to be made at a certain production volume/condition, and each batch's characteristic might be different, therefore so does the yield of the protein or cell mass.

[0008] Therefore, there is a need for a novel method for make these different proteins and/or intermediates in large quantity than what is currently known to one ordinary skilled in the art.

SUMMARY

[0009] The present disclosure relates to methods to increase fermentation yield for protein production.

[0009a] In an aspect, the present disclosure provides a method to increase recombinant protein production in a prokaryote host cell, comprising: performing a recombinant protein fermentation including the steps of: carrying out seeding (time S_0) of a culture of procaryote host cell engineered to inducibly express a recombinant protein and transferring the culture into one or more fermenters each containing a culture medium; measuring a dissolved oxygen (DO) level, an agitation rate, and a pH in the one or more fermenters; supplying a carbon source feeding solution to any of the one or more fermenters, individually, whenever condition(s) (i) and/or (ii) are met: (i) said DO level exceeds above 35% to 45% and said agitation rate exceeds 300 to 1000 rpm; (ii) said agitation rate exceeds 400 to 700 rpm and said pH exceeds 7.0 to 7.4; and supplying a nitrogen source to any of the one or more fermenters in which induction of expression of the recombinant protein has been initiated, individually, at about I_0 (time at initiation of induction) or I_1 or both; optionally, supplying a nitrogen source to any of the one or more fermenters, individually, at one or more time points selected from about S_5, S_6, S_7, S_8, S_9 and S_{10}.

[0009b] In another aspect, the present disclosure provides a program-controlled mechanism or equipment comprising: a. an apparatus equipped with one or more sensors capable of measuring the oxygen level, pH, and/or agitation of culture medium in a fermentation reactor in a fermentation process in a periodic or continuous mode; and b. a feeding solution injection system integrated within said program-controlled mechanism that is programmed to provide one or more feeding solutions to a fermentation reactor based on measured oxygen levels, pH and/or agitation in the culture medium in a periodic or continuous interval.

[0009c] In another aspect, the present disclosure provides a method for automatically regulating glucose concentration, oxygen level, pH and/or agitation during fermentation, comprising: a. measuring parameters of oxygen level, pH and/or agitation of the fermentation with one or more sensors; b. transmitting the measured parameters to a program-controlled

mechanism; and c. automatically adjusting the injection rate, volume of a feeding solution and agitation based on the measured parameters.

[0010] In an example, the present disclosure relates to a method to increase recombinant protein production in a prokaryote host cell, comprising performing one or more fed-batch recombinant protein fermentation. The fed-batch recombinant protein fermentation includes the steps of: carrying out seeding (time S_0) of a culture of procaryote host cell engineered to inducibly express a recombinant protein and transferring the culture into one or more fermenters each containing a culture medium; measuring a dissolved oxygen (DO) level, an agitation rate, and a pH in the one or more fermenters; supplying a carbon source feeding solution to any of the one or more fermenters using a program-controlled mechanism, individually, whenever condition(s) (i) and/or (ii) are met: (i) said DO level exceeds above about 35% to 45% and said agitation rate exceeds about 300 to 1000 rpm; (ii) said agitation rate exceeds about 400 to 700 rpm and said pH exceeds about 7.0 to 7.4; and supplying a nitrogen source to any of the one or more fermenters in which induction of expression of the recombinant protein has been initiated, individually, at about I_0 (time at initiation of induction) or I_1 or both; optionally, supplying a nitrogen source to any of the one or more fermenters, individually, at one or more time points selected from about S_5, S_6, S_7, S_8, S_9 and S_{10}. In another example, the dissolved oxygen (DO) level, agitation rate, and pH are continuously and/or periodically monitored at a given time interval.

[0011] In an example, the present disclosure relates to a method to increase recombinant protein production in a prokaryote host cell, comprising performing one or more fed-batch recombinant protein fermentation. The fed-batch recombinant protein fermentation includes the steps of: carrying out seeding (time S_0) of a culture of procaryote host cell engineered to inducibly express a recombinant protein and transferring the culture into one or more fermenters each containing a culture medium; measuring a dissolved oxygen (DO) level, an agitation rate, and a pH in the one or more fermenters; supplying a carbon source feeding solution to any of the one or more fermenters, whenever condition(s) (i) and/or (ii) are met: (i) said DO level exceeds above about 35% to 45% and said agitation rate exceeds about 300 to

1000 rpm; (ii) said agitation rate exceeds about 400 to 700 rpm and said pH exceeds about 7.0 to 7.4; and supplying a nitrogen source to any of the one or more fermenters in which induction of expression of the recombinant protein has been initiated, individually, at about I_0 (time at initiation of induction) or I_1 or both; optionally, supplying a nitrogen source to any of the one or more fermenters, individually, at one or more time points selected from about S_5, S_6, S_7, S_8, S_9 and S_{10}. In another example, the dissolved oxygen (DO) level, agitation rate, and pH are continuously and/or periodically monitored at a given time interval.

[0012] In another example, the present disclosure relates to a method to increase recombinant protein production in a prokaryote host cell, comprising performing one or more batch recombinant protein fermentation. The batch recombinant protein fermentation includes the steps of: carrying out seeding (time S_0) of a culture of procaryote host cell engineered to inducibly express a recombinant protein and transferring the culture into one or more fermenters each containing a culture medium; measuring a dissolved oxygen (DO) level, an agitation rate, and a pH in the one or more fermenters; supplying a carbon source feeding solution to any of the one or more fermenters condition(s) (i) and/or (ii) are met: (i) said DO level exceeds above about 35% to 45% and said agitation rate exceeds about 300 to 1000 rpm; (ii) said agitation rate exceeds about 400 to 700 rpm and said pH exceeds about 7.0 to 7.4; and supplying a nitrogen source to any of the one or more fermenters in which induction of expression of the recombinant protein has been initiated, individually, at about I_0 (time at initiation of induction) or I_1 or both; optionally, supplying a nitrogen source to any of the one or more fermenters, individually, at one or more time points selected from about S_5, S_6, S_7, S_8, S_9 and S_{10}. In another example, the dissolved oxygen (DO) level, agitation rate, and pH are continuously and/or periodically monitored at a given time interval.

[0013] In an example, the present disclosure relates to a method to increase recombinant protein production in a prokaryote host cell, comprising performing one or more batch recombinant protein fermentation. The fed-batch recombinant protein fermentation includes the steps of: carrying out seeding (time S_0) of a culture of procaryote host cell engineered to inducibly express a recombinant protein and transferring the culture into one or more fermenters each containing a culture medium; measuring a dissolved oxygen (DO) level, an agitation rate, and a pH in the one or more fermenters; supplying a carbon source feeding solution to any of the one or more fermenters using a program-controlled mechanism, individually, whenever condition(s) (i) and/or (ii) are met: (i) said DO level exceeds above about 35% to 45% and said agitation rate exceeds about 300 to 1000 rpm; (ii) said agitation rate exceeds about 400 to 700 rpm and said pH exceeds about 7.0 to 7.4; and supplying a nitrogen source to any of

the one or more fermenters in which induction of expression of the recombinant protein has been initiated, individually, at about I_0 (time at initiation of induction) or I_1 or both; optionally, supplying a nitrogen source to any of the one or more fermenters, individually, at one or more time points selected from about S_5, S_6, S_7, S_8, S_9 and S_{10}. In another example, the dissolved oxygen (DO) level, agitation rate, and pH are continuously and/or periodically monitored at a given time interval.

[0014] In one example, in the method to increase recombinant protein production in a prokaryote host cell, in condition (i), said DO level exceeds above about 35% to 40% (e.g., about 35, 36, 37, 38, 39, or 40%) and said agitation rate exceeds about 300 to 700 rpm (e.g., about 300, 350, 400, 450, 500, 550, 600, 650, or 700 rpm), optionally about 300 to 500 rpm. In another example, said DO level exceeds above about 35% to 40% (e.g., about 35, 36, 37, 38, 39, or 40%) and said agitation rate exceeds about 300 to 500 rpm, optionally, about 300 to 400 rpm, 350 to 450 rpm, or 350 to 500 rpm. In some examples, said DO level exceeds above about 35% and said agitation rate exceeds about 300 to 500 rpm. In another example, said DO level exceeds above about 35% and said agitation rate exceeds about 300 to 400 rpm, about 400 to 500 rpm, about 350 to 400 rpm, about 400 to 450 rpm, about 450 to 500 rpm, about 300 rpm, about 350 rpm, about 400 rpm, about 450 rpm, or about 500 rpm. In one example, the said DO level exceeds above about 40% and said agitation rate exceeds about 300 to 500 rpm. In yet another example, said DO level exceeds above about 40% and said agitation rate exceeds about 300 to 400 rpm, about 400 to 500 rpm, about 350 to 400 rpm, about 400 to 450 rpm, about 450 to 500 rpm, about 300 rpm, about 350 rpm, about 400 rpm, about 450 rpm, or about 500 rpm.

[0015] In one example, in the method to increase recombinant protein production in a prokaryote host cell, in condition (ii), said agitation rate exceeds about 500 to 600 rpm and said pH exceeds about 7.0 to 7.4 (e.g., about 7.0, 7.1, 7.2, 7.3 or 7.4); optionally, said pH exceeds about 7.0 to 7.2 (e.g., about 7.0, 7.1, or 7.2) and said agitation rate exceeds about 500 rpm, 550 rpm, or 600 rpm. In another example, said agitation rate exceeds about 600 rpm and said pH exceeds about 7.0 to 7.2; optionally, said pH exceeds about 7.2. In yet another example, said agitation rate exceeds about 500 to 600 rpm and said pH exceeds about 7.2 to 7.4; optionally, said pH exceeds about 7.4 and said agitation rate exceeds about 500 rpm, 550 rpm, or 600 rpm.

[0016] In one example, in the method to increase recombinant protein production in a prokaryote host cell, the nitrogen source is supplied to the one or more fermenters, individually, at about I_0 and one or more time points selected from about S_5, S_6, S_7, S_8, S_9 and S_{10} (e.g., S_5,

S_6, S_7, S_8, S_9, S_{10}, S_5 and S_7, S_5 and S_8, S_6 and S_9, S_6 and S_{10}, S_5, S_7, and S_9, or S_6, S_7, and S_{10}); optionally, at about I_0 and one or more time points selected from about S_5, S_6, S_7, and S_8 (e.g., S_5, S_6, S_7, S_8, S_5 and S_7, S_5 and S_8, S_6 and S_8, S_6 and S_7, S_5, S_7, and S_8, or S_6, S_7, and S_8).

[0017] In another example, in the method to increase recombinant protein production in a prokaryote host cell, the nitrogen source is supplied to the one or more fermenters, individually, at about I_1 and one or more time points selected from about S_5, S_6, S_7, S_8, S_9 and S_{10} (e.g., S_5, S_6, S_7, S_8, S_9, S_{10}, S_5 and S_7, S_5 and S_8, S_6 and S_9, S_6 and S_{10}, S_5, S_7, and S_9, or S_6, S_7, and S_{10}); optionally, at about I_1 and one or more time points selected from about S_5, S_6, S_7, and S_8 (e.g., S_5, S_6, S_7, S_8, S_5 and S_7, S_5 and S_8, S_6 and S_8, S_6 and S_7, S_5, S_7, and S_8, or S_6, S_7, and S_8).

[0018] In yet another example, in the method to increase recombinant protein production in a prokaryote host cell, the nitrogen source is supplied to the one or more fermenters, individually, at about I_0 and about I_1 and one or more time points selected from about S_5, S_6, S_7, S_8, S_9 and S_{10} (e.g., S_5, S_6, S_7, S_8, S_9, S_{10}, S_5 and S_7, S_5 and S_8, S_6 and S_9, S_6 and S_{10}, S_5, S_7, and S_9, or S_6, S_7, and S_{10}); optionally, at about I_0 and about I_1 and at one or more time points selected from about S_5, S_6, S_7, and S_8 (e.g., S_5, S_6, S_7, S_8, S_5 and S_7, S_5 and S_8, S_6 and S_8, S_6 and S_7, S_5, S_7, and S_8, or S_6, S_7, and S_8).

[0019] In one example, a carbon source feeding solution is supplied at least once to at least one fermenter during the fed-batch recombinant protein fermentation. In some examples, the carbon source feeding solution is supplied two or more separate times to at least one fermenter during the fed-batch recombinant protein fermentation. In one example, the carbon source feeding solution is a glucose feeding solution, fructose feeding solution, galactose feeding solution, pyruvate feeding solution, or any combination thereof.

[0020] In one example, in the method to increase recombinant protein production in a prokaryote host cell, the nitrogen source is supplied to the one or more fermenters, individually, at about I_0 and/or about I_1 and one or more time points selected from about S_5, S_6, S_7, S_8, S_9 and S_{10} (e.g., S_5, S_6, S_7, S_8, S_9, S_{10}, S_5 and S_7, S_5 and S_8, S_6 and S_9, S_6 and S_{10}, S_5, S_7, and S_9, or S_6, S_7, and S_{10}); optionally, at about I_0 and/or about I_1 and one or more time points selected from about S_5, S_6, S_7, and S_8 (e.g., S_5, S_6, S_7, S_8, S_5 and S_7, S_5 and S_8, S_6 and S_8, S_6 and S_7, S_5, S_7, and S_8, or S_6, S_7, and S_8); and the carbon source feeding solution is supplied at least once or two or more separate times to the one or more fermenters during the fed-batch recombinant protein fermentation as triggered by either or both of conditions (i) and (ii). In an example of the method, the nitrogen source can be supplied to the one or more fermenters, individually, at about I_0 or about I_1 and one or more time points selected from

about S_5, S_6, S_7, and S_8 (e.g., S_5, S_6, S_7, S_8, S_5 and S_7, S_5 and S_8, S_6 and S_8, S_6 and S_7, S_5, S_7, and S_8, or S_6, S_7, and S_8); and the carbon source feeding solution can be supplied at least once or two or more separate times to the one or more fermenters during the fed-batch recombinant protein fermentation as triggered by either or both of conditions (i) and (ii).

[0021] In one example, in the method to increase recombinant protein production in a prokaryote host cell, the carbon source feeding solution is a glucose feeding solution. In one example, a cumulative total of about 800 to 1500g of glucose is supplied to at least one fermenter, per about 5 L fermenter capacity, by the end of the fed-batch recombinant protein fermentation. In some examples, a cumulative total of about 6000 to 9000g of glucose is supplied to at least one fermenter by the end of the fed-batch recombinant protein fermentation, the at least one fermenter having about 40 L capacity.

[0022] In one example, in the method to increase recombinant protein production in a prokaryote host cell, the glucose feeding solution supplied to the one or more fermenters, individually, includes D-(+)-Glucose; optionally, the glucose feeding solution supplied to the one or more fermenters, individually, contains about 600 to 900 g/L D-(+)-Glucose Anhydrous. In one example, the glucose feeding solution supplied to the one or more fermenters, individually, contains H_3BO_3, $CaCl_2.2H_2O$, $CuSO_4.5H_2O$, $MnSO_4.H_2O$, $(NH_4)_6MO_7O_{24}.4H_2O$, EDTA, $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$, 12N (37%) HCl, and/or $MgSO_4.7H_2O$; optionally, about 20 to 600 g/L MgSO4.7H_2O. In another example, the carbon source is added in a continuously fashion, or in a batch fashion, depending on the culture conditions such as dissolved oxygen (DO) level, an agitation rate, and a pH levels.

[0023] In one example, in the method to increase recombinant protein production in a prokaryote host cell, the nitrogen source supplied to the one or more fermenters, individually, is a yeast extract, peptone, soytone, urea, tryptone, or any combinations thereof; optionally, the nitrogen source is a yeast extract solution. In some examples, the yeast extract solution supplied to the one or more fermenters, individually, has a concentration of about 100 to 600 g/L, 200 to 300 g/L, 300 to 400 g/L, 400 to 500 g/L, 500 to 600 g/L, 150 to 200 g/L, 200 to 250 g/L, 250 to 300 g/L, 300 to 350 g/L, 350 to 400 g/L, 400 to 450 g/L, 450 to 500 g/L, 100 g/L, 150 g/L, 200 g/L, 250 g/L, 300 g/L, 350 g/L, 400 g/L, 428 g/L, 450 g/L, 500 g/L, or 600 g/L. The total volume of nitrogen source can be adjusted based on fermentation scale.

[0024] In one example, in the method to increase recombinant protein production in a prokaryote host cell, about 40 to 150 mL of the yeast extract solution is supplied to the one or more fermenters each time, individually; optionally about 300 to 1500 mL of the yeast extract

solution is supplied to the one or more fermenters each time, individually. In another example, about 45 to 60 mL of the yeast extract solution is supplied to the one of more fermenters, individually, if supplied at any time point selected from about S_5, S_6, S_7, S_8, S_9 and S_{10}; and about 90 to 120 ml of the yeast extract solution is supplied to the one of more fermenters, individually, if supplied at about I_0 or I_1. In one example, about 350 to 500 mL of the yeast extract solution is supplied to the one of more fermenters, individually, if supplied at any time point selected from about S_5, S_6, S_7, S_8, S_9 and S_{10}; and about 700 to 1200 mL of the yeast extract solution is supplied to the one of more fermenters, individually, if supplied at about I_0 or I_1.

[0025] In some examples, in the method to increase recombinant protein production in a prokaryote host cell, the nitrogen source supplied to the one or more fermenters, individually, contains potassium, optionally in the form of K_2HPO_4 and/or KH_2PO_4; or wherein potassium, optionally in the form of K_2HPO_4 and/or KH_2PO_4, is supplied to the one or more fermenters, individually, at about the same time the nitrogen source is supplied. In one example, the nitrogen source contains about 1.0 to 3.0 M of potassium, or wherein potassium is supplied to reach a final potassium concentration of about 35 to 40 mM in the one or more fermenters, individually.

[0026] In an example of the method to increase recombinant protein production in a prokaryote host cell, the one or more fermenters, individually, can have about 5 L to 200 L or larger capacity (e.g., about 5 L, 10 L, 20 L, 30 L, 40 L, 50 L, 80 L, 100 L, 150 L, 200 L, or larger than 200 L); optionally, the one or more fermenters each has about a 5 L capacity; optionally, the one or more fermenters each has about a 40 L capacity; optionally, the one or more fermenters, individually, has 1 to 200 L of the medium.

[0027] In another example, the concentration of each ingredient of the present disclosure can remain unchanged when the fermentation size is scaled upwards or downwards (e.g., about 5 L, 10 L, 20 L, 30 L, 40 L, 50 L, 80 L, 100 L, 150 L, 200 L, or larger than 200 L). In an example, the total volume of each ingredient, such as carbon and/or nitrogen source, may be adjusted depending on the fermentation size. Nonlimiting example include that for a 5L to 40L scale up, the volume required is eight times for 40L fermentation as compared to 5L. Additionally, or alternatively, customary adjustments on parameters are also contemplated in the present disclosure based on fermentation size.

[0028] In one example, in the method to increase recombinant protein production in a prokaryote host cell, wherein said program-controlled mechanism for supplying a carbon

source feeding solution includes one or more sensors which measure the DO level, pH, redox measurement, and/or carbon amount in the one or more fermenters, and wherein said program-controlled mechanism automatically injects the carbon source feeding solution into the one or more fermenters.

[0029] In one example, in the method to increase recombinant protein production in a prokaryote host cell, the fed-batch recombinant protein fermentation is performed with an inoculum ratio of about 6.0 to 8.5%; optionally, about 6.5 to 7%, 7.0 to 7.5%, 7.5 to 8%, or 8.0 to 8.5%; optionally, about 6.25%, 6.3%, 6.4%, 6.5%, 6.67%, 6.7%, 6.8%, 6.95%, 7.0%, 7.24%, 7.3%, 7.33%, 7.48%, 7.5%, 7.6%, 7.76%, 7.8%, 7.9%, 8%, 8.14%, or 8.4%. In an example of the method, the inoculum ratio can be about 6.0 to 8.5%; optionally, about 6.5 to 7%, 7.0 to 7.5%, 7.5 to 8%, or 8.0 to 8.5%; optionally, about 6.25%, 6.3%, 6.4%, 6.5%, 6.67%, 6.7%, 6.8%, 6.95%, 7.0%, 7.24%, 7.3%, 7.33%, 7.48%, 7.5%, 7.6%, 7.76%, 7.8%, 7.9%, 8%, 8.14%, or 8.4%.

[0030] In one example, in the method to increase recombinant protein production in a prokaryote host cell, induction of expression of the recombinant protein is initiated when OD_{600} in the culture in a fermenter reaches about 40 to 80, optionally, about 40 to 50, 50 to 60, 60 to 70, 70 to 80, 55 to 60, 60 to 65, 65 to 70, 70 to 75, 75 to 80, 40, 45, 49, 52, 55, 60, 66, 67, 68, 69, 70, 71, 72, 75, 78, or 80. In one example of the method, expression of the recombinant protein is induced by adding Isopropyl-beta-D-1-thiogalactopyranoside (IPTG), optionally, about 3 to 5 mL of about 0.70 to 0.85 mM of IPTG.

[0031] In an example of the method to increase recombinant protein production in a prokaryote host cell, the recombinant protein can be any of VX-001, AVE-9633, AC-9301, NY-ESO-1 vaccine, erythropoietin, EPO-Fc, CNTO-528, AMG-114, JR-013, Factor XIII, IFN-α2b, Pro-IFN-α2b, anti-PD1 antibody, and GCSF or a combination thereof.

[0032] In an example of the method to increase recombinant protein production in a prokaryote host cell, the prokaryote host cell comprises an *E. coli* cell. In one example, the *E. coli* is BL-21 strain, *E. coli* BLR-Codon Plus (DE3)-RIL, *E. coli* BLR-Codon Plus (DE3), or *E. coli* BL21-CodonPlus (DE3)-RIL.

[0033] In one example, the method to increase recombinant protein production in a prokaryote host cell further includes a step of collecting the culture of *E. coli* host cell or a sample of the culture in the one or more fermenters after induction of expression of the recombinant protein, individually. In one example, the method further comprising processing inclusion bodies in the collected culture of *E. coli* cell or sample to obtain a crude

protein extract. In one example, the step of inclusion body processing includes: resuspending the *E. coli* cells in the collected culture or sample in a buffer; homogenizing the *E. coli* cells one or more times at about 800 to 1200 bar, thereby obtaining a cell mixture; centrifuging the cell mixture to form a cell pellet utilizing a batch centrifuging with a centrifugation speed about 20,000 to 30,000 rpm; alternatively, the centrifuge step can be carried our using a continuous centrifuging flow method with a feeding rate about 30-60 ml/min, and with a centrifugation speed about 20,000 to 30,000 rpm; collecting the cell pellet; and processing the cell pellet to obtain a crude protein extract. In an example of the method, the culture or sample can be collected after or at about I_4 to I_8 (e.g., after or at about I_4, I_5, I_6, I_7, or I_8). In one example, the total time for the fed-batch recombinant protein fermentation includes about 10 to 95 hours or more, optionally about 14 ± 1, 16 ± 1, 24 ± 1, 30 ± 1, 36 ± 1, 48 ± 1, 60 ± 1, 72 ± 1, 84, ± 1, or 96 ± 1 hours.

[0034] In one example, the method to increase recombinant protein production in a prokaryote host cell further includes determining yield of the expressed recombinant protein in the crude protein extract, wherein the yield is increased by at least about 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least about 62.7%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 100%, at least 110%, at least 120%, at least 130%, at least 140%, at least 150%, at least 160%, at least 170%, at least 167%, at least 180%, at least 190%, at least 200%, at least 210%, at least 220%, at least 230%, at least 240%, at least 250%, at least 260%, at least 270%, at least 280%, at least 290%, at least 300%, at least 350%, at least 383%, at least 400%, at least 450%, at least 500%, at least 550%, at least 600%, at least 650%, at least 700%, at least 748%, at least 750%, at least 800%, at least 9 folds, at least 10 folds, at least 20 folds or more, as compared to another fermentation method. In one example, the yield is determined for a fermenter having about a 40 L capacity and the yield is increased by at least about 100% to 200%, 100%, 105%, 110%, 115%, 120%, 125%, 130%, 135%, 140%, 145%, 150%, 155%, 160%, 165%, 170%, 175%, 180%, 190%, or 200%, as compared to the yield from a fermenter having about a 5 L capacity in the other fermentation method. In one example, the recombinant protein is GCSF, Pro-IFN-α2b, and/or anti-PD1 monoclonal antibody.

[0035] In one example, the method to increase recombinant protein production in a prokaryote host cell further includes determining yield of the expressed recombinant protein in the crude protein extract, wherein the yield is about 5 to 10 g of the recombinant protein in a fermenter having about a 5 L capacity; optionally, wherein the yield is about 20 to 30 g of

the recombinant protein in a fermenter having about a 40 L capacity; optionally, the recombinant protein is GCSF, Pro-IFN-α2b, and/or anti-PD1 monoclonal antibody.

[0036] In one example, the method to increase recombinant protein production in a prokaryote host cell further comprises the following steps performed before the fed-batch recombinant protein fermentation: obtaining a codon optimized nucleic acid encoding the recombinant protein; transfecting said codon optimized nucleic acid into the prokaryotic host cell; and polarizing said prokaryotic host cell and culturing said prokaryotic host cell at about 36-37º C overnight or about 12-18 hours, thereby resulting in a whole cell broth which includes the transfected codon optimized nucleic acid.

[0037] In another example, described herein is a program-controlled mechanism or equipment comprising: (a) an apparatus equipped with one or more sensors capable of measuring the oxygen level, pH, and/or agitation of culture medium in a fermentation reactor in a fermentation process; and (b) a feeding solution injection system integrated within said program-controlled mechanism that is programmed to provide one or more feeding solutions to a fermentation reactor based on measured oxygen levels, pH and/or agitation in the culture medium. In an example, the mechanism or equipment can measure and/or feed solution for a periodical interval (e.g., every 30 minutes for up to 96 hours or longer) or in a continuous fashion.

[0038] In one example of the program-controlled mechanism or equipment, the one or more sensors transmit real-time measurements to the program-controlled mechanism; optionally, the program-controlled mechanism or equipment further comprises a control unit configured to receive sensor measurements and automatically adjust the injection rate and volume of the feeding solution based on the measured parameters. In another example, the program-controlled mechanism or equipment further comprises a control unit configured to receive sensor measurements and automatically adjust the injection rate and volume of the feeding solution based on the measured parameters. In one example, feeding solution injection system comprises a reservoir, a metering pump, and a distribution system to deliver the feeding solution into the fermentation process; optionally, the feeding solution injection system is programed to include predetermined setpoints and control algorithms to modify glucose levels, dissolved oxygen, and/or pH within the fermentation process in the culture medium.

[0039] In yet another example, this disclosure includes a method for automatically regulating glucose concentration, oxygen level, pH and/or agitation during fermentation, comprising: (a)

measuring parameters of oxygen level, pH and/or agitation of the fermentation with one or more sensors; (b) transmitting the measured parameters to a program-controlled mechanism; and (c) automatically adjusting the injection rate, volume of a feeding solution and agitation based on the measured parameters. In another example, the method for measuring, transmitting, and/or adjusting injection rate can be performed in a periodical fashion (e.g., every 30 minutes for up to 96 hours, or longer), or in a continuous fashion.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] For a more complete understanding of the features and advantages of the present disclosure, reference is now made to the detailed description herein along with the accompanying figures and in which:

[0041] Figure 1 shows a structure referred to as proline-IFNα2b or also known as Pro-hIFN alpha 2b

[0042] Figure 2 shows an image of an SDS-PAGE of Pro-hIFN alpha 2b at various stages of post IPTG induction using a manufacturing method disclosed in the present disclosure. The arrow denotes the size and expected band location. Lane M: Marker; Lane RS: Pro-hIFN alpha 2b drug reference standard (2µg); Lane 0: initial time Isopropyl-beta-D-1-thiogalactopyranoside (IPTG) used for protein induction at time = 0hr; Lane 1: IPTG post initial induction time at about 1.0hr; Lane 2: IPTG post initial induction time at about 2.0hr; Lane 3: IPTG post initial induction time at about 3.0hr, Lane 4: IPTG post initial induction time at about 4.0hr; Lane 5: IPTG post initial induction time at about 5.0hr. Y axis denotes size.

[0043] Figure 3 shows an image of an SDS-PAGE performed on protein Pro-hIFN alpha 2b after purification. The arrow denotes the size and expected band location. M stands for Marker, RS stands for Pro-hIFN alpha 2b drug reference standard (2µg), and SA stands for purified protein sample (5.0µg loading).

[0044] Figure 4 shows a HPLC graph on purity of Pro-hIFN alpha 2b using a fermentation method as disclosed in the present disclosure. Peak 1 denotes for Pro-hIFN alpha 2b. Peak 2 is solvent peak. X-axis is retention time in minutes. Y-axis denotes amount.

[0045] Figure 5 denotes an amino acid sequence of GCSF with Methionine at the N terminus.

[0046] Figure 6 denotes an amino acid sequence of GCSF.

[0047] Figure 7 denotes the amino acid sequence of an anti-PD-1 monoclonal antibody.

What is claimed is:

1. A method to increase recombinant protein production in a prokaryote host cell, comprising:

performing a recombinant protein fermentation including the steps of:

carrying out seeding (time S_0) of a culture of procaryote host cell engineered to inducibly express a recombinant protein and transferring the culture into one or more fermenters each containing a culture medium;

measuring a dissolved oxygen (DO) level, an agitation rate, and a pH in the one or more fermenters;

supplying a carbon source feeding solution to any of the one or more fermenters, individually, whenever condition(s) (i) and/or (ii) are met:

(i) said DO level exceeds above 35% to 45% and said agitation rate exceeds 300 to 1000 rpm;

(ii) said agitation rate exceeds 400 to 700 rpm and said pH exceeds 7.0 to 7.4; and

supplying a nitrogen source to any of the one or more fermenters in which induction of expression of the recombinant protein has been initiated, individually, at about I_0 (time at initiation of induction) or I_1 or both;

optionally, supplying a nitrogen source to any of the one or more fermenters, individually, at one or more time points selected from about S_5, S_6, S_7, S_8, S_9 and S_{10}.

2. The method according to claim 1, wherein (i) said DO level exceeds above 35% to 40% and said agitation rate exceeds 300 to 700 rpm.

3. The method according to claim 1, wherein (ii) said agitation rate exceeds 500 to 600 rpm and said pH exceeds 7.0 to 7.4.

4. The method according to claim 1, wherein the nitrogen source is supplied, individually, at about I_0 or I_1 both, and at one or more time points selected from about S_5, S_6, S_7, S_8, S_9 and S_{10}.

5. The method according to claim 1, wherein a carbon source feeding solution is supplied at least once to at least one fermenter during the recombinant protein fermentation; optionally,

the carbon source feeding solution is a glucose feeding solution, fructose feeding solution, galactose feeding solution, pyruvate feeding solution, or any combination thereof.

6. The method according to claim 5, wherein a cumulative total of 800 to 1500g of glucose is supplied to at least one fermenter, per about 5 L fermenter capacity, by the end of the recombinant protein fermentation.

7. The method according to claim 1, wherein the nitrogen source supplied to the one or more fermenters, individually, comprises potassium.

8. The method according to claim 1, wherein the nitrogen source supplied to the one or more fermenters, individually, comprises a yeast extract, peptone, soytone, urea, tryptone, or any combinations thereof.

9. The method of claim 8, wherein 40 to 150 mL of a yeast extract solution is supplied the one or more fermenters each time, individually; optionally 300 to 1500 mL of a yeast extract solution is supplied to the one or more fermenters each time, individually.

10. The method according to claim 1, wherein the recombinant protein fermentation is performed with an inoculum ratio of 6.0 to 8.5%.

11. The method according to claim 1, wherein said recombinant protein comprises VX-001, AVE-9633, AC-9301, NY-ESO-1 vaccine, erythropoietin, EPO-Fc, CNTO-528, AMG-114, JR-013, Factor XIII, IFN-α2b, Pro-IFN-α2b, anti-PD1 antibody, and/or GCSF.

12. The method according to claim 1, wherein said prokaryote host cell comprises an *E. coli* BLR-Codon Plus (DE3) cell.

13. The method according to claim 1, further comprising a step of collecting the culture of prokaryote host cell or a sample of the culture in the one or more fermenters after induction of expression of the recombinant protein, individually.

14. The method according to claim 13, further comprising processing inclusion bodies in the collected culture of prokaryote cell or sample to obtain a crude protein extract.

15. The method according to claim 13, wherein the culture or sample are collected after or at about I_4 to I_8.

16. The method according to claim 14, further comprising determining yield of the expressed recombinant protein in the crude protein extract, wherein the yield is increased by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 62.7%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 100%, at least 110%, at least 120%, at least 130%, at least 140%, at least 150%, at least 160%, at least 170%, at least 167%, at least 180%, at least 190%, at least 200%, at least 210%, at least 220%, at least 230%, at least 240%, at least 250%, at least 260%, at least 270%, at least 280%, at least 290%, at least 300%, at least 350%, at least 383%, at least 400%, at least 450%, at least 500%, at least 550%, at least 600%, at least 650%, at least 700%, at least 748%, at least 750%, at least 800%, at least 9 folds, at least 10 folds, at least 20 folds or more, as compared to another fermentation method.

17. The method according to claim 16, wherein the yield is determined for a fermenter having about a 40 L capacity and the yield is increased by at least 100% to 200%, 100%, 105%, 110%, 115%, 120%, 125%, 130%, 135%, 140%, 145%, 150%, 155%, 160%, 165%, 170%, 175%, 180%, 190%, or 200%, as compared to the yield of a fermenter having about a 5 L capacity in the other fermentation method.

18. The method according to claim 14, further comprising determining yield of the expressed recombinant protein in the crude protein extract, wherein the yield is 5 to 10 g of the recombinant protein in a fermenter having about a 5 L capacity; optionally, wherein the yield is 20 to 30 g of the recombinant protein in a fermenter having about a 40 L capacity.

19. A program-controlled mechanism or equipment comprising:

a. an apparatus equipped with one or more sensors capable of measuring the oxygen level, pH, and/or agitation of culture medium in a fermentation reactor in a fermentation process in a periodic or continuous mode; and

b. a feeding solution injection system integrated within said program-controlled mechanism that is programmed to provide one or more feeding solutions to a

fermentation reactor based on measured oxygen levels, pH and/or agitation in the culture medium in a periodic or continuous interval.

20. A method for automatically regulating glucose concentration, oxygen level, pH and/or agitation during fermentation, comprising:

a. measuring parameters of oxygen level, pH and/or agitation of the fermentation with one or more sensors;

b. transmitting the measured parameters to a program-controlled mechanism; and

c. automatically adjusting the injection rate, volume of a feeding solution and agitation based on the measured parameters.