control of perfusion or HIPCOP" are used to describe the process by which cells control their own rate of perfusion. Volumetric productivities of equal to about, or greater than, 1 gram/L/day for moderate specific productivity cell lines are achieved with very modest medium volumes, comparatively simple bioreactor operations, and a batch length that fits in a standard fed-batch window. The volumetric productivity achieved is more than double what one might achieve with the same cell line in an optimized fed-batch culture.

The subject technology relates to a continuous perfusion culture process comprising monitoring pH in a cell culture in perfusion bioreactor with a pH controller or sensor, activating a medium perfusion pump delivering fresh medium and a permeate perfusion pump removing a nearly equivalent volume of permeate when the pH increases above a setpoint or predetermined value, and deactivating the medium perfusion pump and permeate perfusion pump when the pH decreases below a setpoint or predetermined value. In one aspect, the subject technology relates to a continuous perfusion culture process, including: (a) monitoring pH in a cell culture with a pH sensor; (b) delivering fresh medium and removing permeate when the pH is above a predetermined value; and (c) deactivating the medium delivery and the permeate removal when the pH decreases below the predetermined value. In essence, the pH change in the cell culture medium is a result of the cell metabolism which then triggers the perfusion process. Alternatively, the method may be described as a cell-controlled perfusion process without a need to measure glucose concentration. The pH trigger for turning on and off the perfusion pumps, fresh medium and permeate pumps, is set at a predetermined value. This predetermined value is about pH 7 (e.g., between 6.8-7.4). The medium comprises glucose, L-lactate and a specified ratio of amino acid to glucose. According to this subject technology, the activation and deactivation of the medium perfusion pump and the permeate perfusion pump may occur simultaneously or independently. Also, the permeate may be cell-free or may contain cells.

The described method also comprises adding L-lactate to a fresh perfusion medium used in the continuous culture process. In an embodiment, the L-lactate present in the perfusion medium is in an amount of about 0.1 g/L to about 7.0 g/L. More preferably, the L-lactate present in the perfusion medium is in an amount of about 1 to about 4 g/L, about 1 to about 3 g/L, or

Thus, the subject technology also relates to a perfusion culture process, including: (a) monitoring pH in a cell culture in a perfusion bioreactor with a pH sensor; (b) delivering fresh medium and removing permeate when the pH is above a predetermined value; and (c) deactivating the medium delivery and the permeate removal when the pH is below the predetermined value. In one or more embodiments related, directly or indirectly, to the above to each other, the fresh medium includes L-lactate; the L-lactate is present in the fresh medium in an amount of about 0.1 g/L to 7.0 g/L; the L-lactate is present in the fresh medium in an amount of about 1 to 4 g/L; the L-lactate is present in the fresh medium in an amount of about 1 to 3 g/L; the L-lactate is present in the fresh medium in an amount of about 1 to 2.5 g/L; alternatively or in addition to L-lactate, additional sodium bicarbonate is added to the perfusion medium or the perfusion bioreactor in an amount of about 1 to about 2.5 g/L such that the total sodium bicarbonate in the culture medium is about 2 to about 5.5 g/L and wherein the additional sodium bicarbonate is delivered to the perfusion bioreactor when the pH is above the predetermined value; the additional sodium bicarbonate is added to the perfusion bioreactor such that 1 molar carbonate enters the perfusion bioreactor at a rate of 8.7 mL per 1 liter of perfusion medium utilized; alternatively or in addition to L-lactate, any other physiologically acceptable base such as sodium carbonate, potassium carbonate, or the like is added to the perfusion bioreactor in an amount that would provide an upward influence on the pH in a manner similar to that which would occur by the addition of L-lactate; the fresh medium includes: (a) glucose; (b) L-lactate and/or additional sodium bicarbonate; and (c) amino acids; the fresh medium includes: (a) between about 0.5 to about 40 g/L glucose; (b) between about 0.1 to about 7 g/L L-lactate and/or between about 2 to about 5.5 g/L sodium bicarbonate; and (c) amino acids in a mole-of-glucose-to-mole-of-amino-acids ratio of between about 0.25 to about 1.0 ; the fresh medium includes glucose in an amount equal to about 70 mM of amino acids and about 5.3 grams of glucose per liter of medium; the fresh medium includes glucose at an amino-acids-(in mM)-to-glucose-(in g/L) ratio selected from the group consisting of about 60 to about 4.2; about 90 to about 8; about 100 to about 12; about 120 to about 13; about 240 to about 42 and about 380 to about 70 per liter of medium; the predetermined pH value is about pH 7 or is about 6.8 to about 7.4; a measurement of glucose concentration in the cell culture or addition of glucose to the cell culture by a glucose pump is not required.

The subject technology also relates to a method for achieving rapid cell growth in a perfusion culture process, comprising: (a) monitoring pH in a cell culture in a perfusion bioreactor with a pH sensor; (b) delivering fresh medium and removing permeate when the pH is above a predetermined value; and (c) deactivating the medium delivery and the permeate removal when the pH is below the predetermined value. In another aspect, the subject technology relates to method for achieving rapid cell growth in a continuous perfusion culture process, comprising: monitoring pH in a cell culture with a pH sensor; delivering fresh medium and removing permeate when the pH is above a predetermined value; and (c) deactivating the medium delivery and the permeate removal when the pH is below the predetermined value. In one or more embodiments related, directly or indirectly, to the above to each other, the fresh medium comprises L-lactate; L-lactate is in an amount of about 0.1 g/L to 7.0 g/L; alternatively or in addition to L-lactate, additional sodium bicarbonate is added to the perfusion bioreactor in an amount of about 1 to about 2.5 g/L such that the total sodium bicarbonate in the culture medium is about 2 to about 5.5 g/L and wherein the additional sodium bicarbonate is delivered to the perfusion bioreactor when the pH is above the predetermined value; the additional sodium bicarbonate is added to the perfusion bioreactor such that 1 molar carbonate enters the perfusion bioreactor at a rate of 8.7 mL per 1 liter of perfusion medium utilized; alternatively or in addition to L-lactate, any other physiologically acceptable base such as sodium carbonate, potassium carbonate, or the like is added to the perfusion bioreactor in an amount that would provide an upward influence on the pH in a manner similar to that which would occur by the addition of L-lactate; the fresh medium includes: (a) glucose; (b) L-lactate and/or additional sodium bicarbonate; and (c) amino acids; the fresh medium includes: (a) between about 0.5 to about 40 g/L glucose; (b) between about 0.1 to about 7 g/L L-lactate and/or between about 2 to about 5.5 g/L sodium bicarbonate; and (c) amino acids in a mole-of-glucose-to-mole-of-amino-acids ratio of between about 0.25 to about 1.0 ; the fresh medium includes glucose in an amount equal to about 70 mM of amino acids and about 5.3 grams of glucose per liter of medium; the fresh medium includes glucose at an amino-acids-(in mM)-to-glucose-(in g/L) ratio selected from the group consisting of about 60 to about 4.2; about 90 to about 8; about 100 to about 12; about 120 to about 13; about 240 to about 42 and about 380 to about 70 per liter of medium; the predetermined pH value is about pH 7 or is about 6.8 to about 7.4; a measurement of glucose

concentration in the cell culture or addition of glucose to the cell culture by a glucose pump is not required; a viable cell density of about 60 x 10^6/mL is achieved within 4 days.

The subject technology also relates to a hybrid perfusion/fed-batch culture process. In another aspect, the subject technology relates to a hybrid culture process, comprising: the continuous perfusion culture process as defined herein as the first culture process. This process comprising using the continuous perfusion process described above in combination with a fed-batch process. According to the subject technology, the process would

run in perfusion mode for 4 to 6 days prior to being switched to fed-batch mode for an additional 4 to 8 days. By using this hybrid mode, volumetric productivity of equal to about or greater than 1.0 gram/L/day is achieved. In this embodiment, the continuous perfusion culture process includes steps (a) monitoring pH in a cell culture with a pH sensor; (b) delivering fresh medium and removing permeate when the pH is above a predetermined value; (c) and deactivating the medium delivery and the permeate removal when the pH is below the predetermined value.

The subject technology also relates to a continuous perfusion bioreactor which consists of two significantly different phases. The first phase includes the initial continuous phase as mentioned above (ramp up of cell density and perfusion rate using HIPCOP) utilizing a comparably dilute medium, followed by a second phase of perfusion in which the perfusion rates are significantly reduced by utilization of a highly concentrated perfusion medium. By using this two-phase perfusion system, volumetric productivities of equal to about or greater than 1.5 grams/L/day is achieved while using very modest volumes of perfusion medium. No significant cell bleed (removal of cells from the bioreactor) occurs in the examples presented in this application.

The subject technology also relates to the use of a diluent liquid that is added to the bioreactor during the later stages of the perfusion culture when the concentrated perfusion medium is being added to the bioreactor. In one embodiment, such a diluent liquid is a solution of saline of appropriate concentration. Because the long-distance transport of liquid nutrient medium can incur many difficulties (cost of transport, maintenance of sterility, temperature control) there is significant value in using highly concentrated perfusion medium in an industrial setting. Such medium allows for perfusion rates as low as 0.05-0.30 reactor volumes per day for the concentrated perfusion medium. In a related embodiment, in such a bioreactor system it is necessary to flush product material out of the bioreactor, particularly if a continuous downstream process is linked directly to capture the continuously delivered upstream harvest material (e.g., the material coming through the hollow fiber cell retention system), and if the protein being produced is highly labile.

The subject technology also relates to a hybrid culture process, including a first continuous perfusion culture process followed by a second continuous perfusion culture process, wherein the first continuous perfusion culture process includes steps (a) monitoring pH

in a cell culture with a pH sensor; (b) delivering fresh medium and removing permeate when the pH is above a predetermined value; (c) and deactivating the medium delivery and the permeate removal when the pH is below the predetermined value.; and wherein the second continuous perfusion culture process includes steps of (a) adding a concentrated medium; and (b) adding a diluent. In a related embodiment, the hybrid culture process optionally includes an intervening fed-batch step. In this embodiment, the first continuous perfusion culture process is carried out first followed by a fed-batch step which is then followed by the second continuous perfusion culture process. In one or more embodiments related, directly or indirectly, to the above to each other, the continuous perfusion culture process is the first culture process; the continuous perfusion culture process is followed by a fed-batch process; the continuous perfusion culture process is followed by a second continuous perfusion culture process; the second continuous perfusion culture process comprises the steps of: (a) adding a concentrated medium; and (b) adding a diluent; the concentrated medium comprises 600 millimolar amino acids, 90 grams/liter glucose, 0 g/L sodium L-lactate; the diluent is selected from the group consisting of saline and water; the saline or water normalizes osmotic strength of culture medium to between about 0 to 250 and/or 250 to 350 mOsm/kg; the saline comprises 2.0 g/L sodium bicarbonate, 2.4 g/L polyvinyl alcohol, 20 mM potassium chloride, and 80 mM sodium chloride; the hybrid culture process results in volumetric productivity of greater than 1 gram cells/L/day; the hybrid culture process is performed in a culture volume of about 50 and about 150 L; the culture volume is about 70 L.

The subject technology also relates to a bioreactor for conducting a continuous perfusion culture process, wherein the continuous perfusion culture process includes steps of: (a) monitoring pH in a cell culture in a perfusion bioreactor with a pH sensor; (b) delivering fresh medium and removing permeate when the pH is above a predetermined value; (c) and deactivating the medium delivery and the permeate removal when the pH is below the predetermined value. In another aspect, the subject technology relates to a bioreactor for conducting a continuous perfusion culture process, wherein the continuous perfusion culture process comprises the steps of: (a) monitoring pH in a cell culture with a pH sensor; (b) delivering fresh medium and removing permeate when the pH is above a predetermined value; and (c) deactivating the medium delivery and the permeate removal when the pH is below the predetermined value. In one or more embodiments related, directly or indirectly, to

the above to each other, the fresh medium comprises: (a) glucose; (b) L-lactate; and (c) amino acids; L-lactate is in an amount of about 0.1 g/L to 7.0 g/L ; alternatively or in addition to L-lactate, additional sodium bicarbonate is added to the perfusion bioreactor in an amount of about 1 to about 2.5 g/L such that the total sodium bicarbonate in the culture medium is about 2 to about 5.5 g/L and wherein the additional sodium bicarbonate is delivered to the perfusion bioreactor when the pH

is above the predetermined value; the additional sodium bicarbonate is added to the perfusion bioreactor such that 1 molar carbonate enters the perfusion bioreactor at a rate of 8.7 mL per 1 liter of perfusion medium utilized; alternatively or in addition to L-lactate, any other physiologically acceptable base such as sodium carbonate, potassium carbonate, or the like is added to the perfusion bioreactor in an amount that would provide an upward influence on the pH in a manner similar to that which would occur by the addition of L-lactate; the fresh medium includes: (a) glucose; (b) L-lactate and/or additional sodium bicarbonate; and (c) amino acids; the fresh medium includes: (a) between about 0.5 to about 40 g/L glucose; (b) between about 0.1 to about 7 g/L L-lactate and/or between about 2 to about 5.5 g/L sodium bicarbonate; and (c) amino acids in a mole-of-glucose-to-mole-of-amino-acids ratio of between about 0.25 to about 1.0 ; the fresh medium includes glucose in an amount equal to about 70 mM of amino acids and about 5.3 grams of glucose per liter of medium; the fresh medium includes glucose at an amino-acids-(in mM)-to-glucose-(in g/L) ratio selected from the group consisting of about 60 to about 4.2; about 90 to about 8; about 100 to about 12; about 120 to about 13; about 240 to about 42 and about 380 to about 70 per liter of medium; the predetermined pH value is about pH 7 or is about 6.8 to about 7.4; a measurement of glucose concentration in the cell culture or addition of glucose to the cell culture by a glucose pump is not required.

In another aspect, the subject technology relates to a perfusion culture process, comprising: (a) monitoring pH in a cell culture in a perfusion bioreactor with a pH sensor; (b) delivering fresh medium to the perfusion bioreactor and removing permeate when the pH is above a predetermined value; and (c) deactivating the medium delivery and the permeate removal when the pH is below the predetermined value.

In another aspect, the subject technology relates to a method for achieving rapid cell growth in a perfusion culture process, comprising: (a) monitoring pH in a cell culture in a perfusion bioreactor with a pH sensor; (b) delivering fresh medium to the perfusion bioreactor and removing permeate when the pH is above a predetermined value; and (c) deactivating the medium delivery and the permeate removal when the pH is below the predetermined value.

In another aspect, the subject technology relates to a bioreactor for conducting a continuous perfusion culture process, wherein the continuous perfusion culture process comprises the steps of: (d) monitoring pH in a cell culture in a perfusion bioreactor with a pH sensor; (e) delivering fresh medium to the perfusion bioreactor and removing permeate when the pH is above a predetermined value; and (f) deactivating the medium delivery and the permeate removal when the pH is below the predetermined value.

In another aspect, the subject technology relates to a protein of interest produced by a method comprising: (a) culturing cells comprising a gene that encodes the protein of interest in a perfusion cell culture bioreactor under conditions that allow production of the protein of interest comprising: i. monitoring pH in the cell culture bioreactor with a pH sensor; ii. delivering fresh medium and removing permeate when the pH is above a predetermined value; and iii. deactivating the medium delivery and the permeate removal when the pH is below the predetermined value, (b) harvesting the protein of interest from the cell culture bioreactor.

The subject technology also relates to a protein of interest produced by a method including: (a) culturing cells comprising a gene that encodes the protein of interest in a perfusion cell culture bioreactor under conditions that allow production of the protein of interest including: (i) monitoring pH in the cell culture in a perfusion bioreactor with a pH sensor; (ii) delivering fresh medium and removing permeate when the pH is above a predetermined value; and (iii) deactivating the medium delivery and the permeate removal when the pH is below the predetermined value, (b) harvesting the protein of interest from the cell culture bioreactor. In one or more embodiments related, directly or indirectly, to the above to each other, the fresh medium includes: (a) glucose, (b) L-lactate, and (c) amino acids; L-lactate is in an amount of about 0.1 g/L to 7.0 g/L; alternatively or in addition to L-lactate, additional sodium bicarbonate is added to the perfusion bioreactor in an amount of about 1 to about 2.5 g/L such that the total sodium bicarbonate in the culture medium is about 2 to about 5.5 g/L and wherein the additional sodium bicarbonate is delivered to the perfusion bioreactor when the pH is above the predetermined value; the additional sodium bicarbonate is added to the perfusion bioreactor such