, including proteins such as antibody products. “Mannosylation,” as used herein, refers to the presence of mannose sugar units at the terminus of the attached glycan on a particular molecule, such as a particular antibody product. In a preparation of antibody product, the level of mannosylation is the percentage of antibody molecules that possess a mannose sugar unit present at the terminus of the attached glycan.

Glycosylation can be measured by a number of methods including mass spectrometry and high pressure liquid chromatography (HPLC). Since there can be some variation between the methods used, in one embodiment the percentage is measured using a Time of Flight, Liquid Chromatograph Mass Spectrometer (TOF LC/MS) system. The antibodies are reduced and then loaded directly onto a liquid chromatograph mass spectrometer (LC/MS), such as an Agilent 6230B TOF LC/MS system (without the need for a PNGase F digestion), with the reduced antibodies being passed through a reverse phase de-salting column on the HPLC prior to being injected into the time of flight mass spectrometer.

Other suitable methods include those described in, for example, Tay and Butler, 2015, J. Biol. Methods 2:19 Mishra et al, 2020, J. Biotechnology X 5: 100015, the disclosure of which is incorporated by reference herein in its entirety and in particular for the disclosed methods of glycosylation determination. In one of these described methods, glycans can be removed using PNGase F and dried. They are then labelled using 2-AB labelling and analysed using hydrophilic interaction liquid chromatography - HPLC (HILIC-HPLC).

Detected species are then analysed to determine the percentage of glycosylated antibody. Measurements are usually replicated to improve measurement precision.

In one embodiment, the percentage glycosylation is calculated with respect to only N-linked glycan species, such as N-linked glycan species that are linked to the Fc domain of an antibody. N-linked glycan species include G0, G0F, G0FLys, G0F-GlcNAc, G1F, G2F, G1F + NeuAc, G2F + NeuAc, G2F + 2NeuAc, Man5, Man6, Man7, Man8, and Man9.

In one embodiment, afucosylation is measured as a percentage based on G0/(Sum of all glycan species).

In one embodiment, galactosylation is measured as a percentage based on (Sum of G1F, G2F, G1FNeuAc, G2FNeuAc, G2F2NeuAc)/(Sum of all glycan species).

In one embodiment, mannosylation is measured as a percentage based on (Sum of M5, M6, M7, M8, M9)/(Sum of all glycan species).

Measurement of the amount of change in glycosylation of an antibody product prepared according to the present methods are made using methods known in the art, such as

mass spectrometry analysis, including liquid chromatography -mass spectrometry, as well as other methods. As also described above, since there can be some variation between the methods used, in one embodiment the percentage is measured using a TOF LC/MS system: the antibodies are reduced and then loaded directly onto a liquid chromatograph mass spectrometer (LC-MS), such as an Agilent 6230B TOF LC/MS system (without the need for a PNGase F digestion), with the reduced antibodies being passed through a reverse phase de-salting column on the HPLC prior to being injected into the time of flight mass spectrometer.

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Comparison of the amount of glycosylation from one protein population to another provides the percentage change in glycosylation and is generally provided relative to a population of antibodies. That is, the measurement of a percent change in glycosylation from one antibody population compared to another is generally calculated based on an amount of antibody produced that is on the order of about 0.5 g/L or more, rather than on an individual antibody basis. Suitably, the amount of antibody produced using the methods described herein is about 1 g/L or more, suitably 5 g/L or more, or about 10 g/L or more. Thus, in embodiments herein where there is a change in glycosylation of at least 0.5%, the change is measured relative to a total amount of antibody that is about 0.5 g/L or more.

In some embodiments, the disclosure provides a process for making biological products. A production process for making biological products includes a cell population which is producing the biological product, along with a production medium or buffer, that suitably includes the necessary reagents and supplements, including suitable nutrient media, to support the cell proliferation and production of the desired biological product.

As used herein, a cell culture means a population of cells within a nutrient media.

In embodiments, the cells are eukaryotic cells, e.g., mammalian cells. The mammalian cells can be for example human or rodent or bovine cell lines or cell strains. Examples of such cells, cell lines or cell strains are e.g. mouse myeloma (NSO)-cell lines, Chinese hamster ovary (CHO)-cell lines, HT1080, H9, HepG2, MCF7, MDBK Jurkat, NIH3T3, PC12, BHK (baby hamster kidney cell), VERO, SP2/0, YB2/0, Y, C127, L cell, COS, e.g., COS1 and COS7, QC1-3, HEK-293, VERO, PER.C6, HeLA, EBI, EB2, EB3, oncolytic or hybridoma-cell lines. Preferably the mammalian cells are CHO-cell lines. In one embodiment, the cell is

a CHO cell. In one embodiment, the cell is a CHO-K1 cell, a CHO-K1 SV cell, a DG44 CHO cell, a DUXB11 CHO cell, a CHOS, a CHO GS knock-out cell, a CHO FUT8 GS knock-out cell, a CHOZN, or a CHO-derived cell. The CHO GS knock-out cell (e.g., GSKO cell) is, for example, a CHO-K1 SV GS knockout cell. The CHO FUT8 knockout cell is, for example, the Potelligent® CHOK1 SV (Lonza Biologics, Inc.). Eukaryotic cells can also be avian cells, cell lines or cell strains, such as for example, EBx® cells, EB14, EB24, EB26, EB66, or EBv13.

In some embodiments, the biological product may be an antibody or antibody product. As used herein, an “antibody product” and “antibody” are used interchangeably, with antibody product being the result of an antibody production process. As used herein, the terms “antibody” and “immunoglobulin” can be used interchangeably and refer to a polypeptide or group of polypeptides that include at least one binding domain that is formed from the folding of polypeptide chains having three-dimensional binding spaces with internal surface shapes and charge distributions complementary to the features of an antigenic determinant of an antigen. An antibody typically has a tetrameric form, with two pairs of polypeptide chains, each pair having one “light” and one “heavy” chain. The variable regions of each light/heavy chain pair form an antibody binding site. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies between the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains (CH). Each light chain has a variable domain at one end (VL) and a constant domain (CL) at its other end, wherein the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Light chains are classified as either lambda chains or kappa chains based on the amino acid sequence of the light chain constant region.

Immunoglobulin molecules can be of any isotype (e.g., IgG, IgE, IgM, IgD, IgA and IgY), subisotype (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or allotype (e.g., Gm, e.g., G1m(f, z, a or x), G2m(n), G3m(g, b, or c), Am, Em, and Km(l, 2 or 3)). Immunoglobulins include, but are not limited to, monoclonal antibodies (mAb) (including full-length monoclonal antibodies), polyclonal antibodies, multispecific antibodies formed from at least two different epitope binding fragments (e.g., bispecific antibodies), CDR- grafted, human antibodies, humanized antibodies, camelised antibodies, chimeric antibodies, anti-idiotypic (anti-id) antibodies, intrabodies, and desirable antigen binding fragments thereof, including

recombinantly produced antibody fragments. Examples of antibody fragments that can be recombinantly produced include, but are not limited to, antibody fragments that include variable heavy- and light-chain domains, such as single chain Fvs (scFv), single-chain antibodies, Fab fragments, Fab' fragments, F(ab')₂ fragments. Antibody fragments can also include epitope-binding fragments or derivatives of any of the antibodies enumerated above.

Antibody product includes antibody conjugates in which the antibody is conjugated to a small molecule via a linker molecule. In suitable embodiments, the antibody product is a monoclonal antibody (mAb) and more suitably is a therapeutic antibody product.

In some embodiments, the biological product is prepared from a cell culture, the cell culture is grown in a cell culture media, and the disclosure controls the culture media supplement to control the fucosylation, mannosylation and galactosylation properties of the biological product. In some embodiments, the biological product is an antibody product.

Production Process

As described herein, the antibody production process is suitably carried out in a bioreactor - a vessel suitable for the cultivation of producer cells that express the antibody of interest. Since the bioreactor is typically being used at a production scale, or a pilot scale prior to being scaled up for production, the bioreactor used in the production process typically has a volume of at least 10 L although smaller bioreactors may be used to test the process e.g. the AMBR® 250 system which has a volume of 100 to 250 mL. Accordingly, in exemplary embodiments, the bioreactor can have a volume between about 100 mL and about 50,000 L. Non-limiting examples include a volume of 100 mL, 250 mL, 500 mL, 750 mL, 1 liter, 2 liters, 3 liters, 4 liters, 5 liters, 6 liters, 7 liters, 8 liters, 9 liters, 10 liters, 15 liters, 20 liters, 25 liters, 30 liters, 40 liters, 50 liters, 60 liters, 70 liters, 80 liters, 90 liters, 100 liters, 150 liters, 200 liters, 250 liters, 300 liters, 350 liters, 400 liters, 450 liters, 500 liters, 550 liters, 600 liters, 650 liters, 700 liters, 750 liters, 800 liters, 850 liters, 900 liters, 950 liters, 1000 liters, 1500 liters, 2000 liters, 2500 liters, 3000 liters, 3500 liters, 4000 liters, 4500 liters, 5000 liters, 6000 liters, 7000 liters, 8000 liters, 9000 liters, 10,000 liters, 15,000 liters, 20,000 liters, and/or 50,000 liters. Suitable reactors can be multi-use, single use, disposable, or non-disposable and can be formed of any suitable material including metal alloys such as stainless steel (e.g., 316L or any other suitable stainless steel) and Inconel, plastics, and/or glass.

In the embodiment illustrated in FIG. 1, a bioreactor system in accordance with the present disclosure includes a bioreactor 10. The bioreactor 10 comprises a hollow vessel or

container that includes a bioreactor volume 12 for receiving a cell culture within a fluid growth medium, a rotatable shaft 14 coupled to an agitator such as dual impellers 16 and 18, a sparger 20, and a baffle 22. The rotatable shaft 14 can be coupled to a motor 24 for rotating the shaft 14 and the impellers 16 and 18. The sparger 20 is in fluid communication with a gas supply 48 for supplying gases to the bioreactor 10, such as carbon dioxide, oxygen and/or air. In addition, the bioreactor system can include various probes for measuring and monitoring pressure, foam, pH, dissolved oxygen, dissolved carbon dioxide, and the like. The bioreactor 10 includes a bottom port 26 connected to an effluent 28 for withdrawing materials from the bioreactor continuously or periodically. In addition, the bioreactor 10 includes a plurality of top ports, such as ports 30, 32, and 34. Port 30 is in fluid communication with a first fluid feed 36, port 32 is in fluid communication with a second feed 38 and port 34 is in fluid communication with a third feed 40. The feeds 36, 38 and 40 are for feeding various different materials to the bioreactor 10, such as a nutrient media.

As shown in FIG. 1, the bioreactor can be in communication with multiple nutrient feeds. In this manner, a nutrient media can be fed to the bioreactor containing only a single nutrient for better controlling the concentration of the nutrient in the bioreactor during the process. In addition or alternatively, the different feed lines can be used to feed gases and liquids separately to the bioreactor.

In addition to ports on the top and bottom of the bioreactor 10, the bioreactor can include ports located along the sidewall. For instance, the bioreactor 10 shown in FIG. 1 includes ports 44 and 46.

Ports 44 and 46 are in communication with a monitoring and control system that can maintain optimum concentrations of one or more parameters in the bioreactor 10 for propagating cells or otherwise producing a biological product. In the embodiment illustrated, for example, port 44 is associated with a pH sensor 52, while port 46 is associated with a dissolved oxygen sensor 54. The pH sensor 52 and the dissolved oxygen sensor 54 are in communication with a controller 60. The system of the present disclosure can be configured to allow for the determination and the measurements of various parameters within a cell culture contained within the bioreactor 10. Some of the measurements can be made in line, such as pH and dissolved oxygen. Alternatively, however, measurements can be taken at line or off line. For example, in one embodiment, the bioreactor 10 can be in communication with a sampling station. Samples of the cell culture can be fed to the sampling station for taking various measurements. In still another embodiment, samples of the cell culture can be removed from the bioreactor and measured off line.

Media

As used herein, a nutrient media refers to any fluid, compound, molecule, or substance that can increase the mass of a bioproduct, such as anything that may be used by an organism to live, grow or otherwise add biomass. For example, a nutrient feed can include a gas, such as oxygen or carbon dioxide that is used for respiration or any type of metabolism. Other nutrient media can include carbohydrate sources. Carbohydrate sources include complex sugars and simple sugars, such as glucose, maltose, fructose, galactose, and mixtures thereof. A nutrient media can also include an amino acid. The amino acid may comprise, glycine, alanine, valine, leucine, isoleucine, methionine, proline, phenylalanine, tryptophan, serine, threonine, asparagine, glutamine, tyrosine, cysteine, lysine, arginine, histidine, aspartic acid and glutamic acid, single stereoisomers thereof, and racemic mixtures thereof. The term “amino acid” can also refer to the known non-standard amino acids, e.g., 4-hydroxyproline, ϵ -N,N,N-trimethyllysine, 3-methylhistidine, 5-hydroxylysine, O-phosphoserine, γ -carboxyglutamate, γ -N-acetyllysine, ω -N-methylarginine, N-acetylserine, N,N,N-trimethylalanine, N-formylmethionine, γ -aminobutyric acid, histamine, dopamine, thyroxine, citrulline, ornithine, β -cyanoalanine, homocysteine, azaserine, and S-adenosylmethionine. In some embodiments, the amino acid is glutamate, glutamine, lysine, tyrosine or valine.

The nutrient media can also contain one or more vitamins. Vitamins that may be contained in the nutrient media include group B vitamins, such as B12. Other vitamins include vitamin A, vitamin E, riboflavin, thiamine, biotin, and mixtures thereof. The nutrient media can also contain one or more fatty acids and one or more lipids. For example, a nutrient media feed may include cholesterol, steroids, and mixtures thereof. A nutrient media may also supply proteins and peptides to the bioreactor. Proteins and peptides include, for instance, albumin, transferrin, fibronectin, fetuin, and mixtures thereof. A growth medium within the present disclosure may also include growth factors and growth inhibitors, trace elements, inorganic salts, hydrolysates, and mixtures thereof. Trace elements that may be included in the growth medium include trace metals. Examples of trace metals include cobalt, nickel, and the like.

Glycan Profile Supplements

The glycosylation profile of a biological product can be controlled by adding specific supplements to the nutrient media. These supplements can include a hexose sugar, a metal ion

cofactor, an amino monosaccharide, and an alpha-mannosidase inhibitor. Together, these supplements can be used to change the levels of galactosylation, mannosylation, and afucosylation.

Uniquely, the amino monosaccharide is capable of mitigating the effects of the alpha-mannosidase inhibitor. This effect is surprising and has not been documented in literature before. There are no known mechanisms by which to explain this phenomenon.

As described above, the glycosylation profile of the biological product is dependent on the relative amounts of this hexose sugar, the metal ion cofactor, the amino monosaccharide, and alpha-mannosidase inhibitor present in the nutrient media. In order to determine the relative concentrations of these supplements needed in the nutrient media to achieve a desired glycosylation profile, a predictive model is developed to correlate the effects of concentration of each supplement on the glycosylation profile.

The predictive model represents a functional relationship that encapsulates how media supplements impact the resulting glycosylation profile. Provided with concentrations of media supplements, the model produces estimates of the future glycosylation profile. Such models can be built from prior reference data, first principle/mechanistic relationships or hybrid simulation strategies that combine both. The predictive model can use various multivariate methods in predicting the future glycosylation profile. In one embodiment, a predictive model can be trained from collected reference data comprised of media concentrations of the aforementioned supplements and the associated percent galactosylation, mannosylation and afucosylation levels. In the simplest case, multiple linear regression can be employed on this reference data, using inputs of media supplement concentrations (and/or interactions thereof) that are determined to be statistically significant via analysis of variance analyses, to create a functional relationship between the media supplement concentrations and the glycosylation profile. Other methods could also be used to develop relationships between media supplement concentrations and glycosylation profiles, including but not limited to: partial least squares, neural networks, extreme gradient boosted trees, random forests, support vector machines and the like.

In one embodiment, the predictive model uses media concentrations of the hexose sugar, metal ion cofactor, amino monosaccharide and alpha-mannosidase inhibitor to predict the future percent galactosylation, mannosylation and afucosylation levels. The predictive model can be incorporated into an optimization strategy to determine the concentrations of each supplement required in the media to minimize deviations from the pre-determined target glycosylation profile in terms of percent galactosylation, mannosylation and afucosylation.

Changes to the current media concentrations of each supplement can be made based upon the optimization output and the resulting percent galactosylation, mannosylation, and afucosylation levels recorded. The recorded percent galactosylation, mannosylation, and afucosylation levels can be compared to the pre-determined target levels and differences between them employed to compensate for errors in the predictive model(s).

In another embodiment, the predictive model can be used to control the glycan profile to a target profile using a feedback controller, such as a model predictive controller. In this case, a dynamic predictive model is constructed to predict the glycan profile at multiple future time points. Such a model can be constructed by a variety of different strategies. In one embodiment, the model can use the media supplement concentrations, manipulatable culture conditions (pH, temperature and the like) and glycan profile values from prior time points to predict the glycan profile at a future time point. This model can be extended into a multi-step ahead predictor by using the output prediction of the glycan profile values along with prescribed variations in the media supplement concentrations and culture conditions, such as would be determined by a control strategy, to predict future glycan profile outputs. In another embodiment, reactor mass balances are employed to generate a set of differential equations that govern culture conditions, such as viable cell concentration, metabolite concentrations and the like. In this embodiment, media supplement conditions, manipulatable culture conditions (pH, temperature, etc.) and recorded/simulated culture conditions can be used to create a model that predicts unknown terms in the differential equations, such as growth rate and the cell-specific consumption/production rate of each metabolite. Alternatively, unknown terms in the differential equations can be replaced with assumed mechanistic relationships, such as Monod-type equations or the like, that are parameterized and optimized to best fit prior reference data. In the hybrid model of the first formulation, provided with initial culture conditions and prescribed values for future media supplement variations and changes in manipulatable culture conditions, the predictive model can be used to determine the unknown parameters in the differential equations, enabling simulation of the differential equations to a future time point. In this simulation, the model-predicted parameters are held constant between time points. At a future time point, the simulated culture conditions are employed with the prescribed variations in the media supplements and manipulatable culture conditions, such as would be determined by a control strategy, to determine new values for the unknown parameters in the differential equations and the process repeats. A second predictive model can be developed that employs media supplement concentrations, manipulatable culture conditions and recorded/simulated culture

conditions to predict the glycan profile, enabling a continuous prediction of the time evolution of the glycan profile. In some embodiments, soft sensors, such as those developed from Raman spectra, can be used in this embodiment to replace physical measurements of cell culture metabolites required for the initialization of the simulation.

As described above, in one embodiment, the system and method of the present disclosure are directed to regulating glycan profiles using a manipulated set of variables. In one embodiment, a model predictive controller can prescribe the values for the manipulated variables over a control horizon from knowledge of the desired glycan profile and prior values of the recorded manipulated variables and glycan profile. The model predictive controller can employ the dynamic model developed from historical process data to determine the values for the manipulated variables that will result in the glycan profile reaching the desired values in the future. Glycan profile predictions are generated in a multi-step fashion from the predictive model/simulation over the prediction horizon from a sequence of values for the manipulated variables over the control horizon. Optimal values for the manipulated variables are determined over the control horizon to minimize an objective function involving the deviation of the model output predictions from the desired trajectory over the prediction horizon. Once the optimal sequence of manipulated variables is determined, in one embodiment, only the first of these values can be employed in the bioreactor. In this manner, at the next sampling instant, the glycan profile is measured and the process repeats. Because the recorded, rather than predicted, glycan profile is employed in each subsequent optimization cycle, the prediction errors that can accumulate in a multi-step prediction/simulation are limited in their impact in the controller implementation.

In one embodiment, the design of a model predictive controller can include specifying a number of design parameters to compute the objective function optimized during the controller operation. For example, in one embodiment, the objective function may be represented by:

$$J = \sum_{i=1}^P \sum_{j=1}^{n_o} \{w_{i,j}^y (\hat{y}_j(t+i) - r_j(t+i))\}^2 + \sum_{j=1}^{n_{mv}} \sum_{i=1}^P \left\{ \frac{w_{i,j}^u}{s_j^u} (u_j(t+i) - u_j(t+i-1)) \right\}^2$$

wherein:

- P is the number of days in the prediction horizon
- n_o is the number of glycan outputs
- $\hat{y}_j$ is the predicted value of glycan j from the predictive model
- r_j is the value of glycan j for the desired reference trajectory

- $w_{i,j}^y$ is the weighting to be applied to the difference between the predicted output and the reference trajectory for each instant (i) in the prediction horizon for each glycan (j)
- n_{mv} is the number of manipulated variables
- u_j is the value of manipulated variable j at a particular instant
- $w_{i,j}^{\Delta u}$ is the weighting applied to the difference between subsequent manipulated variable values for manipulated variable j on the i^{th} prediction horizon instant
- s_j^u is a scaling factor for the j^{th} manipulated variable, to handle differences in scales between the manipulated variables

In one embodiment, the coefficients on the right side of the above equation can be set to zero to provide the following simplified equation

$$J = \sum_{i=1}^P \sum_{j=1}^{n_o} \{w_{i,j}^y (\hat{y}_j(t+i) - r_j(t+i))\}^2$$

where: P is the number of days in the prediction horizon; n_o is the number of glycan outputs, $\hat{y}_j$ is the predicted value of glycan j from the predictive model; r_j is the value of glycan j for the desired reference trajectory; $w_{i,j}^{\Delta u}$ is the weighting to be applied to the difference between the predicted output and the reference trajectory for each time instant, for each glycan, over the prediction horizon.

The objective function penalizes differences in the predicted outputs from the reference trajectory values. Different weightings can be employed across the instants of the prediction horizon if concern exists regarding multi-step prediction accuracy of the predictive model far into the future. The optimal values for the manipulated variables over the control horizon are achieved by minimizing the objective function with respect to both bound and rate constraints on the manipulated variables.

Glycosylation control

In exemplary embodiments, the amount of increase of glycosylation resulting from the methods described herein is at least a 0.5% increase, or in other embodiments, at least a 0.6% increase, at least a 0.7% increase, at least a 0.8% increase, at least a 0.9% increase, at least a 1% increase, at least a 1.1% increase, at least a 1.2% increase, at least a 1.3% increase, at least a 1.4% increase, at least a 1.5% increase, at least a 1.6% increase, at least a 1.7% increase, at least a 1.8% increase, at least a 1.9% increase, at least a 2.0% increase, at least a

2.1% increase, at least a 2.2% increase, at least a 2.3% increase, at least a 2.4% increase, at least a 2.5% increase, at least a 2.6% increase, at least a 2.7% increase, at least a 2.8% increase, at least a 2.9% increase, at least a 3.0% increase, or an increase of 0.5% to about 2.0%, an increase of about 0.5% to about 1.5%, or an increase of about 0.5% to about 1.0%.

In another embodiment, the process is used to control the level of glycosylation to match a predetermined level (target value). Accordingly in one embodiment a method is provided for matching the glycosylation of a recombinantly produced antibody to a previously obtained target glycosylation percentage for the same antibody, the method comprising culturing cells that express the antibody in a bioreactor; controlling the addition of the hexose sugar, metal ion cofactor, amino monosaccharide and alpha-mannosidase inhibitor to the bioreactor during the antibody production process to obtain the expressed antibody with the target percentage glycosylation .

Typically, the level of glycosylation is controlled to within +/- 0.05%, +/- 0.10%, +/- 0.15%, +/- 0.20%, +/- 0.25%, +/- 0.30%, +/- 0.35%, +/- 0.40%, +/- 0.45%, +/- 0.50%, +/- 0.75%, +/- 1%, +/- 1.50%, +/- 1.75%, +/- 2%, +/- 5%, or +/- 8% of the desired target value (where the target value is a range, the variation is with respect to the midpoint of the range). In some embodiments, the level of glycosylation is controlled to within +/- 0.25%. In some embodiments, the level of glycosylation is controlled to within +/- 0.5%.

In some embodiments, the present disclosure provides a process or method for control of glycosylation of a biological product having a predetermined target glycosylation profile. In some embodiments, the process comprises: providing a predictive model for glycosylation of said biological product dependent on concentrations of a hexose sugar, a metal ion cofactor, an amino monosaccharide, and an alpha-mannosidase inhibitor in a nutrient media, said predictive model including information relating to said pre-determined target glycosylation profile of said biological product;

growing cells in said nutrient media, said cells capable of producing said biological product;

measuring a first measured glycosylation profile of said biological product;

inputting said first measured glycosylation profile into said predictive model,

said predictive model calculating concentrations of a hexose sugar, a metal ion cofactor, an amino monosaccharide and an alpha-mannosidase inhibitor required in said nutrient media in order to change said first measured glycosylation profile in a direction toward said pre-determined target glycosylation profile;

creating a media supplement based on the concentrations of a hexose sugar, a metal ion cofactor, an amino monosaccharide, and an alpha-mannosidase inhibitor calculated by said predictive model; and

adding said media supplement to said nutrient media.

In some embodiments, the predetermined target glycosylation profile is a percentage or a range of percentages of glycosylation of the product. In some embodiments, the predetermined target glycosylation profile is inputted to and stored in the predictive model in advance, based on the measurement of the glycosylation of the product from a smaller sized manufacture bioreactor or an experimental bioreactor or other device, where the product has a high quality in regard to its glycosylation, such as having a low fucosylation. In some embodiments, the predetermined target glycosylation profile was measured by mass spectroscopy and HPLC, or any other suitable method, on the high quality product obtained from the smaller sized manufacture bioreactor or the experimental bioreactor.

A cell for producing the biological product is cultured in a nutrient media in a bioreactor. The cell can be a mammalian cell described herein, the bioreactor can be a large sized manufacturing bioreactor as shown in FIG. 1, and the cell can include an exogenous gene encoding the biological product. The biological product can be a monoclonal antibody. During the culturing process, the biological product is expressed, and preferably secreted to the cell media. The glycosylation profile of the product in the culture media is measured and inputted into the predictive model. The measured glycosylation profile may deviate from the predetermined target glycosylation profile. In some embodiments, the predictive model compares the measured glycosylation profile and the predetermined target glycosylation profile. When the measure glycosylation profile is out of the range of the predetermined target glycosylation profile, or deviates from the predetermined target glycosylation profile greater than a threshold value, such as 0.25%, the predictive model then predicts the concentrations of the hexose sugar (e.g. galactose), the metal ion cofactor (e.g. manganese), the amino monosaccharide (e.g. glucosamine), and the alpha-mannosidase inhibitor (e.g. kifunensine) that are able to change the glycosylation profile of the product toward the predetermined glycosylation profile. The four types of components are also named critical process parameters (CPPs) because their concentrations are critical for the quality of the produced product. After prediction, the predictive model then instructs or controls the bioreactor to prepare a media supplement comprising the CPPs based on the predicted concentrations of CPPs required, and adds the medial supplement to the culture media.

In some embodiments, the hexose sugar is galactose, the metal ion cofactor is manganese, the amino monosaccharide is glucosamine, and the alpha-mannosidase inhibitor is kifunensine.

In some embodiments, the step of measuring glycosylation profiles of the product, predicting the concentrations of the CPPs required to change the glycosylation profile to the predetermined glycosylation profile, preparing a media supplement comprising the predicted concentrations of CPPs, and adding the prepared media supplement to the culture media is repeated in the culture process. In certain embodiments, after the step of measuring glycosylation profile and before the step of predicting the concentration of the CPP required, the process further comprises a step of comparing the measured glycosylation profile to the predetermined glycosylation profile. If the measured glycosylation profile deviates from the predetermined glycosylation profile, the process continues to the step of prediction. If the measured glycosylation profile does not deviate from the predetermined glycosylation profile, the predictor stops the process and waits for the next input of the measured glycosylation profile.

Example 1 – Modulations of Afucosylation, Galactosylation, and Mannosylation with the Addition of Galactose + Manganese, Glucosamine, and Kifunensine

Controllability Study

CHO GS-KO cells are cultured in chemically defined culture medium supplemented with different chemicals at different concentrations to test the impact of those chemicals on the N-linked glycan profile of the product produced by the cultures. The product produced by this cell line is a model IgG antibody.

Experiment Flow:

1. Prepare culture media with supplemented chemicals
2. Culture cells in media from (1)
3. Harvest mAb produced in cultures and measure glycan profile of purified, reduced, and de-salted mAb products
4. Determine statistical impact of chemical supplements on glycan product quality

Preparation of media with supplemented chemicals:

The chemicals that are supplemented to the medium included:

- None (Control)
- Galactose + Manganese
- Glucosamine
- Kifunensine

Concentrated stock solutions of galactose, manganese, glucosamine, and kifunensine are prepared and added into culture media. Specific volumes of the stock solutions are supplemented to the culture media to generate the conditions in Table 1. Conditions are mixed in a variety of combinations as part of the execution of a full factorial DoE to generate the model herein.

Table 1:

Condition	Supplement
Culture Medium (Control)	None
Culture Medium + Galactose+ Manganese	0.5 g/L Galactose 1 uM Manganese Chloride
Culture Medium + Glucosamine	4.98 mM Glucosamine Hydrochloride
Culture Medium + Kifunensine	200 nM Kifunensine

Cell Culture:

GS-KO cells are inoculated at a density of 5×10^5 cells/mL into vented shake flasks containing a mixture of media from Table 1. Cultures are grown for five days in a temperature, CO₂, and humidity-controlled incubator. Samples are taken immediately after inoculation and on the harvest day to monitor the culture health.

Product Harvest & Glycan Measurement:

MAB product is harvested from 5-day shake flask cultures and purified through Protein A capture. Purified mAb is prepared for glycan analysis by reducing mAb to separate heavy and light chains. Reduced mAb is injected onto an LC-MS where the reduced mAb is de-salted by passing through a reverse-phase de-salting column on the LC prior to injection into a time of flight mass spectrometer (TOF MS) (Agilent 6230B). Three injections per sample are performed for technical replicates.

Glycan Data Analysis and Statistical Analysis:

Protein Metrics Software is used to process the resulting LC-MS data and the relative abundance of each glycan species is reported. The glycan species measured in processing include: G0, G0F, G0FLys, G0F-GlcNAc, G1F, G2F, G1F + NeuAc, G2F + NeuAc, G2F + 2NeuAc, Man5, Man6, Man7, Man8, and Man9. The relative abundance of these species is used to determine the percent glycosylation.

The supplement conditions tested in the full factorial DoE are categorically encoded for use in an analysis of variance (ANOVA) analysis to determine statistically significant terms. The analysis of variance technique is performed between the categorically encoded conditions and the recorded changes in percent galactosylation, mannosylation and afucosylation using the `anova_lm` function in the python statsmodels package to determine if mAb produced in cultures fed supplemented media significantly differ from mAb produced in cultures fed unsupplemented medium (control). This analysis is also used to investigate the presence of combinatorial effects associated with co-supplementation of the media supplements tested in these experiments. The resulting p values calculated from the ANOVA analyses are employed to determine variables that have a statistically significant relationship with the outputs, with a threshold p value of 0.1 used for statistical significance. For the purposes of determining system controllability, coefficients of terms with p values less than the threshold are retained, while those with p values exceeding the threshold are set to zero. The resulting coefficients are arranged in a process gain matrix (K) to relate changes in the input factors to the changes in the glycan profile via:

$$\Delta y = K \Delta u$$

where Δu and Δy represent the change in the input factors and output glycan profiles, respectively. System controllability is determined by determining the rank of the process gain matrix, with a full rank matrix indicating a controllable system. Statistically significant coefficients from this analysis are presented in Table 2.

Results/Discussion:

Supplementation of culture medium with the tested supplements results in changes to the baseline glycan profile (see Table 2).

Table 2

Condition	Result	Significance
Culture Medium	<u>Baselines</u> Galactosylation: 45.09%	N/A (Control)

	Mannosylation: 0.29% Afucosylation: 2.78%	
Culture Medium + Galactose, Manganese	Galactosylation: +21.25% Mannosylation: N/A Afucosylation: N/A	Galactosylation: p=0.00 Mannosylation: p=0.73 Afucosylation: p=0.18
Culture Medium + Glucosamine	Galactosylation: -32.64% Mannosylation: -0.25% Afucosylation: -2.17%	Galactosylation: p=0.00 Mannosylation: p=0.08 Afucosylation: p=0.00
Culture Medium + Kifunensine	Galactosylation: -36.27% Mannosylation: +76.24% Afucosylation: +38.18%	Galactosylation: p=0.00 Mannosylation: p=0.00 Afucosylation: p=0.00
Culture Medium + Galactose, Manganese, Glucosamine	Galactosylation: N/A Mannosylation: N/A Afucosylation: N/A	Galactosylation: p=0.78 Mannosylation: p=0.42 Afucosylation: p=0.21
Culture Medium + Galactose, Manganese, Kifunensine	Galactosylation: -6.12% Mannosylation: N/A Afucosylation: N/A	Galactosylation: p=0.08 Mannosylation: p=0.66 Afucosylation: p=0.17
Culture Medium + Glucosamine, Kifunensine	Galactosylation: +28.60% Mannosylation: -32.79% Afucosylation: -31.10%	Galactosylation: p=0.05 Mannosylation: p=0.08 Afucosylation: p=0.00
Culture Medium + Galactose, Manganese, Glucosamine, Kifunensine	Galactosylation: N/A Mannosylation: N/A Afucosylation: N/A	Galactosylation: p=0.29 Mannosylation: p=0.45 Afucosylation: p=0.17

Quantities in the results table represent differences from the baseline glycan profile produced by the unsupplemented culture medium. Additionally, the results of conditions treated with more than one supplement represent differences from the baseline glycan profile plus the summation of the effects of the individual conditions.

For example, the %galactosylation results of the Galactose, Manganese, Kifunensine condition (-6.12%) represent the difference between the results and the summation of the baseline galactosylation (45.09%), the galactosylation from the Galactose, Manganese condition (+21.25%), and the Kifunensine condition (-36.27%). This implies that although the individual effects of galactose, manganese, and kifunensine would suggest the result should be ~30.07% galactosylation, the result is actually 6.12% lower than that, ~23.95%.

Example 2 – Further Modulations of Afucosylation, Galactosylation, and Mannosylation with the Addition of Galactose + Manganese, Glucosamine, and Kifunensine

Controllability Studies

Further experimentation is performed using the methods described in the Controllability Study section above. These experiments consist of other cell clones cultured and supplemented using the same methods, including GS Xceed® CHOK1SV GS-KO® cell line. The results of this study provide information on the impact of clone and product on the efficacy of the supplements discussed herein. The clones are created using a different insertion method than the clone used for the data collection thus far. One of these clones produces a different product than the clone used for the data collection thus far, while the other produces the same product. The completion of these studies proves broad applicability of the model system described herein.

Verification Study

Concentrated stock solutions of galactose, manganese, glucosamine, and kifunensine are prepared and added into culture media. Specific volumes of the stock solutions are supplemented to the culture media to generate the conditions in Table 3. Conditions in Table 3 are identified by using the model generated herein to estimate concentrations of the supplements needed to achieve a target glycan profile.

Table 3:

Condition	Supplement
Culture Medium (control)	None
Model Recipe for 60% galactosylation, 5% mannosylation, and 8% afucosylation	0.196 g/L Galactose 1 uM Manganese Chloride 0.183 mM Glucosamine Hydrochloride 16.18 nM Kifunensine
Model Recipe for 30% galactosylation, 25% mannosylation, and 15% afucosylation	0.385 g/L Galactose 1 uM Manganese Chloride 0.111 mM Glucosamine Hydrochloride 15.84 nM Kifunensine
Model Recipe for 50% galactosylation, 5% mannosylation, and 8% afucosylation	0 g/L Galactose 0 uM Manganese Chloride 0.52 mM Glucosamine Hydrochloride 68.08 nM Kifunensine

Exemplary Embodiments:

Embodiment 1 is a process for the control of glycosylation of a biological product having a pre-determined target glycosylation profile, comprising:
providing a predictive model for glycosylation of said biological product, said predictive model including data selected from concentration of a hexose sugar, a metal ion cofactor, an

amino monosaccharide, and an alpha mannosidase inhibitor in a nutrient media, said predictive model configured to predetermine a target glycosylation profile of said biological product;

growing cells in said nutrient media for producing said biological product;

measuring a first measured glycosylation profile of said biological product;

inputting said first measured glycosylation profile into said predictive model, said predictive model calculating concentrations of hexose sugar, the metal ion cofactor, the amino monosaccharide, and the alpha mannosidase inhibitor required in said nutrient media, in order to modulate said first measured glycosylation profile to said pre-determined target glycosylation profile; and

creating a media supplement based on the concentration of hexose sugar, the metal ion cofactor, the amino monosaccharide and the alpha mannosidase inhibitor calculated by said predictive model; and

adding said media supplement to said nutrient media.

Embodiment 2 includes the process of Embodiment 1, wherein said concentration of said amino monosaccharide is used to reduce mannosylation caused by said alpha mannosidase inhibitor.

Embodiment 3 includes the process of any preceding Embodiments, further comprising the step of sampling said biological product in said nutrient media after addition of said media supplement, measuring a second glycosylation profile of said biological product, inputting said second glycosylation profile into said predictive model to calculate a second media supplement, and adding said second media supplement to said nutrient media.

Embodiment 4 includes the process of any preceding Embodiments, wherein said alpha mannosidase inhibitor is kifunensine.

Embodiment 5 includes the process of any preceding Embodiments, wherein said hexose sugar is a precursor for UDP-Gal.

Embodiment 6 includes the process of any preceding Embodiments, wherein said precursor for UDP-Gal is galactose.

Embodiment 7 includes the process of any preceding Embodiments, wherein said metal ion cofactor is a cofactor for a galactosyltransferase.

Embodiment 8 includes the process of any preceding Embodiments, wherein said cofactor for a galactosyltransferase is manganese.

Embodiment 9 includes the process of any preceding Embodiments, wherein said amino monosaccharide is a competitor for UTP.

Embodiment 10 includes the process of any preceding Embodiments, wherein said competitor for UTP is glucosamine.

Embodiment 11 includes the process of any preceding Embodiments, wherein said competitor for UTP is N-acetylglucosamine.

Embodiment 12 includes the process of any preceding Embodiments, wherein said predictive model provides an output that modulates fucosylation, galactosylation and mannosylation of said biological product.

Embodiment 13 includes the process of any preceding Embodiments, wherein said biological product is an antibody.

Embodiment 14 includes the process of any preceding Embodiments, wherein said cells are mammalian cells, more preferably Chinese hamster ovary cells.

Embodiment 15 includes the process of any preceding Embodiments, further comprising, after the step of inputting said first measured glycosylation profile into said predictive model: comparing the first measured glycosylation profile to the pre-determined target glycosylation profile,

wherein when the first measured glycosylation profile is increased by a threshold value as compared to the pre-determined target glycosylation profile, continues to the step of calculating concentrations, and wherein in when the first measured glycosylation profile is increased by less than the threshold value as compared to the pre-determined target glycosylation profile, waiting for a second measurement of the glycosylation profile.

Embodiment 16 includes the process of Embodiment 15, wherein the threshold value is 1% to 5%.

Embodiment 17 includes the process of Embodiment 15, wherein the threshold value is 2%.

Embodiment 18 includes a process for the control of glycosylation of a biological product having a pre-determined target glycosylation profile, comprising:
providing a predictive model for glycosylation of said biological product, said predictive model including data selected from concentration of a hexose sugar, a cofactor for an enzyme in the Leloir pathway, a molecule capable of reducing the levels of UDP-Gal, and an alpha mannosidase inhibitor in a nutrient media, said predictive model configured to predetermine a target glycosylation profile of said biological product;
growing cells in said nutrient media for producing said biological product;
measuring a first measured glycosylation profile of said biological product;
inputting said first measured glycosylation profile into said predictive model, said predictive model calculating concentrations of hexose sugar, the cofactor for an enzyme in the Leloir pathway, the molecule capable of reducing the levels of UDP-Gal, and the alpha mannosidase inhibitor required in said nutrient media, in order to modulate said first measured glycosylation profile to said pre-determined target glycosylation profile; and
creating a media supplement based on the concentration of hexose sugar, the cofactor for an enzyme in the Leloir pathway, the molecule capable of reducing the levels of UDP-Gal, and the alpha mannosidase inhibitor calculated by said predictive model; and
adding said media supplement to said nutrient media.

Embodiment 19 includes the process of any of the preceding claims, wherein said hexose sugar is a precursor for UDP-Gal; said cofactor for an enzyme in the Leloir pathway is a cofactor for a galactosyltransferase; said molecule capable of reducing the levels of UDP-Gal is a competitor for UTP; and said alpha mannosidase inhibitor is kifunensine.

Embodiment 20 includes the process of any of the preceding claims, wherein said precursor for UDP-Gal is galactose; said cofactor for a galactosyltransferase is manganese; and said competitor for UTP is glucosamine or N-acetylglucosamine.

CLAIMS

What is claimed is:

1. A process for the control of glycosylation of a biological product having a pre-determined target glycosylation profile, comprising:
providing a predictive model for glycosylation of said biological product, said predictive model including data selected from concentration of a hexose sugar, a metal ion cofactor, an amino monosaccharide, and an alpha mannosidase inhibitor in a nutrient media, said predictive model configured to predetermine a target glycosylation profile of said biological product;
growing cells in said nutrient media for producing said biological product;
measuring a first measured glycosylation profile of said biological product;
inputting said first measured glycosylation profile into said predictive model, said predictive model calculating concentrations of hexose sugar, the metal ion cofactor, the amino monosaccharide, and the alpha mannosidase inhibitor required in said nutrient media, in order to modulate said first measured glycosylation profile to said pre-determined target glycosylation profile;
creating a media supplement based on the concentration of hexose sugar, the metal ion cofactor, the amino monosaccharide and the alpha mannosidase inhibitor calculated by said predictive model; and
adding said media supplement to said nutrient media.
2. The process of claim 1, wherein said concentration of said amino monosaccharide is used to reduce mannosylation caused by said alpha mannosidase inhibitor.
3. The process of any of the preceding claims further comprising the step of sampling said biological product in said nutrient media after addition of said media supplement, measuring a second glycosylation profile of said biological product, inputting said second glycosylation profile into said predictive model to calculate a second media supplement, and adding said second media supplement to said nutrient media.
4. The process of any of the preceding claims, wherein said alpha mannosidase inhibitor is kifunensine.
5. The process of any of the preceding claims, wherein said hexose sugar is a precursor

for UDP-Gal.

6. The process of any of the preceding claims, wherein said precursor for UDP-Gal is galactose.
7. The process of any of the preceding claims, wherein said metal ion cofactor is a cofactor for a galactosyltransferase.
8. The process of any of the preceding claims, wherein said cofactor for a galactosyltransferase is manganese.
9. The process of any of the preceding claims, wherein said amino monosaccharide is a competitor for UTP.
10. The process of any of the preceding claims, wherein said competitor for UTP is glucosamine.
11. The process of any of the preceding claims, wherein said competitor for UTP is N-acetylglucosamine.
12. The process of any of the preceding claims, wherein said predictive model provides an output that modulates fucosylation, galactosylation and/or mannosylation of said biological product.
13. The process of any of the preceding claims, wherein said biological product is an antibody.
14. The process of any of the preceding claims, wherein said cells are mammalian cells, more preferably Chinese hamster ovary cells.
15. The process of any of the preceding claims, further comprising, after the step of inputting said first measured glycosylation profile into said predictive model:
comparing the first measured glycosylation profile to the pre-determined target glycosylation profile,

wherein when the first measured glycosylation profile is increased by a threshold value as compared to the pre-determined target glycosylation profile, continues to the step of calculating concentrations, and wherein in when the first measured glycosylation profile is increased by less than the threshold value as compared to the pre-determined target glycosylation profile, waiting for a second measurement of the glycosylation profile.

16. The process of claim 15, wherein the threshold value is 1% to 5%.

17. The process of claim 15, wherein the threshold value is 2%.

18. A process for the control of glycosylation of a biological product having a pre-determined target glycosylation profile, comprising:
providing a predictive model for glycosylation of said biological product, said predictive model including data selected from concentration of a hexose sugar, a cofactor for an enzyme in the Leloir pathway, a molecule capable of reducing the levels of UDP-Gal, and an alpha mannosidase inhibitor in a nutrient media, said predictive model configured to predetermine a target glycosylation profile of said biological product;
growing cells in said nutrient media for producing said biological product;
measuring a first measured glycosylation profile of said biological product;
inputting said first measured glycosylation profile into said predictive model, said predictive model calculating concentrations of hexose sugar, the cofactor for an enzyme in the Leloir pathway, the molecule capable of reducing the levels of UDP-Gal, and the alpha mannosidase inhibitor required in said nutrient media, in order to modulate said first measured glycosylation profile to said pre-determined target glycosylation profile; and
creating a media supplement based on the concentration of hexose sugar, the cofactor for an enzyme in the Leloir pathway, the molecule capable of reducing the levels of UDP-Gal, and the alpha mannosidase inhibitor calculated by said predictive model; and
adding said media supplement to said nutrient media.

19. The process of any of the preceding claims, wherein said hexose sugar is a precursor for UDP-Gal; said cofactor for an enzyme in the Leloir pathway is a cofactor for a galactosyltransferase; said molecule capable of reducing the levels of UDP-Gal is a competitor for UTP; and said alpha mannosidase inhibitor is kifunensine.

20. The process of any of the preceding claims, wherein said precursor for UDP-Gal is galactose; said cofactor for a galactosyltransferase is manganese; and said competitor for UTP is glucosamine or N-acetylglucosamine.