

CLAIMS

1. A process for producing an inoculum for a subsequent cell culture production process comprising:

introducing a cell culture into a perfusion bioreactor;

feeding a nutrient media at a flow rate to the perfusion bioreactor and withdrawing fluid media from the perfusion bioreactor;

determining a biovolume fraction over time within said perfusion bioreactor using a biomass sensor, the biomass sensor being in communication with a controller; wherein said biovolume fraction is the volume of the perfusion bioreactor that is inside the cell membranes of the cells, expressed as a percentage or fraction (mL biovolume/mL bioreactor); and

wherein said biomass sensor is calibrated to measure biovolume fraction; and

adjusting the nutrient media flow rate into the perfusion bioreactor based on said biovolume fraction sensed by the biomass sensor, the controller being configured to adjust the media flow rate based on information received from the biomass sensor, wherein the nutrient media flow rate is adjusted based on the following relationship:

$$P = K \cdot \phi$$

wherein K is the biovolume specific perfusion rate (mL feed/mL biovolume/day);

ϕ is the biovolume fraction sensed by the biomass sensor; and

P is the perfusion rate expressed in mL feed/mL bioreactor/day.

2. A process according to claim 1, wherein the biomass sensor is a capacitance sensor.

3. A process according to claim 1 or claim 2, wherein the controller is configured to increase the flow rate of the nutrient media as the biovolume increases.

4. A process according to any one of the preceding claims, further comprising the step of determining the amount of fluid media within the perfusion bioreactor and based on the amount, selectively increasing or decreasing a rate at which the fluid media is withdrawn from the perfusion bioreactor.

5. A process according to claim 4, wherein the amount of fluid media within the perfusion bioreactor is determined by weighing the perfusion bioreactor using a weighing device.
6. A process according to claim 5, wherein the weighing device is in communication with the controller, and based on weight information from the weighing device, the controller is configured to control a pumping device in fluid communication with the perfusion bioreactor for selectively increasing or decreasing the rate at which fluid media is withdrawn.
7. A process according to claim 4, wherein the amount of fluid media within the perfusion bioreactor is determined by measuring volume.
8. A process according to any one of the preceding claims, wherein the cell density in the cell culture in the perfusion bioreactor increases over time such as by at least 60% per day.
9. A process according to any one of the preceding claims, wherein the biomass sensor determines the biovolume within the perfusion reactor at least every 6 hours.
10. A process according to any one of the preceding claims, wherein the perfusion bioreactor has a volume of from 10L to 4000L.
11. A process according to any one of the preceding claims, wherein after an incubation period, the process further includes transferring the cell culture from the perfusion bioreactor to a second bioreactor, the second bioreactor having a volume that is larger than a volume of the perfusion bioreactor, the volume ratio between the perfusion bioreactor and the second bioreactor being from 1:3 to 1:40, such as from 1:4 to 1:10.
12. A process according to claim 11, wherein the cell culture continues to grow in the second bioreactor in a fed batch-type manner.
13. A process according to claim 11 or 12, wherein the cell culture remains in the perfusion bioreactor from 3 days to 12 days and remains in the second bioreactor for less than 12 days, such as less than 10 days.
14. A process according to any one of the preceding claims, wherein the cell culture reaches a cell density within the perfusion bioreactor of greater than $10 \times$

10^6 cells/ mL, such as greater than 30×10^6 cells/mL, such as greater than 50×10^6 cells/mL, such as greater than 70×10^6 cells/mL.

15. A process according to any one of the preceding claims, wherein the cell culture reaches a cell density within the perfusion bioreactor of 100×10^6 cells/mL or greater.

16. A process according to any one of the preceding claims, wherein the cell culture comprises mammalian cells.