PREDICTIVE CELL-BASED FED-BATCH PROCESS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims benefit of U.S. Provisional Patent Application No. 63/221,174 filed July 13, 2021, U.S. Provisional Patent Application No. 63/221,183 filed July 13, 2021, and U.S. Provisional Patent Application No. 63/221,197 filed July 13, 2021. The entire contents of each of which are hereby incorporated by reference herein.

BACKGROUND

[0002] Cell culture productivity relies on optimization of culture medium management to enable high cell densities and productivity. Nutrient feeding is an important parameter for process optimization in fed-batch cell feeding processes. High cell density processes can require substantial amounts of nutrients, and the daily requirements can vary with cell type and/or cell density. One known complex nutrient feeding strategy in fed-batch processes involves providing feed to a bioreactor based on a fixed percentage of bioreactor weight or bioreactor volume. Alternative cell culture processes which can result in an increase in cell culture productivity are desirable.

SUMMARY

[0003] Improved materials and methods for the prediction of daily nutrient target requirements for a given cell line is addressed by the present disclosure. The disclosed technology relates to a method of controlling a nutrient feed in a cell culture process, specifically, delivery of complex feed nutrients that is based on number of cells. Volume of feed per day can be proportional to a predicted change in integrated viable cells (IVC) from the present feeding day to the next feeding day. A per cell factor (PCF) is developed herein. The volume of feed per day can be set equal to a product of the PCF and change in IVC from the present feeding day to the next feeding day. The PCF can be further refined to vary over time during a fed-batch process.

[0004] The disclosed technology also relates to a method of executing a cell-based fed-batch bioreactor run. A quantity of feed may be provided to a bioreactor based at least in part on an estimated feed consumption rate (q_s) and based at least in part on a predicted change in integrated viable cell (IVC) number, the predicted IVC number being from a present feeding

interval to a future feeding interval.

[0004a] In one aspect, the disclosed technology relates to a method of executing a cell-based fed-batch bioreactor run, the method comprising: providing a quantity of feed to a bioreactor based at least in part on an estimated feed consumption rate (q_s) and based at least in part on a predicted change in integrated viable cell (IVC) number from a present feeding interval to a future feeding interval.

[0005] The change in IVC number can be predicted as follows: an IVC number of a present feeding interval can be determined, an IVC number of a previous feeding interval can be determined, a growth rate can be estimated based on the viable cell density of the present feeding interval and the viable cell density of the previous feeding interval, an IVC number for a following feeding interval can be based at least in part on the estimated growth rate, and the change in IVC can be set equal to the IVC number for the following feeding interval minus the IVC number for the present feeding interval.

[0006] Providing the quantity of feed to a bioreactor can be based at least in part on a Per Cell Factor (PCF). The PCF can be proportionate to the estimated feed consumption rate (q_s).

[0007] The PCF can vary over time during the cell-based fed-batch bioreactor run. In one example, the PCF does not increase over time during the cell-based fed-batch bioreactor run. In one example, the PCF is approximately equal to 0.002 g/cell × day for culture days 2 through 3 of the cell-based fed-batch bioreactor run; the PCF is approximately equal to 0.00175 g/cell × day for culture days 4 through 6 of the cell-based fed-batch bioreactor run; the PCF is approximately equal to 0.0015 g/cell × day for culture days 7 through 8 of the cell-based fed-batch bioreactor run; the PCF is approximately equal to 0.0012 g/cell × day for culture days 9 through 10 of the cell-based fed-batch bioreactor run; and the PCF is approximately equal to 0.0007 g/cell × day for culture days 11 through 13 of the cell-based fed-batch bioreactor run.

[0008] The quantity of feed can be provided to the bioreactor such that for each feed interval during the fed-bath bioreactor run, an amount of metabolite added to the bioreactor during each respective feed interval run is approximately equal to an amount of metabolite consumed within the bioreactor during each respective feed interval.

[0009] The cell-based fed-batch bioreactor run can be completed such that between about 20% and about 50% of a volume of the bioreactor consists of feed at the completion of the cell-based fed-batch bioreactor run.

[0010] Providing the quantity of feed can include providing a complex nutrient feed comprising at least one of, but not limited to: alanine, arginine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, hydroxy-L-proline, serine, threonine, tryptophan, tyrosine , valine, amino

acid di- and tri-peptides, B vitamins, carbohydrates, lipids, antioxidants, growth factors and trace elements

[0011] The quantity of feed can be provided to the bioreactor in an initial stage having a first plurality of feeding intervals and a subsequent final stage having a second plurality of feeding intervals. The quantity of feed can increase from one feeding interval to a subsequent feeding interval for each of the first plurality of feeding intervals of the initial stage. The quantity of feed can decrease from one feeding interval to a subsequent feeding interval for each of the second plurality of feeding intervals of the final stage.

[0012] The cell-based fed-batch bioreactor run can be completed such that a viable cell density in the bioreactor is approximately 3 to approximately 5 times a resulting viable cell density of a volume-based fed-batch bioreactor run executed under identical conditions, but for feeding strategy, as the cell-based fed-batch bioreactor run.

[0013] The estimated feed consumption rate (q_s) and IVC can each be based on a reduced-scale study of the cell-based fed-batch bioreactor run. The estimated feed consumption rate (q_s) and IVC can each be based on about a 0.25 L study of the cell-based fed-batch bioreactor run.

[0014] The disclosed technology also relates to a method of developing a cell-based fed-batch feeding schedule. A fed-batch bioreactor run can be executed on a cell line in which feeds are added at a regular time interval. For each regular time interval, a normalized feed per cell value can be calculated as follows: for each regular time interval calculate an interval feed per cell value that is a feed quantity fed at the respective time interval divided by a change in an integrated viable cell (IVC) number from a previous time interval to the respective time interval, determine a maximum interval feed per cell value of the calculated interval feed per cell values, and for each regular time interval set the normalized feed per cell value equal to the interval feed per cell value for the respective time interval divided by the maximum interval feed per cell value. For each regular time interval, a normalized IVC number can be calculated as follows: for each regular time interval estimate a daily IVC number based at least in part on an estimated IVC number of the previous time interval and a change in viable cells from the previous time interval to the respective time interval, determine a maximum daily IVC number of the estimated daily IVC numbers, and for each regular time interval set the normalized IVC number to the estimated daily IVC number divided by the maximum daily IVC number. A balanced feed time interval can be selected from the regular time intervals such that the normalized feed per cell value is

approximately equal to the normalized IVC number for the balanced feed time interval. A per cell factor (PCF) can be set equal to the interval feed per cell value of the balanced feed time interval. The cell-based fed-batch feeding schedule can be developed such that feed amounts are determined for each feeding interval and each respective feed amount is based at least in part on the PCF and is proportional to a predicted change in IVC number from a present feeding interval to a future feeding interval.

[0014a] Hence, in another aspect, the disclosed technology relates to a method of developing a cell-based fed-batch feeding schedule, the method comprising: executing a fed-batch bioreactor run on a cell line in which feeds are added at a regular time interval; calculating, for each regular time interval, a normalized feed per cell value as follows: for each regular time interval, calculating an interval feed per cell value that is a feed quantity fed at the respective time interval divided by a change in an integrated viable cell (IVC) number from a previous time interval to the respective time interval, determining a maximum interval feed per cell value of the calculated interval feed per cell values, and for each regular time interval, setting the normalized feed per cell value equal to the interval feed per cell value for the respective time interval divided by the maximum interval feed per cell value; calculating, for each regular time interval, a normalized IVC number as follows: for each regular time interval, estimating a daily IVC number based at least in part on an estimated IVC number of the previous time interval and a change in viable cells from the previous time interval to the respective time interval, determining a maximum daily IVC number of the estimated daily IVC numbers, and for each regular time interval, setting the normalized IVC number to the estimated daily IVC number divided by the maximum daily IVC number; selecting a balanced feed time interval from the regular time intervals such that the normalized feed per cell value is approximately equal to the normalized IVC number for the balanced feed time interval; setting a per cell factor (PCF) equal to the interval feed per cell value of the balanced feed time interval; and developing the cell-based fed-batch feeding schedule such that feed amounts are determined for each feeding interval and each respective feed amount is based at least in part on the PCF and is proportional to a predicted change in IVC number from a present feeding interval to a future feeding interval.

[0015] At least four fed-batch bioreactor runs can be executed on the cell line in which feeds are added at the regular time interval. For each regular time interval of each of the fed-batch bioreactor runs, the normalized feed per cell value can be calculated. For each regular time interval

of each of the fed-batch bioreactor runs, the normalized IVC number can be calculated. For each fed-batch bioreactor run, the balanced feed time interval can be selected. For each fed-batch bioreactor run, the PCF can be set equal to the respective interval feed per cell value of the respective balanced feed time interval. An average PCF can be determined that is an average of the PCFs of the at least four fed-batch bioreactor runs. The cell-based fed-batch feeding schedule can be developed such that feed amounts are determined for each feeding interval and each respective feed amount is equal to the average PCF multiplied by the predicted change in IVC number.

[0016] A first cell-based fed-batch bioreactor run can be executed according to the fed-batch feeding schedule. An updated PCF can be determined based at least in part on said PCF and based at least in part on the first cell-based fed-batch bioreactor run. The updated PCF can result in an updated fed-batch feeding schedule that results in a decreased total amount of feed added at an end of a second cell-based fed-batch bioreactor run compared to the total amount of feed added at an end of the first cell-based fed-batch bioreactor run. Reducing the amount of feed added while meeting cell nutritional requirements will lower the culture osmolality providing a more optimal environment for cell growth and recombinant protein production.

[0017] The first cell-based fed-batch bioreactor run can be executed according to the fed-batch feeding schedule. A variable PCF and a variable fed-batch feeding schedule can be determined based at least in part on said PCF and based at least in part on the first cell-based fed-batch bioreactor run.

[0018] The PCF can vary over time during the cell-based fed-batch bioreactor run. In one example, the PCF does not increase over time during the cell-based fed-batch bioreactor run.

In one example, the PCF is approximately equal to 0.002 mL/cell × day for culture days 2 through 3 of the cell-based fed-batch bioreactor run; the PCF is approximately equal to 0.00175 mL/cell × day for culture days 4 through 6 of the cell-based fed-batch bioreactor run; the PCF is approximately equal to 0.0015 mL/cell × day for culture days 7 through 8 of the cell-based fed-batch bioreactor run; the PCF is approximately equal to 0.0012 mL/cell × day for culture days 9 through 10 of the cell-based fed-batch bioreactor run; and the PCF is approximately equal to 0.0007 mL/cell × day for culture days 11 through 13 of the cell-based fed-batch bioreactor run.

[0019] The cell-based fed-batch feeding schedule is developed at a reduced scale to provide feed targets that are transferrable across scales. The cell-based fed-batch feeding schedule is developed at about a 0.25 L scale and is transferrable across fed-batch production processes utilizing about 250 liter (L) and about 1,000 L bioreactors respectively.

[0020] The disclosed technology also relates to a nutrient feed control system. The nutrient feed control system can include a viable cell density measurement system, a future viable cell density prediction system, a per cell factor (PCF) system, and a feed calculation system. The viable cell density measurement system can be configured to determine density of viable cells in a production reactor. The future viable cell density prediction system can be configured to receive the viable cell density measurement and can be configured to estimate a density of viable cells in a future feed time interval based at least in part on the viable cell density measurement. The PCF system can be configured to provide a PCF value. The feed calculation system can be configured to calculate an amount of feed to provide to the production reactor based at least in part on the density of viable cells in the future feed time interval and based at least in part on the PCF value.

[0020a] In another aspect, the disclosed technology relates to a nutrient feed control system comprising: a viable cell density measurement system configured to determine density of viable cells in a production reactor; a future viable cell density prediction system configured to receive the viable cell density measurement and configured to estimate a density of viable cells in a future feed time interval based at least in part on the viable cell density measurement; a per cell factor (PCF) system configured to provide a PCF value; and a feed calculation system configured to calculate an amount of feed to provide to the production reactor based at least in part on the density of viable cells in the future feed time interval and based at least in part on the PCF value.

[0021] The PCF system can further be configured to determine a balanced feed time interval at which metabolites consumed in the bioreactor are approximately equal to metabolites provided to the bioreactor during the balanced feed time interval. The PCF system can further be configured to determine a feed per cell value for the balanced feed time interval. The PCF system can further be configured to determine the PCF value based at least in part on the feed per cell value for the balanced feed time interval.

[0022] In one example, the nutrient feed control system can further include a processor and non-transitory computer-readable medium in communication with the processor with