The applicant seeks to provide cell culture methods and systems which address at least these problems to create time efficient cell culture methods and systems which requires less human intervention during cell production.

Summary

In one aspect, provided herein is a method of providing a seed train comprising:
culturing mammalian cells in a chemostat, turbidostat or auxostat,
performing continuous culturing using the chemostat, turbidostat or auxostat.

In another aspect, provided herein is a chemostat, turbidostat or auxostat for use in the method described herein.

In one aspect, provided herein is a cell culture system, comprising a chemostat, turbidostat or auxostat as described herein and a bioreactor.

According to a first example of the disclosure, there is provided a method of providing a seed train comprising:
culturing mammalian cells in a chemostat or turbidostat,
performing continuous culturing using the chemostat or turbidostat.

In one example, the method may further comprise varying the dilution rate.

In one example, the dilution rate may be about 0.026 days $^{-1}$. In one example, the dilution rate is about 0.28 days^{-1} to about 1.11 days^{-1}, optionally wherein the dilution rate is 0.026 hours^{-1}.

In one example, the cell density and/or nutrient concentration and/or metabolite levels may be kept constant over time.

In one example, the cells may be maintained in the exponential phase.

In one example, the chemostat or turbidostat may have a volume of between about 0.05 litres to about 1 litre.

In one example, the chemostat or turbidostat may have a volume of between about 0.1 litres to about 50 litres.

In one example, the chemostat or turbidostat may have a volume of between about 0.5 litres to about 25 litres.

In one example, the chemostat or turbidostat may have a volume of between about 1 litre to about 10 litres.

In one example, the chemostat or turbidostat may comprise an inflow line to allow fresh culture medium to be continuously supplied to the culture vessel, an outflow line to allow fresh culture medium to be continuously removed to the culture vessel.

In one example, the method may further comprise the step of seeding the chemostat or turbidostat with about 50,000 to about 150,000 cells/mL.

In one example, the method may further comprise the step of seeding the chemostat or turbidostat with about 50,000 to about 100,000 cells/mL.

According to a second example of the disclosure, there is provided a chemostat or turbidostat for use in the disclosure.

According to a third example of the disclosure, there is provided a medium exchange method for use in cell culture, comprising the steps of:
culturing cells in a vessel,
gravitational settling separation, and
media exchange.

In one example, the vessel may comprise an agitator, an internal dip-tube affixed to outflow line to allow culture medium to be removed from the culture vessel, an inflow line to allow fresh culture medium to be supplied to the culture vessel.

In one example, the method may further comprise the step of removing agitation by deactivating the agitator.

In one example, the method may further comprise the step of concentrating the cells using gravitational settling separation to form a concentrated cell aggregation.

In one example, the step of removing agitation by deactivating the agitator may be controlled by a biomass detector and/or timer and/or metabolite detector.

In one example, the step of media exchange comprises extending a dip-tube into said vessel.

In one example, the dip-tube may extend up to about 95% of vessel height.

In one example, the dip-tube may extract growth media from the vessel following the step of gravitational settling separation.

In one example, the vessel may be replenished with fresh media following the step of media extraction.

In one example, the method may further comprise a step of controlling heat and/or gas sparging.

In one example, wherein the vessel has a volume of between about 1 litre to about 50,000 litres.

In one example, the vessel may have a volume of between about 50 litres to about 10,000 litres.

In one example, the vessel may have a volume of between about 100 litres to about 1,000 litres.

In one example, the cells may have a high propensity to form aggregates.

In one example, the cells may be genetically modified.

In one example, the cells may be eukaryotic.

In one example, the cells may be mammalian.

In one example, the gravitational settling separation may be performed for between about 5 minutes to about 3 hours.

In one example, the gravitational settling separation may be performed for between about 20 minutes to about 2 hours.

According to a fourth example of the disclosure, there is provided a bioreactor comprising an inflow line to allow fresh culture medium to be supplied to the culture vessel, an outflow line to allow fresh culture medium to be removed to the culture vessel, and a vessel, wherein the vessel comprises an agitator and an internal dip-tube in fluid communication with said outflow line.

In one example, the bioreactor further comprising a biomass detector and/or timer and/or metabolite detector.

In one example, the dip-tube may be extendable.

In one example, the dip-tube may extend between about 70% to about 95% of vessel height.

In one example, the bioreactor may further comprise a device to control heat and/or gas sparging.

In one example, the device may be reversibly activated.

In one example, the bioreactor may have a volume of between about 1 litre to about 50,000 litres.

In one example, the bioreactor may have a volume of between about 50 litres to about 10,000 litres.

In one example, the bioreactor may have a volume of between about 100 litres to about 1,000 litres.

In one example, the bioreactor may be for culturing cells which have a high propensity to form aggregates.

In one example, the cells may be genetically modified.

In one example, the cells may be eukaryotic.

In one example, the cells may be mammalian.

According to a fifth example of the disclosure, there is provided a cell culture method comprising:

a method of providing a seed train according to the disclosure, and

a medium exchange method according to the disclosure.

In one example of the cell culture method, the method of providing a seed train may be performed in a chemostat or turbidostat and the medium exchange method may be performed at a predetermined time point after incubation of the cells from the seed train in chemostat or turbidostat have been cultured in a separate additional bioreactor.

According to a fifth example of the disclosure, there is provided a cell culture system, comprising a chemostat or turbidostat according to the disclosure and a bioreactor according to the disclosure.

Brief description of figures

The invention will now be described with reference to the accompanying non-limiting figures.

Figure 1: Schematic of unit operations and FTE requirements and FTE requirements for typical seed train to 600L terminal bioreactor.

Figure 2: Cell density, % viability and cumulative doubling time for batch reactors R6 and R9.

Figure 3: Cell density during 42-day chemostat, showing 2 steady stat periods and key actions.

Figure 4: Cell viability and average cell diameter during 42-day chemostat

Figure 5: Images of cells at 4x, 10x and 20x magnification on day 14 of the chemostat.

Figure 6: Schematic delineating the transfer of cells during 600L seed train, using chemostat and batch reactors at the 3L scale.

Figure 7: Cell density, % viability and cumulative doubling time for 20L batch reactors R11 (seeded from chemostat and batch reactor) and R14 (seeded from chemostat alone).

Claims

1. A method of providing a seed train comprising:
   culturing mammalian cells in a chemostat, turbidostat or auxostat,
   performing continuous culturing using the chemostat, turbidostat or auxostat.

2. The method according to claim 1, further comprising varying the dilution rate.

3. The method according to claim 2, wherein the dilution rate is 0.28 $days^{-1}$ to 1.11 $days^{-1}$, optionally wherein the dilution rate is 0.026 $hours^{-1}$.

4. The method according to any of the preceding claims, wherein the cell density and/or nutrient concentration and/or metabolite levels are kept constant over time.

5. The method according to any of the preceding claims, wherein the cells are maintained in the exponential phase.

6. The method according to any of the preceding claims, wherein the chemostat, turbidostat or auxostat has a volume of between 0.05 litres to 1 litre.

7. The method according to any of the preceding claims, wherein the chemostat, turbidostat or auxostat has a volume of between 0.1 litres to 50 litres.

8. The method according any one of claims 1 to 6, wherein the chemostat, turbidostat or auxostat has a volume of between 0.5 litres to 25 litres.

9. The method according any one of claims 1 to 6, wherein the chemostat, turbidostat or auxostat has a volume of between 1 litre to 10 litres.

10. The method according to any of the preceding claims, wherein the chemostat, turbidostat or auxostat comprises an inflow line to allow fresh culture medium to be continuously supplied to the culture vessel, an outflow line to allow fresh culture medium to be continuously removed to the culture vessel.

11. The method according to any of the preceding claims, further comprising the step of seeding the chemostat, turbidostat or auxostat with 50,000 to 150,000 cells/mL.

12. The method according any one of claims 1 to 10, further comprising the step of seeding the chemostat, turbidostat or auxostat with 50,000 to 100,000 cells/mL.

13. A chemostat, turbidostat or auxostat for use in the method of claims 1 to 10.

14. A cell culture system, comprising a chemostat, turbidostat or auxostat according to claim 13 and a bioreactor.