

Cell culturing systems and methods

Field of invention

- 5 The invention relates to systems and methods for cell culture. More particularly, the invention relates to systems and methods for increasing cell volume and yield in an optimal timeframe.

Background

- 10 Providing cells at an optimal state and at an optimal density is time-consuming and labour intensive. Making cell culture commercially viable requires creating a necessary cell density in as short a timeframe as possible. Time consuming human interventions into the process in order to achieve this can reduce the efficiency of cell culture.
- 15 One example is creating enough cells to be used as a starter culture for a production sized bioreactor. This is often referred to as a seed train. A seed train requires culturing a small number of cells until they are at a sufficient cell density and in an exponential phase of growth such that they are in an optimal state to be used as the starter culture for the production bioreactor. This is a time and labour-intensive exercise and the cells are only in
- 20 the exponential phase for a small window. Once the cells have been transferred from the seed train to the larger production vessel, the bioreactor used for the seed train must be cleaned prior to it being used again. There is therefore a need to optimise this process and more efficiently provide cells in an exponential phase ready for use to seed the larger production reactor.
- 25 A further issue with current cell culture methods and systems is that nutrients in growth media are depleted over time and need to be replaced to achieve high density cell cultures. The state-of-the-art technologies used for cell clarification are typically centrifugation, perfusion or filtration-based and therefore the process requires additional equipment. These
- 30 techniques are therefore labour intensive and time consuming. In addition, the mechanical intervention into the culture system also leads to cell damage and cell death which can be counter productive and increase culturing time.

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.

5 Summary of the invention

According to a first aspect of the invention, there is provided a method of providing a seed train comprising:

- culturing mammalian cells in a chemostat or turbidostat,
- 10 performing continuous culturing using the chemostat or turbidostat.

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

- 15 In one embodiment, the dilution rate may be about 0.026 days^{-1} . In one embodiment, 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} .

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

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

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

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

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

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

In one embodiment, 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.

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

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

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According to a third aspect of the invention, 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.

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In one embodiment, 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.

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

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

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

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

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

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

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

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

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

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

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

In one embodiment, the cells may be genetically modified.

25 In one embodiment, the cells may be eukaryotic.

In one embodiment, the cells may be mammalian.

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

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

According to a fourth aspect of the invention, 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
5 outflow line.

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

10 In one embodiment, the dip-tube may be extendable.

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

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

In one embodiment, the device may be reversibly activated.

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

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

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

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

In one embodiment, the cells may be genetically modified.

In one embodiment, the cells may be eukaryotic.
35

In one embodiment, the cells may be mammalian.

According to a fifth aspect of the invention, there is provided a cell culture method comprising:

- 5 a method of providing a seed train according to the invention, and
 a medium exchange method according to the invention.

In one embodiment 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
10 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 aspect of the invention, there is provided a cell culture system, comprising a chemostat or turbidostat according to the invention and a bioreactor according
15 to the invention.

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
20 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
25 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.
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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
35 (seeded from chemostat and batch reactor) and R14 (seeded from chemostat alone).

Figure 8: BP_TB_006 'improved' cell growth profile

Figure 9: BP_TB_006 'improved' waste metabolite and nutrient profile.

5 Figure 10: BP_TB_005 'typical' cell growth curve

Figure 11: BP_TB_005 Waste metabolite and nutrient curves

Figure 12: Further examples of LPH growth curves

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Detailed description

15 In one aspect of the invention, there is provided 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. A chemostat is a bioreactor to which fresh medium is continuously added, while culture liquid containing left over nutrients, metabolic end products and microorganisms is continuously removed at the same rate to keep the culture volume constant. A turbidostat operates in a similar fashion to
20 a chemostat but maintains a constant turbidity of the culture. An auxostat is also similar to a chemostat but uses feedback from a measurement taken on the growth chamber to control the media flow rate, maintaining the measurement at a constant.

25 Traditionally, a seed train will be run in batch mode and cells transferred to the following process (typically increasing in vessel volume) once cells reach an acceptable density. Typically, cells will only be in an acceptable state of optimal growth kinetics for cell transfer for a short period of time before nutrients begin to deplete or metabolites build up and cell growth kinetics decline. Additionally, there is a risk of cells not growing under acceptable kinetics and therefore will not be able to be used as a cell source.

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Operating under chemostat removes the risk of cells not growing under acceptable kinetics as well as keeping the growth kinetics at the most optimal. Nutrients are able to be kept at a level sufficient for cell growth while simultaneously metabolites are removed and are kept at low levels as to not hinder cell proliferation. Chemostat culture also operates as a closed
35 system. It achieves these benefits with minimal intervention which decreases the labour-

intensive hours required to gain a healthy cell source compared to a traditional seed train. Additionally, the lack of human intervention results in reduced microbacterial contamination of the media which may be otherwise introduced by routine handling. As such, antibiotic free media may be used.

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Therefore, a chemostat, turbidostat or auxostat can be used as an efficient seed train because fresh media being constantly added and depleted media constantly removed maintains the cells at an exponential phase of growth. This means that cells are always ready to be taken as a seed culture for a larger production bioreactor.

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A chemostat requires fresh media to be continually fed at the same rate that culture is removed from the bioreactor. The rate at which culture is replaced with fresh media is called the dilution rate. As live cells will be being removed from the bioreactor as the cells are still proliferating it is important to ensure the dilution rate is not so high that culture is being removed faster than cells proliferating and a washout occurs where all cells eventually leave the system. In one embodiment, the dilution rate is about 0.026 days⁻¹. In one embodiment, 561 mL/day of fresh media is being added and 561 mL/day of culture is leaving the system. In one embodiment, the dilution rate is about 0.026 hours⁻¹. In one embodiment, the dilution rate is about 0.624 days⁻¹. In one embodiment, the method further comprises varying the dilution rate. In one embodiment, the dilution rate is about 0.05 days⁻¹ to about 0.011 days⁻¹, about 0.049 days⁻¹, about 0.048 days⁻¹, about 0.047 days⁻¹, about 0.047 days⁻¹, about 0.046 days⁻¹, about 0.045 days⁻¹, about 0.044 days⁻¹, about 0.043 days⁻¹, about 0.042 days⁻¹, about 0.041 days⁻¹, about 0.04 days⁻¹, about 0.039 days⁻¹, about 0.038 days⁻¹, about 0.037 days⁻¹, about 0.036 days⁻¹, about 0.035 days⁻¹, about 0.034 days⁻¹, about 0.033 days⁻¹, about 0.032 days⁻¹, about 0.031 days⁻¹, about 0.030 days⁻¹, about 0.030 days⁻¹, 0.029 days⁻¹, 0.028 days⁻¹, 0.027 days⁻¹, 0.026 days⁻¹, about 0.025 days⁻¹, 0.024 days⁻¹, 0.023 days⁻¹, 0.022 days⁻¹, 0.021 days⁻¹, about 0.020 days⁻¹, about 0.019 days⁻¹, about 0.018 days⁻¹, about 0.017 days⁻¹, about 0.016 days⁻¹, about 0.015 days⁻¹, about 0.014 days⁻¹, about 0.013 days⁻¹, about 0.012 days⁻¹, about 0.011 days⁻¹, about 0.01 days⁻¹. In one embodiment, the dilution rate is about 0.28 days⁻¹ to about 1.11 days⁻¹. In one embodiment, the dilution rate is about 0.10 days⁻¹, about 0.12 days⁻¹, about 0.14 days⁻¹, about 0.16 days⁻¹, about 0.18 days⁻¹, about 0.20 days⁻¹, about 0.22 days⁻¹, about 0.24 days⁻¹, about 0.26 days⁻¹, about 0.28 days⁻¹, about 0.30 days⁻¹, about 0.32 days⁻¹, about 0.34 days⁻¹, about 0.36 days⁻¹, about 0.38 days⁻¹, about 0.40 days⁻¹, about 0.42 days⁻¹, about 0.44 days⁻¹, about 0.46 days⁻¹, about 0.48 days⁻¹, about 0.50 days⁻¹, about 0.52 days⁻¹, about 0.54 days⁻¹, about 0.56 days⁻¹, about 0.58 days⁻¹, about 0.60 days⁻¹,

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about 0.62 days⁻¹, about 0.64 days⁻¹, about 0.66 days⁻¹, about 0.68 days⁻¹, about 0.70 days⁻¹,
about 0.72 days⁻¹, about 0.74 days⁻¹, about 0.76 days⁻¹, about 0.78 days⁻¹, about 0.80 days⁻¹,
about 0.82 days⁻¹, about 0.84 days⁻¹, about 0.86 days⁻¹, about 0.88 days⁻¹, about 0.90 days⁻¹,
about 0.92 days⁻¹, about 0.94 days⁻¹, about 0.96 days⁻¹, about 0.98 days⁻¹, about 1.00 days⁻¹,
5 about 1.02 days⁻¹, about 1.04 days⁻¹, about 1.06 days⁻¹, about 1.08 days⁻¹, about 1.10 days⁻¹,
about 1.12 days⁻¹, about 1.14 days⁻¹, about 1.16 days⁻¹, about 1.18 days⁻¹, about 1.20 days⁻¹,
about 1.30 days⁻¹, about 1.40 days⁻¹, about 1.50 days⁻¹, about 1.60 days⁻¹, about 1.70 days⁻¹,
about 1.80 days⁻¹, about 1.90 days⁻¹, about 2.00 days⁻¹. In one embodiment the dilution rate
is about 0.0115 hours⁻¹ to about 0.0463 hours⁻¹. In one embodiment, the dilution rate is about
10 0.005 hours⁻¹, about 0.0075 hours⁻¹, about 0.01 hours⁻¹, about 0.0115 hours⁻¹, about 0.012
hours⁻¹, about 0.014 hours⁻¹, about 0.016 hours⁻¹, about 0.018 hours⁻¹, about 0.020 hours⁻¹,
about 0.022 hours⁻¹, about 0.024 hours⁻¹, about 0.026 hours⁻¹, about 0.028 hours⁻¹, about
0.030 hours⁻¹, about 0.032 hours⁻¹, about 0.034 hours⁻¹, about 0.036 hours⁻¹, about 0.038
hours⁻¹, about 0.040 hours⁻¹, about 0.042 hours⁻¹, about 0.044 hours⁻¹, about 0.046 hours⁻¹,
15 about 0.050 hours⁻¹, about 0.060 hours⁻¹, about 0.070 hours⁻¹, about 0.080 hours⁻¹, about
0.090 hours⁻¹, about 0.10 hours⁻¹.

In some embodiment, cells are seeded into the bioreactor and the system will be dynamic
with the cell density increasing over time. Eventually, a steady state will be reached where
20 cell density and nutrient and metabolite levels do not change over time. At any point the only
intervention needed is to replenish the fresh media reservoir and to harvest the effluent
vessel. In one embodiment, the cell density and/or nutrient concentration and/or metabolite
levels are kept constant over time. In one embodiment, the cell density is about 0.05×10^6
cells/ml to about 200×10^6 cells/ml, about 1×10^6 cells/ml, about 2×10^6 cells/ml, about 3×10^6
25 cells/ml, about 4×10^6 cells/ml, about 5×10^6 cells/ml, about 10×10^6 cells/ml, about 20×10^6
cells/ml, about 30×10^6 cells/ml, about 40×10^6 cells/ml, about 50×10^6 cells/ml, about 60×10^6
cells/ml, about 70×10^6 cells/ml, about 80×10^6 cells/ml, about 90×10^6 cells/ml, about 100×10^6
cells/ml, about 110×10^6 cells/ml, about 120×10^6 cells/ml, about 130×10^6 cells/ml, about
140 $\times 10^6$ cells/ml, about 150×10^6 cells/ml, about 160×10^6 cells/ml, about 170×10^6 cells/ml,
30 about 180×10^6 cells/ml, about 190×10^6 cells/ml, about 200×10^6 cells/ml. In one embodiment,
the chemostat, turbidostat or auxostat may be operated for about 42 days. In one
embodiment, the chemostat, turbidostat or auxostat may be operated for long periods of time
as long as sterility is maintained. In one embodiment, the chemostat, turbidostat or auxostat
may be operated for about 1 day, for about 5 days, for about 10 days, for about 15 days, for
35 about 20 days, for about 25 days, for about 30 days, for about 35 days, for about 40 days, for

about 45 days, for about 50 days, for about 55 days, for about 60 days, for about 3 months, for about 3.5 months, for about 4 months, for about 4.5 months, for about 5 months, for about 5.5 months, for about 6 months.

- 5 To culture the cells, different chemostat, turbidostat or auxostat of different sizes and designs may be used. For example, in some embodiments, the culture volume of the chemostat, turbidostat or auxostat is about 0.05 litres to about 1 litre, 0.075 litres to about 1 litre, 0.1 litres to about 1 litre, 0.2 litres to about 1 litre, 0.25 litres to about 1 litre, about 0.5 litres, about 1 litre, about 1.5 litres, about 2 litres, about 3 litres, about 4 litres, about 5 litres, about 6 litres, about 7 litres, about 8 litres, about 9 litres, about 10 litres, about 15 litres, about 20 litres, about 25 litres, about 30 litres, about 35 litres, about 40 litres, about 45 litres, about 50 litres, about 55 litres, about 60 litres, about 65 litres, about 70 litres, about 75 litres, about 80 litres, about 85 litres, about 90 litres, about 95 litres, about 100 litres, about 150 litres, about 200 litres, about 250 litres, about 300 litres, about 350 litres, about 400 litres, about 450 litres, about 500 litres, about 550 litres, about 600 litres, about 650 litres, about 700 litres, 750 litres, about 800 litres, about 850 litres, about 900 litres, about 950 litres, or about 1,000 litres. In some embodiments, the culture volume of the vessel is between about 0.1 litres to about 0.25 litres, about 0.1 litres to about 0.5 litres, about 0.1 litres to about 1 litres, about 0.1 litres to about 1.5 litres, about 0.1 litres to about 2 litres, about 0.1 litres to about 2.5 litres, about 0.1 litres to about 3 litres, about 0.1 litres to about 3.5 litres, about 0.1 litres to about 4 litres, about 0.1 litres to about 4.5 litres, about 0.1 litres to about 5 litres, about 0.1 litres to about 7.5 litres, about 0.1 litres to about 10 litres, about 0.1 litres to about 15 litres, about 0.1 litres to about 20 litres, about 0.1 litres to about 25 litres, about 0.1 litres to about 30 litres, about 0.1 litres to about 35 litres, about 0.1 litres to about 45 litres, about 0.1 litres to about 50 litres, about 0.5 litres to about 1 litres, about 0.5 litres to about 1.5 litres, about 0.5 litres to about 2 litres, about 0.5 litres to about 2.5 litres, about 0.5 litres to about 3 litres, about 0.5 litres to about 3.5 litres, about 0.5 litres to about 4 litres, about 0.5 litres to about 4.5 litres, about 0.5 litres to about 5 litres, about 0.5 litres to about 7.5 litres, about 0.5 litres to about 10 litres, about 0.5 litres to about 15 litres, about 0.5 litres to about 20 litres, 0.5 litres to about 25 litres, about 1 litre to about 2 litres, about 1 litre to about 3 litres, about 1 litres to about 4 litres, about 1 litre to about 5 litres, about 1 litre to about 6 litres, about 1 litre to about 7 litres, about 1 litre to about 8 litres and about 1 litre to about 9 litres, or about 1 litre to about 10 litres.

In one embodiment, 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.

- 5 In some embodiments, gravitational settling separation is performed for between about 5 minutes to about 3 hours, about 5 minutes to about 2 hours, about 5 minutes to about 1 hours, about 5 minutes to about 30 minutes, 5 minutes to about 20 minutes, about 20 minutes to about 2 hours, about 20 minutes to about 1 hours, about 20 minutes to about 45 minutes, 20 minutes to about 30 minutes, about 30 minutes to about 3 hours, about 30 minutes to about 2 hours, about 30 minutes to about 1 hours.

- 15 In one embodiment, the method of providing a seed train further comprising the step of seeding the chemostat, turbidostat or auxostat with about 50,000 to about 150,000 cells/mL, about 50,000 to about 150,000 cells/mL. Low concentrations of cells as a starter culture for the chemostat or turbidostat has been found to be advantageous.

According to an aspect of the invention, there is provided a chemostat, turbidostat or auxostat for use in the above-mentioned method of providing a seed train.

- 20 According to one aspect, the invention may relate to a medium exchange method for use in cell culture, comprising the steps of culturing cells in a vessel, gravitational settling separation, and media exchange. The use of such a method allows for 'light partial harvesting' of the cells being cultured in the vessel and is advantageous because the method does not exert as much stress onto the cells as centrifugation-based cell clarification methods and the method is referred to henceforth as 'light partial harvesting'.

- 30 Commonly used techniques for cell clarification are typically centrifugation or filtration-based which requires additional equipment and can lead to damage of the cells. The 'light partial harvest' achieves high levels of cell retention by exploiting the cells' propensity to settle in stationary liquid under the force of gravity. The only additional equipment required is a straight or fractionated internal dip-tube that is used to draw spent media from the reactor while preventing cell clumps from leaving the reactor.

- 35 In one embodiment, the cells used in the medium exchange method have a high propensity to form aggregates. For example, suspension-adapted cells that have a high propensity to

form aggregates and settle in the absence of power-input. The suspension adapted cells were previously adherent cells but were adapted for suspension cell culture. They retain certain features of adherent cells, such as increased aggregation. In one embodiment, the cells have increased amounts of factors involved in aggregation, such as integrins. In one
5 embodiment, the cells are genetically modified. For example, the cells may be immortalised. In one embodiment, the cells are genetically edited. In one embodiment, the cells are eukaryotic. In one embodiment, the cells are mammalian. For example, suspension-adapted cultured meat cells could be used in the method of the present invention. Growing cultured meat cells in suspension cultures can be advantageous over growing them in adherent
10 cultures because cells can be grown in large quantities.

The 'light partial harvest' technique may be carried out at scheduled intervals throughout culture, or in response to metabolite accumulation. The 'light partial harvest' therefore allows
15 cells to grow to higher densities (or concentrations) than would otherwise be possible through simple batch or fed-batch culture, and therefore can be regarded as a bioprocess intensification strategy. It is surprising that the invention performs as well as it does as convention thinking is that it would take too long for the cells to settle and that the cells would be damaged or killed through lack of oxygenation because the agitator is turned off during
20 cell settling. However, this is not the case and the inventors have demonstrated that the cells are not adversely affected by the process. In fact, the process provides an advantage to the cells by providing fresh media without the potential for contamination. The lack of intervention by human hand in the sealed system of the bioreactor is highly beneficial to preventing bacterial contamination.

25 Additionally, through intensifying the cell cultivation process the load imposed on the downstream process (DSP) is reduced, which is advantageous from an economic perspective.

30 According to a further aspect of the invention, 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 (Fig. 1). The bioreactor may be used in the aforementioned method of the
35 invention described above.

The method of the invention and the associated bioreactor are therefore advantageous because it is highly efficient and efficacious (able to achieve high levels of cell retention) while being scalable (in terms of operation, function, and financial viability) and being non-damaging to cells.

In one embodiment, the vessel of the bioreactor comprises 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 some embodiment, the agitation in the vessel may be removed by deactivating the agitator. Deactivation of the agitator causes the cells in the vessel to begin to aggregate and sink to the lower parts of the vessel due to gravity over a set period of time. The method therefore comprises the step of concentrating the cells using gravitational settling separation to form a concentrated cell aggregation.

Deactivating the agitator may be controlled by a biomass detector and/or timer and/or metabolite detector. As culture time progresses, nutrients in the media will be consumed and, eventually, the culture media will not be able to sustain continued growth of the cell in the exponential phase. Therefore, a metabolite detector may be used to determine when the nutrients are being depleted and deactivate the agitator such that gravitational settling separation occurs and the vessel is ready for removal of media depleted of nutrients and replenishment with fresh media. Alternatively, a biomass detector or probe may be used to ascertain the correct amount of time required for settling based upon biomass measurements. Alternatively, this could be done at set time points using a timer.

In one embodiment, the step of media exchange comprises extending a dip-tube into said vessel. In some embodiments, the dip-tube is a dip-tube of a specific geometry and length to remove the spent media without drawing cell aggregates into the effluent flow. In certain embodiments, the dip-tube extends up to about 95% of vessel height. In one embodiment, the dip-tube extends from the headplate to 10%-30% in vessel height above the base of the bioreactor. The dip-tube serves to remove spent media from the reactor. The internal dip-tube may be sized according to the geometry of the vessel in use. In some embodiments, the dip-tube should extend somewhere between 70% to 95% along the length (height) of the vessel from the headplate.

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The dip-tube can be used to extract growth media from the vessel following the step of gravitational settling separation. Fresh media may be used to replenish the vessel following the step of media extraction using the dip-tube.

- 5 The method of the invention may further comprise a step of controlling heat and/or gas sparging. During the 'light partial harvest' it is necessary to prevent any disruption to the settling cells, hence gas sparging may be turned off in some embodiments. Further, during the 'light partial harvest' temperature control loops which provide heat to the culture may be turned off in some embodiments. In some embodiments, under settling conditions, the
- 10 reactor contents may not be mixed, and therefore providing heat during the 'light partial harvest' process could cause overheating and the formation of 'hot-spots' in the reactor. The bioreactor may therefore comprise means to control the heat and/or gas sparging. In one embodiment the bioreactor may comprise a device to control heat and/or gas sparging.
- 15 To culture the cells, different vessels of different sizes and designs may be used. For example, in some embodiments, the culture volume of the vessel is about 0.015 litres, about 0.02 litres, about 0.025 litres, about 0.03 litres, about 0.035 litres, about 0.040 litres, about 0.045 litres, about 0.5 litres, about 1 litre, about 1.5 litres, about 2 litres, about 3 litres, about 4 litres, about 5 litres, about 6 litres, about 7 litres, about 8 litres, about 9 litres, about 10
- 20 litres, about 15 litres, about 20 litres, about 25 litres, about 30 litres, about 35 litres, about 40 litres, about 45 litres, about 50 litres, about 55 litres, about 60 litres, about 65 litres, about 70 litres, about 75 litres, about 80 litres, about 85 litres, about 90 litres, about 95 litres, about 100 litres, about 150 litres, about 200 litres, about 250 litres, about 300 litres, about 350 litres, about 400 litres, about 450 litres, about 500 litres, about 550 litres, about 600 litres,
- 25 about 650 litres, about 700 litres, 750 litres, about 800 litres, about 850 litres, about 900 litres, about 950 litres, about 1,000 litres, about 1,500 litres, 2,000 litres, about 2,500 litres, about 3,000 litres, about 3,500 litres, about 4,000 litres, about 5,000 litres, about 5,500 litres, about 6,000 litres, about 7,000 litres, about 8,000 litres, about 9,000 litres, about 10,000 litres, about 15,000 litres, about 20,000 litres, about 25,000 litres, about 30,000 litres, about
- 30 40,000 litres, about 50,000 litres, about 60,000 litres, about 70,000 litres, about 80,000 litres, about 90,000 litres, or about 100,000 litres. In some embodiments, the culture volume of the vessel is between about 1 litre to about 50 litres, about 1 litre to about 100 litres, about 1 litre to about 150 litres about 1 litre to about 200 litres about 1 litre to about 250 litres, about 1 litre to about 300 litres, about 1 litre to about 400 litres, about 1 litre to about 500 litres, about 1
- 35 litre to about 600 litres, about 1 litre to about 700 litres, about 1 litre to about 800 litres, about

- 1 litre to about 900 litres, about 1 litre to about 1,000 litres, about 1 litre to about 2,000 litres, about 1 litre to about 3,000 litres, about 1 litre to about 4,000 litres, about 1 litre to about 5,000 litres, about 1 litre to about 10,000 litres, about 1 litre to about 20,000 litres, about 1 litre to about 30,000 litres, about 1 litre to about 40,000 litres, 1 litre to about 50,000 litres,
- 5 about 50 litres to about 100 litres, about 50 litres to about 150 litres about 50 litres to about 200 litres about 50 litres to about 250 litres, about 50 litres to about 300 litres, about 50 litres to about 400 litres, about 50 litres to about 500 litres, about 50 litres to about 600 litres, about 50 litres to about 700 litres, about 50 litres to about 800 litres, about 50 litres to about 900 litres, about 50 litres to about 1,000 litres, about 50 litres to about 2,000 litres, about 50 litres
- 10 to about 3,000 litres, about 50 litres to about 4,000 litres, about 50 litres to about 5,000 litres, about 50 litres to about 10,000 litres, 50 litres to about 20,000 litres, about 100 litres to about 150 litres about 100 litres to about 200 litres about 100 litres to about 250 litres, about 100 litres to about 300 litres, about 100 litres to about 400 litres, about 100 litres to about 500 litres, about 100 litres to about 600 litres, about 100 litres to about 700 litres, about 100 litres
- 15 to about 800 litres, about 100 litres to about 900 litres, about 100 litres to about 1,000 litres, about 100 litres to about 2,000 litres, about 100 litres to about 3,000 litres, about 100 litres to about 4,000 litres, about 100 litres to about 5,000 litres, or about 100 litres to about 10,000 litres.
- 20 According to one aspect of the invention, there is provided the use of the bioreactor to culture cells have a high propensity to form aggregates. For example, suspension-adapted cells that have a high propensity to form aggregates and settle in the absence of power-input. In one embodiment, the cells are genetically modified. In one embodiment, the cells are genetically edited. In one embodiment, the cells are eukaryotic. In one embodiment, the cells are
- 25 mammalian. For example, suspension-adapted cultured meat cells could be used in the method of the present invention. Growing cultured meat cells in suspension cultures can be advantageous over growing them in adherent cultures because cells can be grown in large quantities.
- 30
- The term "cultivated meat" is used herein to describe meat grown from *in vitro* animal cell culture distinguished from meat of slaughtered animals. Additional terms that may be used in the Art to describe meat grown from *in vitro* animal cell culture include cultured meat, cell-grown meat, clean meat, lab-grown meat, test tube meat, *in vitro* meat, tube steak, synthetic
- 35 meat, cell-cultured meat, cell grown meat, tissue engineered meat, engineered meat, artificial

meat, and manmade meat. The phrases “cell-based meat”, “slaughter-free cell- based meat”, “*in vitro* produced meat”, “*in vitro* cell-based meat”, “cultured meat”, “slaughter- free cultured meat”, “ *in vitro* produced cultured meat”, “*in vitro* meat”, “*in vitro* cultured meat” and other similar such phrases are interchangeably used herein, and refer to the meat that is generated
5 *in vitro*, starting with cells in culture, and methods which does not involve the slaughter of an animal in order to directly obtain meat from an animal for dietary consumption. The modified cells of the invention may be suitable for human and/or non-human consumption. In some embodiments, the cell-based meat is suitable for consumption by animals, such as domesticated animals. Accordingly, the cellular biomass herein support the growth of “pet
10 food”, e.g. dog food, cat food, and the like.

The terms “animal” and “non-human animal” with reference to animals and cells derived therefrom are used herein interchangeably and refer only to cells of non-human-animals. Cells for use in the invention may be of any other animal origin. However, the cells are not human
15 cells. Cells suitable for use in cellular agriculture are preferably non-human animal cells that provide a source of any dietary protein, fat and/or carbohydrate.

In one embodiment of the aspects of the invention, the modified cell is a primary cell. In another embodiment of the aspects of the invention, the modified cell is a somatic cell. Any somatic
20 cell suitable for use in cellular agriculture, that is the production of animal-sourced foods from cell culture, is within the scope of the invention. For example, the cell may be a fat or muscle cell. For example, the cell may be selected from one or more of the following cell types: myoblast, fibroblast, myofibroblast, adipose derived stem cell, epithelial cell, mesenchymal stem cell, satellite cell or hepatocytes.

25 The cells are cells of non-human animals that are suitable for human and animal consumption. These include animals such as non-human mammals, birds, fish, crustaceans, molluscs, reptiles, amphibians, or insects. Exemplary non-human mammals include those in the genera Bovinae, Camelidae, Canidae, Caprae, Cervidae, Felidae, Equidae, Lagomorphs,
30 Macropodidae, Oves, Rodents, or Suidae. The cells may be cells of any livestock or poultry. The cells may be porcine, bovine (e.g. cattle), ovine, caprine, avine, or piscine. The cell may be shrimp, prawn, crab, crayfish, and/or lobster. In one embodiment, the animal is a pig or bovine (e.g. cattle).

The animal used in various aspects of the invention may be of an animal species used in agriculture. An animal species used in is an animal farmed for human. Such animals are listed above. In a preferred embodiment, they include pig, bovine (e.g. cattle), poultry (e.g. chicken, turkey, duck, geese), sheep, goat, Equidae, fish, crustaceans or mollusc.

5

The method of this aspect of the invention may be used on a continuous cell culture system of a batch cell culture system.

According to one aspect of the invention, there is provided a cell culture method comprising a method of providing a seed train according to the above-mentioned method and a medium exchange method according to the above mentioned method.

In one embodiment, the method of providing a seed train is performed in a chemostat, turbidostat or auxostat and the medium exchange method is performed at a predetermined time point after incubation of the cells from the seed train in chemostat, turbidostat or auxostat have been cultured in a separate additional bioreactor. A combination of using a chemostat, turbidostat or auxostat to provide a seed train and a 'light partial harvesting method as described above creates an advantageous technique for cell culture which limits human intervention and makes the entire process less labour intensive and time consuming.

The combined technique also ensures that cell damage during the process is limited and high cell density can be achieved without the drawbacks of mechanical process being used for cell clarification.

According to a further aspect of the invention, there is provided a cell culture system, comprising a chemostat, turbidostat or auxostat as described above and a bioreactor as described above.

"and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example, "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein. Unless context dictates otherwise, the descriptions and definitions of the features set out above are not limited to any particular aspect or embodiment of the invention and apply equally to all aspects and embodiments which are described.

35

Examples

Continuous seed train (chemostat)

Upstream bioprocess seed trains are time-consuming and labour-intensive activities. They
5 require operators to manage culture vessels at a range of scales (erlenmeyers to large-scale
bioreactors). Seed trains also possess points of increased risk, during the material transfer
across scales (e.g. when cells are thawed and transferred to culture vessels under a
biological safety cabinet).

At Ivy Farm, the typical seed train from Erlenmeyer scale to the 600L pilot plant bioreactor
10 takes the form in Figure 1. Requiring 6 FTE to manage the seed train vessels/bioreactors.

A key factor for successful bioreactor seed trains is ensuring that cells maintain favourable
characteristics, so that they proliferate at each stage of the process. For this we use the
following selection criteria when deciding whether a population of cells will be transferred to
15 the next (larger stage) of the bioreactor process.

Table 1: Cell population selection criteria for cell transfer in seed train

<u>Parameter</u>	<u>Selection criteria</u>
Cell viability (%)	>95%
Doubling time (hrs)	<30 hrs
Cell diameter (micron)	14 to 16
Morphology	Low level of debris

20 During batch cultures, careful timing is required to ensure that cells do not leave their
exponential phase of growth, which is why a cumulative doubling time of <30 hrs is set. For
example, reactor R6 in Figure 2, leaves its exponential phase of growth (from day 3 to 4).
The cumulative doubling time of the cell population no longer fulfils the criteria for transfer
within our seed train. This results in a short operational window (less than a day), in which
25 enough cells are proliferating at the desired growth rate to seed the next bioreactor scale in
the seed train.

To increase the window of operation for seed train cell transfer, a chemostat was constructed. The chemostat was set at a dilution rate of 0.55 L/day (for a 1 L working volume) and was seeded at 0.05×10^6 cells/ml. Although it unconventional to seed a chemostat, at such a low density, we hypothesise that a lower seeding density for the chemostat, maximises the % removal of cells that exhibit a lag phase. The dilution rate used in a chemostat, ensures precise control over the cell population doubling time. When using a dilution of 0.55 vvd (as used in the chemostat shown in Figure 2), only a cell population that can proliferate with a doubling time of 30 hrs can maintain a stationary phase in the cell growth. If the bioreactor cell density continues to increase, regardless of this dilution rate, then they are proliferating at doubling times less than 30 hrs. This widens the window of operation from which an inoculum can be accessed for transfer within a seed train.

Figure 3 demonstrates the ability to maintain a chemostat, with cells at stationary or exponential growth phase for most of the bioreactor run. This was conducted with a dilution rate of 0.55 vvd (equivalent to a doubling time of 30 hrs) for a >40-day period. The chemostat demonstrated a seed train reactor, that provided cells that fulfil the growth kinetics criteria for the 600L seed train, for a 40-day window (rather than <1 days). The cell densities during the chemostat are $>2 \times 10^6$ cells/ml during the stationary phases. The cell density within the chemostat varies, as it was actively used on multiple occasions to seed a 20L reactor. During the chemostat, the cells satisfied the % viability (>95%) criteria and the cell size (14 to 16 micron) criteria for all times in which the cells were in growth or stationary phase. With days 16 to 19 representing the only days in which cell density reduction was observed (due to a deviation in the media storage conditions causing some media component degradation). The morphology of cells is shown in Figure 5. Although cell morphology at this stage is subjective, these cells would pass the morphology criteria, given the presence of viable cells with low levels of cell debris. The continuous removal of media of the chemostat would help to reduce the amount of cell debris present, in addition to providing the nutrient and removal of waste metabolites that provide the cells with a favourable environment.

An example of the chemostat's use in the seed train is given in Figure 6. Cells were transferred from the chemostat (R5) and the batch culture (R9) to two 20L reactors, as part of the seed train to the 600L bioreactor.

Figure 7 demonstrates the growth curves of the 2x 20L reactors seed from the chemostat. These 20L reactors were used to inoculate the 600L reactor.

2: Light Partial Harvest Media Exchange method

Waste metabolites are a constant challenge in cultivated meat cell culture. Specific waste
5 metabolites such as lactate and ammonium inhibit cell proliferation when a specific
concentration is reached during the culture. Waste metabolites are inevitable when culturing
mammalian cells and removing or reducing them can prove an invasive and difficult
challenge. Typical full media exchanges are typically used but are highly invasive and come
with a high contamination and cell loss risk.

10 The light partial harvest (LPH) is an *in-situ* method of exchanging a controlled volume of
media while retaining cells without the need for invasive separation techniques i.e.
centrifugation. Cells are allowed to settle passively within the bioreactor for a maximum of
2.5 hours and the top supernatant fraction of spent media is removed and replaced by fresh
media. This allows for the culture to receive a full media exchange without a significant loss
15 of cells and a low contamination risk.

In the LPH process, all control loops within the bioreactor are switched off (pH control, DO
control, stirrer and temperature) and the cells within the culture are allowed to settle. Cells
will settle for a minimum of 2.5h and settling progression will be closely monitored by a
capacitance biomass probe (Aber Futura). As cells settle the biomass probe will reduce
20 down to its zero'd point indicating that all cells have settled to below the probe and therefore
below the media outlet pipe for exchanging media. The media is then removed at a height
80% the length of the working volume and then replaced with fresh media.

BP_TB_006 was the first attempt at an LPH, Figure 8 shows the cell growth profile indicating
25 the extended growth period enabled by the LPH. Figure 9 shows the decrease in waste
metabolites caused by the media exchange.

Figures 10 and 11 show a typical growth curve and nutrient/metabolite curve for a typical
culture.

30

Other Examples of LPH growth curves are shown in Figure 12

Figures 8-11 show that the LPH is a way of increasing the cell density while significantly
reducing contamination risk and time cells spend outside of the culture vessel. Comparing a
35 typical culture in BP_TB_005 cells peak at just over 1E6 cells/mL but using the LPH method

this peak was managed to be pushed to 5E6 cells/mL. Additionally, ammonia being the most harmful waste metabolite is able to be reduced by 80% allowing cells to continue growing uninhibited.

Claims

1. A method of providing a seed train comprising:
culturing mammalian cells in a chemostat, turbidostat or auxostat,
5 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 about 0.28 days^{-1} to
10 about 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.
- 15 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 about 0.05 litres to about 1 litre.
20
7. The method according to any of the preceding claims, wherein the chemostat,
turbidostat or auxostat has a volume of between about 0.1 litres to about 50 litres.
8. The method according any one of claims 1 to 6, wherein the chemostat, turbidostat or
25 auxostat has a volume of between about 0.5 litres to about 25 litres.
9. The method according any one of claims 1 to 6, wherein the chemostat, turbidostat or
auxostat has a volume of between about 1 litre to about 10 litres.
- 30 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 about 50,000 to about 150,000 cells/mL.
- 5 12. The method according any one of claims 1 to 10, further comprising the step of seeding the chemostat, turbidostat or auxostat with about 50,000 to about 100,000 cells/mL.
13. A chemostat, turbidostat or auxostat for use in the method of claims 1 to 10.
- 10 14. A medium exchange method for use in cell culture, comprising the steps of:
culturing cells in a vessel,
gravitational settling separation, and
media exchange.
- 15 15. The medium exchange method according to claim 14, wherein the vessel comprises 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.
- 20 16. The medium exchange method according to claim 15, further comprising the step of removing agitation by deactivating the agitator.
- 25 17. The medium exchange method according to claim 16, further comprising the step of concentrating the cells using gravitational settling separation to form a concentrated cell aggregation.
- 30 18. The medium exchange method according to claim 16 or claim 17, wherein the step of removing agitation by deactivating the agitator is controlled by a biomass probe and/or timer and/or metabolite detector.
19. The medium exchange method according to any of one of claims 14 to 18, wherein the step of media exchange comprises extending a dip-tube into said vessel.

20. The medium exchange method according to claim 19, wherein the dip-tube extends up to about 95% of vessel height.
- 5 21. The medium exchange method according to claims 19 or 20, wherein the dip-tube extracts growth media from the vessel following the step of gravitational settling separation.
- 10 22. The medium exchange method according to claim 21, wherein the vessel is replenished with fresh media following the step of media extraction.
23. The medium exchange method according to any one of claims 14 to 22, further comprising a step of controlling heat and/or gas sparging.
- 15 24. The medium exchange method according to any one of claims 14 to 23, wherein the vessel has a volume of between about 1 litre to about 50,000 litres.
- 25 25. The medium exchange method according to any one of claims 14 to 23, wherein the vessel has a volume of between about 50 litres to about 10,000 litres.
- 20 26. The medium exchange method according to any one of claims 14 to 23, wherein the vessel has a volume of between about 100 litres to about 1,000 litres.
- 25 27. The medium exchange method according to any one of claims 14 to 26, wherein the cells have a high propensity to form aggregates.
28. The medium exchange method according to any one of claims 14 to 27, wherein the cells are genetically modified.
- 30 29. The medium exchange method according to any one of claims 14 to 28, wherein the cells are eukaryotic.
30. The medium exchange method according to any one of claims 14 to 29, wherein the cells are mammalian.

31. The medium exchange method according to any one of claims 14 to 30, wherein gravitational settling separation is performed for between about 5 minutes to about 3 hours.
- 5 32. The medium exchange method according to any one of claims 14 to 31, wherein gravitational settling separation is performed for between about 20 minutes to about 2 hours.
- 10 33. 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.
- 15 34. The bioreactor according to claim 33, further comprising a biomass detector and/or timer and/or metabolite detector.
35. The bioreactor according to claim 33 or claim 34, wherein the dip-tube is extendable.
- 20 36. The bioreactor according to any one of claims 33 to 35, wherein the dip-tube extends between about 70% to about 95% of vessel height.
37. The bioreactor according to any one of claims 33 to 36, further comprising a device to control heat and/or gas sparging.
- 25 38. The bioreactor according to claim 37, wherein the device is reversibly activated.
39. The bioreactor according to any one of claims 33 to 38, wherein the bioreactor has a volume of between about 1 litre to about 50,000 litres.
- 30 40. The bioreactor according to any one of claims 33 to 38, wherein the bioreactor has a volume of between about 50 litres to about 10,000 litres.
- 35 41. The bioreactor according to any one of claims 33 to 38, wherein the bioreactor has a volume of between about 100 litres to about 1,000 litres.

42. The bioreactor according to any one of claims 33 to 41, for culturing cells which have a high propensity to form aggregates.

43. The use of claim 42, wherein the cells are genetically modified.

44. The use of claim 43, wherein the cells are eukaryotic.

45. The use of claim 44, wherein the cells are mammalian.

46. A cell culture method comprising:
a method of providing a seed train according to claims 1 to 10, and
a medium exchange method according to claims 14 to 32.

47. The cell culture method of claim 50, wherein the method of providing a seed train is performed in a chemostat, turbidostat or auxostat and the medium exchange method is performed at a predetermined time point after incubation of the cells from the seed train in chemostat, turbidostat or auxostat have been cultured in a separate additional bioreactor.

48. A cell culture system, comprising a chemostat, turbidostat or auxostat according to claim 13 and a bioreactor according to claims 33 to 42.