PREDICTING CELL CULTURE PERFORMANCE IN BIOREACTORS

FIELD OF DISCLOSURE

[0001] This disclosure generally relates to methods of *in silico* modeling of bioreactors and the associated biological processes for the purpose of improving bioprocess design and/or operation.

BACKGROUND

[0002] Biotechnology uses living cells to produce valuable biochemical compounds. Different cells can be engineered to produce a wide variety of target biochemical products for medicinal, agricultural, or other uses. Practitioners construct and use bioreactors for growing cell cultures that produce target products along with other metabolic byproducts. Maximizing the target product volume and quality for a given cost of production is one of the main goals of bioreactor design.

[0003] A bioreactor may be configured and controlled to operate in a variety of modes and with different goals. The first goal of the process may be to grow a sufficient amount of cell culture without using more than the necessary amount of substrate nutrients. The type of cellular biomass grown in the bioreactor may depend on an application, with some popular bio-cultures, such as Chinese hamster ovary (CHO) cells, being used for a number of different applications. In one mode of operation of a bioreactor, called a batch mode, growth of a bio-culture may take place without any liquid flowing into or out of the reactor, particularly if the bioreactor control system can maintain good conditions for growth. Good conditions for growth may require appropriate temperature, acidity, and concentrations of substrate metabolites. As the growing biomass consumes substrate metabolites, an input flow may help replenish the metabolites needed for growth, forming a fed-batch configuration of a bioreactor. Metabolic byproducts produced during growth may compromise the optimality of growth conditions. Adding input and output flow streams, forming a continuous bioreactor configuration, may alleviate the negative effects of metabolic byproducts as well as offer more control over the duration of the cell growth stage. A filter may be added to the output stream to prevent removal of cellular biomass, forming a perfusion configuration of the bioreactor.

[0004] Once a sufficient amount of biomass is produced in the bioreactor, the next stage of operation may prioritize generation and collection of a target metabolic product. Such target products may include therapeutic antibodies or other biologics. Inducing cells to shift metabolic energy from growing the biomass to production of the target product often involves introducing a stress factor. The stress may comprise a reduction in optimal host temperature, causing the cells to expend more energy on non-growth metabolism that can, in turn, result in increased target production.

[0005] Optimizing all the stages of the bioreactor operations poses a significant challenge. The many decisions may include when to transition from growth to production, when to begin harvesting, when and in what quantities to supply and remove metabolites, how to control the bioreactor environment. Often, many expensive and time consuming *in vivo* experiments are performed to select operating conditions. Alternative methods for optimizing and controlling bioreactor operations would bring considerable benefits to the industry and the public it serves.

SUMMARY

[0006] A computational method of modeling a bioreactor is used to predict cell culture performance, to improve and/or optimize bioreactor design and/or operation. In one aspect, the method accounts for spatial inhomogeneity and comprises receiving a plurality of current values of process variables, the process variables describing first virtual contents of a first portion of the modeled bioreactor and one or more additional virtual contents of corresponding one or more additional portions of the modeled bioreactor, and the virtual contents including virtual cellular biomass in a virtual extracellular solution. The method also comprises computing, by processing hardware of a computing system, new values of the process variables during a simulated time period. The method computes the new values at least in part by: generating a plurality of constraints on flux rates of metabolic fluxes describing the virtual cellular biomass by modeling one or more effects of at least some of the current values of the process variables on metabolic reaction kinetics; computing the flux rates of the metabolic fluxes by performing flux balance

analysis subject to a metabolic objective and the generated plurality of the constraints on the flux rates; computing rates of change of at least some of the process variables based

at least in part on the computed flux rates; and updating one or more of the current values of the process variables at least in part by integrating one or more of the computed rates of change for a virtual time step within the simulated time period. The method also comprises computing, by the processing hardware, a metric of the computationally modeled bioreactor based on the computed new values of the process variables, and generating, by the processing hardware, and based on the metric of the modeled bioreactor one or more of: i) information displayed to a user via a user interface, ii) a control setting for a real-world bioreactor, or iii) a training set for an artificial intelligence model of a bioreactor. For any method described herein, the method may optionally further comprise culturing mammalian cells in a bioreactor using one or more parameters identified in the modeled bioreactor. By way of example, the mammalian cells may encode a therapeutic protein, and may produce the therapeutic protein when cultured in the bioreactor. Examples of mammalian cells include CHO cells and BHK cells. For conciseness a "bioreactor" may also be referred to herein as a "reactor," and unless explicitly stated otherwise, these terms will be understood to be interchangeable herein.

[0007] In another aspect, non-transitory computer-readable medium stores instructions for computationally modeling a bioreactor, accounting for spatial inhomogeneity, wherein the instructions, when executed by one or more processors, cause the one or more processors to receive a plurality of current values of process variables, the process variables describing virtual contents of a first portion of the modeled bioreactor and one or more additional virtual contents of corresponding one or more additional portions of the modeled bioreactor, and the virtual contents including virtual cellular biomass in a virtual extracellular solution. The instructions also cause the one or more processors to compute, by processing hardware of a computing system, new values of the process variables during a simulated time period. The one or more processors compute the new values at least in part by: generating a plurality of constraints on flux rates of metabolic fluxes describing the virtual cellular biomass by modeling one or more effects of at least some of the current values of the process variables on metabolic reaction kinetics; computing the flux rates of the metabolic fluxes by performing flux balance analysis subject to a metabolic objective and the generated plurality of the constraints on the flux rates; computing rates of change of at least some of the process variables based at least in part on the computed flux rates; and updating one or more of the current values of the process variables at least in part by integrating one or more of the computed rates of change for a virtual time step within the simulated time period. The instructions also cause the one or more processors to compute, by the processing hardware, a metric of the modeled bioreactor based on the computed new values of the process variables, and generate, by the processing hardware, and based on the metric of the modeled bioreactor one or more of: i) information displayed to a user via a user interface, ii) a control setting for a real-world bioreactor, or iii) a training set for an artificial intelligence model of a bioreactor.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] Figure 1 illustrates an exemplary process for computationally modeling a bioreactor, and a corresponding computational model.

[0009] Figure 2 is a graph depicting an exemplary effect of a temperature shift on a stress variable in an implementation of the modeling process of Figure 1.

[0010] Figure 3 illustrates an exemplary computer system that may implement the modeling process of Figure 1.

[0011] Figures 4A and 4B illustrate panels of an exemplary user interface for receiving user inputs.

[0012] Figure 5 illustrates an exemplary user interface for displaying outputs to a user.

[0013] Figure 6 illustrates an exemplary bioreactor control system.

[0014] Figure 7 is a flow chart depicting an exemplary method of computationally modeling a bioreactor.

[0015] Figure 8 illustrates a comparison between a bioreactor model and experimental observations.

[0016] Figures 9A and 9B illustrate a relationship between a metabolic model of an inhomogeneous bioreactor modeled with homogeneous portions and a velocity field that may be computed using computational fluid dynamic (CFD).

What is claimed is:

1. A method of computationally modeling a bioreactor, the method accounting for spatial inhomogeneity, wherein the method comprises:

receiving a plurality of current values of process variables, the process variables describing first virtual contents of ~~at least~~ a first portion of the modeled bioreactor and one or more additional virtual contents of corresponding one or more additional portions of the modeled bioreactor, and the virtual contents including virtual cellular biomass in a virtual extracellular solution;

computing, by processing hardware of a computing system, new values of the process variables during a simulated time period, at least in part by

generating a plurality of constraints on flux rates of metabolic fluxes describing the virtual cellular biomass by modeling one or more effects of at least some of the current values of the process variables on metabolic reaction kinetics,

computing the flux rates of the metabolic fluxes by performing flux balance analysis subject to a metabolic objective and the generated plurality of the constraints on the flux rates,

computing rates of change of at least some of the process variables based at least in part on the computed flux rates, and

updating one or more of the current values of the process variables at least in part by integrating one or more of the computed rates of change for a virtual time step within the simulated time period;

computing, by the processing hardware, a metric of the computationally modeled bioreactor based at least in part on the computed new values of the process variables; and

generating, by the processing hardware, and based on the metric of the modeled bioreactor one or more of i) information displayed to a user via a user interface, ii) a control setting for a real-world bioreactor, or iii) a training set for an artificial intelligence model of a bioreactor.

2. The method of claim 1, wherein the process variables include

a) at least one of: temperature, acidity or one or more variables indicative of total osmolarity of the virtual contents; or

b) one or more variables indicative of concentration of the virtual cellular biomass and one or more variables indicative of extracellular metabolite concentrations in the virtual extracellular solution, optionally wherein the one or more variables indicative of the extracellular metabolite concentrations in the virtual extracellular solution include one or more variables indicative of concentrations of at least one of: oxygen, carbon dioxide, ammonia, glucose, asparagine, glutamine, glycine, or at least one target metabolic product, optionally wherein the process variables additionally include one or more variables indicative of one or more intracellular metabolite concentrations in the virtual cellular biomass.

3. The method of claim 1, wherein the plurality of the constraints on the flux rates includes an upper limit on at least one of i) glucose uptake, ii) glutamine uptake, iii) asparagine uptake, or iv) oxygen uptake or the plurality of the constraints on the rates of change includes a lower limit on an energy consumption rate for cellular maintenance.

4. The method of claim 1, wherein the one or more effects of at least some of the current values of the process variables on the metabolic reaction kinetics includes

a) an effect of a value of at least one of: i) temperature, ii) acidity, or iii) osmolarity on an upper limit of a metabolite uptake rate, optionally wherein modeling the one or more effects of at least some of the current values of the process variables on the metabolic reaction kinetics includes computing the effect of the value of at least one of: i) temperature, ii) acidity, or iii) osmolarity on the upper limit of the metabolite uptake rate by multiplying the upper limit with a correction factor indicative of a reduction in the uptake rate due to non-ideal conditions;

b) an effect of stress on a lower limit of energy consumption rate for cellular maintenance, optionally wherein modeling the effect of stress includes computing the effect based at least in part on a cumulative effect of a temperature shift on the virtual cellular biomass; or

c) an effect of concentration of at least one of metabolic byproducts.

5. The method of claim 1, wherein modeling the one or more effects includes calibrating the modeled effects with experimental data from a real-world cell culture.

6. The method of claim 1, wherein the metabolic objective includes

a) minimizing a ratio of linear combinations of the at least some of the flux rates and squares of flux rates in the flux balance analysis; or

b) minimizing a linear combination of at least some of the flux rates in the flux balance analysis, optionally wherein the linear combination is a sum of the fluxes in the flux balance analysis.

7. The method of claim 1, wherein the metabolic objective includes maximizing energy consumption rate for cellular maintenance or maximizing a rate of growth of the virtual cellular biomass.

8. The method of claim 1, further comprising:

a) receiving one or more variables indicative of flow rates and compositions of one or more virtual input streams into the modeled bioreactor, and wherein updating the at least some of the current values of the process variables includes accounting for the flow rates and the compositions of the one or more virtual input streams into at least one of the first portion or the one or more additional portions of the modeled bioreactor, optionally wherein updating at least some of the current values of the process variables includes solving mass-balance equations; optionally further comprising:

receiving one or more variables indicative of properties of a virtual output filter;

computing compositions of one or more virtual output streams from the modeled bioreactor using the properties of the virtual output filter, and wherein updating the at least some of the current values of the process variables includes accounting for the compositions of the one or more virtual output streams from at least one of the first portion or the one or more additional portions of the modeled bioreactor; or

b) receiving one or more control variables, and updating an affected set of the current values of the process variables at least in part based on the received control variables.

9. The method of claim 1, wherein

a) the metric of the modeled bioreactor includes values of at least one of the process variables at different virtual times within the simulated time period; or

b) the metric of the computationally modeled bioreactor indicates effectiveness or efficiency of the computationally modeled bioreactor in producing a target product.

10. The method of claim 1, wherein

a) the method comprises generating the control setting, and wherein the method further comprises controlling an input to a real-world bioreactor based on the generated control setting, or

b) the method comprises generating the training set for an artificial intelligence model of a bioreactor.

11. The method of claim 1, wherein receiving the plurality of the current values includes at least one of

i) receiving values from a user via a graphical user interface;

ii) receiving values based on measurements of a real bioreactor;

iii) loading values from computer memory and based on previously computed values; or

iv) loading predetermined default values from computer storage.

12. The method of claim 1, wherein:

~~the modeled bioreactor is a spatially heterogeneous bioreactor;~~

~~the at least the portion of the modeled bioreactor is a first portion of the modeled bioreactor;~~

the process variables include ~~are~~ first process variables describing the first virtual contents and one or more additional process variables describing respective virtual contents of the one or more additional virtual contents;

the method further includes receiving a plurality of current values of ~~second~~ the one or more additional process variables~~, the second process variables describing second virtual contents of a second portion of the modeled bioreactor~~; and

computing the new values of the first process variables during the simulated time period is based in part on the received plurality of the current values of the one or more additional~~second~~ process variables.

13. The method of claim 12, wherein computing the new values of the first process variables during the simulated time period includes computing a gradient of at least one of the first process variables based on a value of a corresponding one of the one or more additional~~second~~ process variables.

14. The method of claim 12, further comprising:

determining one or more velocities associated with the first virtual contents ~~of the first portion of the modeled bioreactor~~, optionally wherein determining the one or more velocities is based at least in part on computational fluid dynamics.

15. A non-transitory computer-readable medium storing instructions for computationally modeling a bioreactor, accounting for spatial inhomogeneity, wherein the instructions, when executed by one or more processors, cause the one or more processors to:

receive a plurality of current values of process variables, the process variables describing virtual contents of ~~at least~~ a first portion of the modeled bioreactor and one or more additional virtual contents of corresponding one or more additional portions of the modeled bioreactor, and the virtual contents including virtual cellular biomass in a virtual extracellular solution;

compute, by processing hardware of a computing system, new values of the process variables during a simulated time period, at least in part by

generating a plurality of constraints on flux rates of metabolic fluxes describing the virtual cellular biomass by modeling one or more effects of at least some of the current values of the process variables on metabolic reaction kinetics,

computing the flux rates of the metabolic fluxes by performing flux balance analysis subject to a metabolic objective and the generated plurality of the constraints on the flux rates,

computing rates of change of at least some of the process variables based at least in part on the computed flux rates, and

updating one or more of the current values of the process variables at least in part by integrating one or more of the computed rates of change for a virtual time step within the simulated time period;

compute, by the processing hardware, a metric of the modeled bioreactor based at least in part on the computed new values of the process variables; and

generate, by the processing hardware, and based on the metric of the modeled bioreactor one or more of i) information displayed to a user via a user interface, ii) a control setting for a real-world bioreactor, or iii) a training set for an artificial intelligence model of a bioreactor.

16. The non-transitory computer-readable medium of claim 15, wherein the process variables include

a) at least one of: temperature, acidity or one or more variables indicative of total osmolarity of the virtual contents, or

b) one or more variables indicative of concentration of the virtual cellular biomass and one or more variables indicative of extracellular metabolite concentrations in the virtual extracellular solution, optionally wherein the one or more variables indicative of the extracellular metabolite concentrations in the virtual extracellular solution include one or more variables indicative of concentrations of at least one of: oxygen, carbon dioxide, ammonia, glucose, asparagine, glutamine, glycine, or at least one target metabolic product, optionally wherein the process variables additionally include one or more variables indicative of one or more intracellular metabolite concentrations in the virtual cellular biomass.

17. The non-transitory computer-readable medium of claim 15, wherein the plurality of the constraints on rates of change includes an upper limit on at least one of i) glucose uptake, ii) glutamine uptake, iii) asparagine uptake, or iv) oxygen uptake; or a lower limit on an energy consumption rate for cellular maintenance.

18. The non-transitory computer-readable medium of claim 15, wherein the one or more effects of at least some of the current values of the process variables on the metabolic reaction kinetics includes

a) an effect of a value of at least one of: i) temperature, ii) acidity, or iii) osmolarity on an upper limit of a metabolite uptake rate, optionally wherein modeling the one or more effects of at least some of the current values of the process variables on the metabolic reaction kinetics includes computing the effect of the value of at least one of: i) temperature, ii) acidity, or iii)

osmolarity on the upper limit of the metabolite uptake rate by multiplying the upper limit with a correction factor indicative of a reduction in the uptake rate due to non-ideal condition;

b) an effect of stress on a lower limit of energy consumption rate for cellular maintenance, optionally wherein modeling the effect of stress includes computing the effect based at least in part on a cumulative effect of a temperature shift on the virtual cellular biomass; or

c) an effect of concentration of at least one of metabolic byproducts.

19. The non-transitory computer-readable medium of claim 15, wherein modeling the one or more effects include calibrating the modeled effects with experimental data from a real-world cell culture.

20. The non-transitory computer-readable medium of claim 15, wherein the metabolic objective includes

a) minimizing a linear combination of at least some of the rates of change; or

b) minimizing a linear combination of at least some fluxes in the flux balance analysis, optionally wherein the linear combination is a sum of the fluxes in the flux balance analysis;

c) maximizing energy consumption rate for cellular maintenance; or

d) maximizing a rate of growth of the virtual cellular biomass.

21. The non-transitory computer-readable medium of claim 15, wherein the instructions further cause the one or more processors to:

a) receive one or more variables indicative of flow rates and compositions of one or more virtual input streams into the modeled bioreactor, and wherein updating the at least some of the current values of the process variables includes accounting for the flow rates and the compositions of the one or more virtual input streams into at least one of the first portion or the one or more additional portions of the modeled bioreactor, optionally wherein updating the at least some of the current values of the process variables includes solving mass-balance equations; optionally wherein the instructions further cause the one or more processors to:

receive one or more variables indicative of properties of a virtual output filter;

compute compositions of one or more virtual output streams from the modeled bioreactor using the properties of the virtual output filter, and wherein updating the at least some of the current values of the process variables includes accounting for the compositions of the one or more virtual output streams from at least one of the first portion or the one or more additional portions of the modeled bioreactor; or

b) receive one or more control variables, and update an affected set of the current values of the process variables at least in part based on the received control variables.

22. The non-transitory computer-readable medium of claim 15, wherein

the metric of the modeled bioreactor includes values of at least one of the process variables at different virtual times within the simulated time period; or

the metric of the modeled bioreactor indicates effectiveness or efficiency of the modeled bioreactor in producing a target product.

23. The non-transitory computer-readable medium of claim 15, wherein the instructions

a) cause the one or more processors to generate the control setting, and further cause the one or more processors to control an input to a real-world bioreactor based on the generated control setting; or

b) cause the one or more processors to generate the training set for an artificial intelligence model of a bioreactor.

24. The non-transitory computer-readable medium of claim 15, wherein receiving the plurality of the current values includes at least one of:

i) receiving values from a user via a graphical user interface;

ii) receiving values based on measurements of a real bioreactor;

iii) loading values from computer memory and based on previously computed values; or

iv) loading predetermined default values from computer storage.

25. The non-transitory computer-readable medium of claim 15, wherein:

~~the modeled bioreactor is a spatially heterogeneous bioreactor;~~

~~the at least the portion of the modeled bioreactor is a first portion of the modeled bioreactor;~~

the process variables include~~are~~ first process variables describing the first virtual contents and one or more additional process variables describing respective virtual contents of the one or more additional virtual contents;

the instructions further cause the one or more processors to receive a plurality of current values of the one or more additional~~second~~ process variables~~, the second process variables describing second virtual contents of a second portion of the modeled bioreactor~~; and

computing the new values of the first process variables during the simulated time period is based in part on the received plurality of the current values of the second process variables, optionally wherein computing the new values of the first process variables during the simulated time period includes computing a gradient of at least one of the first process variables based on a value of a corresponding one of the one or more additional~~second~~ process variables.

26. The non-transitory computer-readable medium of claim 25, wherein the instructions further cause the one or more processors to determine one or more velocities associated with the first virtual contents ~~of the first portion of the modeled bioreactor~~, optionally wherein determining the one or more velocities is based at least in part on computational fluid dynamics.