product specifications. There is a need in the art for mitigating or eliminating the incidence of products that do not meet specifications.

SUMMARY

[0006] In one aspect, there is provided a method of producing a recombinant protein comprising (a) fermenting a prokaryotic host cell wherein said prokaryotic host cell has been transformed with a nucleic acid encoding said recombinant protein, and (b) harvesting said recombinant protein under conditions where dissolved oxygen (dO_2) levels are greater than 0%, and (c) purifying said recombinant protein to a filtered bulk for storage (FBS), wherein said filtered bulk does not contain detectable 1,4-dihydroxy-2-naphthoate (DHNA)-recombinant protein adduct, as measured by an ion exchange chromatography (IEC) assay at 310 nm. In one embodiment, in the method described above, the analytical assay is by HPLC, RP HPLC, HIC HPLC, NMR, mass spectrometry, or UV spectroscopy.

[0007] The present disclosure contemplates a method of producing a recombinant protein comprising (a) fermenting a prokaryotic host cell wherein said prokaryotic host cell has been transformed with a nucleic acid encoding said recombinant protein, and (b) harvesting said recombinant protein under conditions where dO_2 levels are greater than 0%, and (c) purifying said recombinant protein to a FBS, wherein said filtered bulk does not contain detectable DHNA-recombinant protein adduct, as measured by an IEC assay at 310 nm, wherein said recombinant protein is a recombinant polypeptide or an isolated antibody.

[0008] The present disclosure contemplates a method of producing a recombinant protein comprising (a) fermenting a prokaryotic host cell wherein said prokaryotic host cell has been transformed with a nucleic acid encoding said recombinant protein, and (b) harvesting said recombinant protein under conditions where dO_2 levels are greater than 0%, and (c) purifying said recombinant protein to a FBS, wherein said filtered bulk does not contain detectable DHNA-recombinant protein adduct, as measured by an IEC assay at 310 nm, wherein the fermentation is scale-independent.

[0009] The present disclosure contemplates a method of producing a recombinant protein comprising (a) fermenting a prokaryotic host cell wherein said prokaryotic host cell has been transformed with a nucleic acid encoding said recombinant protein, and (b) harvesting said recombinant protein under conditions where dO_2 levels are greater than 0%, and (c) purifying said recombinant protein to a FBS, wherein said filtered bulk does not contain detectable DHNA-recombinant protein adduct, as measured by an IEC assay at 310 nm, wherein said prokaryotic host cell is *Escherichia coli (E. coli), Enterobacter, Azotobacter, Erwinia, Bacillus, Pseudomonas, Klebsiella, Proteus, Salmonella, Serratia, Shigella, Rhizobia, Vitreoscilla*, and *Paracoccus*.

[0010] The present disclosure contemplates a method of producing a recombinant protein comprising (a) fermenting a prokaryotic host cell wherein said prokaryotic host cell has been transformed with a nucleic acid encoding said recombinant protein, and (b) harvesting said recombinant protein under conditions where dO_2 levels are greater than 0%, and (c) purifying said recombinant protein to a FBS, wherein said filtered bulk does not contain detectable DHNA-recombinant protein adduct, as measured by an IEC assay

at 310 nm, wherein said dO_2 is maintained at levels greater than 0% continuously throughout the harvest operations of step (b). In one embodiment in the method described above, the harvest operations comprise a homogenization stage. In another embodiment, the dO_2 is maintained at about 30% to about 75% prior to homogenization. In yet another embodiment, the dO_2 is maintained at levels greater than 75% prior to homogenization. In still another embodiment, the dO_2 is maintained at about 50% after homogenization. In another embodiment, the dO_2 is maintained at levels greater than 50% after homogenization. In one embodiment, the dO_2 is maintained for a period of greater than or equal to 1.5 hours. In still another embodiment, the dO_2 is maintained for a period of greater than or equal to 2 hours.

[0011] The present disclosure contemplates a method of producing a recombinant protein comprising (a) fermenting a prokaryotic host cell wherein said prokaryotic host cell has been transformed with a nucleic acid encoding said recombinant protein, and (b) harvesting said recombinant protein under conditions where dO_2 levels are greater than 0%, and (c) purifying said recombinant protein to a FBS, wherein said filtered bulk does not contain detectable DHNA-recombinant protein adduct, as measured by an IEC assay at 310 nm, wherein the dO_2 is maintained with overlay or sparged air, with increased back-pressure, or with agitation (i.e. stirring). In one embodiment, the overlay air is from about 0.4 vvm to about 0.8 vvm. In another embodiment, the overlay air is targeted at 0.6 vvm. In another embodiment, the increased backpressure is between about 1.0 to about 30 psi. In one embodiment, the increased backpressure is targeted at 19 psi. In still another embodiment, the agitation rate is from about 6 Watts/L to about 8 Watts/L. In yet another embodiment, the agitation rate is at least 6 Watts/L. In another embodiment, the agitation rate is targeted at 6 Watts/L.

[0012] In another aspect, there is provided a method of producing a recombinant protein comprising (a) fermenting a *menE* gene-deleted prokaryotic host cell wherein said prokaryotic host cell has been transformed with a nucleic acid encoding said recombinant protein, (b) harvesting said recombinant protein; and (c) purifying said recombinant protein to a filtered bulk for storage (FBS), wherein said filtered bulk does not contain detectable 1,4-dihydroxy-2-naphthoate (DHNA)-recombinant protein adduct, as measured by an ion exchange chromatography (IEC) assay at 310 nm. As a further embodiment to the method described above, the recombinant protein yield is increased by about 20% or greater, by about 30% or greater, by about 40% or greater, by about 50% or greater, by about 60% or greater, as compared to the yield using a control prokaryotic host cell.

[0013] The present disclosure, a method of producing a recombinant protein comprising (a) fermenting a *menE* gene-deleted prokaryotic host cell wherein said prokaryotic host cell has been transformed with a nucleic acid encoding said recombinant protein, (b) harvesting said recombinant protein; and (c) purifying said recombinant protein to a FBS, wherein said filtered bulk does not contain detectable DHNA-recombinant protein adduct, as measured by an IEC assay at 310 nm, is contemplated, wherein the recombinant protein yield is increased by about 20% or greater, by about 30% or greater, by about 40% or greater, by about 50% or greater, by about 60% or greater, as compared to the yield using a control prokaryotic host cell, wherein the fermentation is scale-independent.

[0014] The present disclosure, a method of producing a recombinant protein comprising (a) fermenting a *menE* gene-deleted prokaryotic host cell wherein said prokaryotic host cell has been transformed with a nucleic acid encoding said recombinant protein, (b) harvesting said recombinant protein; and (c) purifying said recombinant protein to a FBS, wherein said filtered bulk does not contain detectable DHNA-recombinant protein adduct, as measured by an IEC assay at 310 nm, is contemplated, wherein the recombinant protein yield is increased by about 20% or greater, by about 30% or greater, by about 40% or greater, by about 50% or greater, by about 60% or greater, as compared to the yield using a control prokaryotic host cell, wherein said recombinant protein is a recombinant polypeptide or an isolated antibody.

[0015] The present disclosure, a method of producing a recombinant protein comprising (a) fermenting a *menE* gene-deleted prokaryotic host cell wherein said prokaryotic host cell has been transformed with a nucleic acid encoding said recombinant protein, (b) harvesting said recombinant protein; and (c) purifying said recombinant protein to a FBS, wherein said filtered bulk does not contain detectable DHNA-recombinant protein adduct, as measured by an IEC assay at 310 nm, is contemplated, wherein the recombinant protein yield is increased by about 20% or greater, by about 30% or greater, by about 40% or greater, by about 50% or greater, by about 60% or greater, as compared to the yield using a control prokaryotic host cell, wherein said prokaryotic host cell is *Escherichia coli (E. coli), Enterobacter, Azotobacter, Erwinia, Bacillus, Pseudomonas, Klebsiella, Proteus, Salmonella, Serratia, Shigella, Rhizobia, Vitreoscilla*, and *Paracoccus*.

BRIEF DESCRIPTION OF THE FIGURES

[0016] Figure 1 shows the COC assay results of three manufacturing runs of a product in which two runs, Run 2 and Run 3, did not meet the expected results for the COC assay. PW = purified water, C = development run control, 1 = Run 1, 2 = Run 2, and 3 = Run 3.

[0017] Figure 2A shows the UV/vis Spectra (10 cm) for Runs 1-3 – Near UV, where Runs 1-3 are represented. New absorbance peaks were observed approximately at 320 nm and at 460 nm which were not apparent for Run 1. Figure 2B shows the UV/vis spectra for Run 3 minus Run 1, in which the difference of the absorbance peaks for Runs 2 and 3 can be distinguished from Run 1.

[0018] Figure 3 shows an IEC assay monitored at 310 nm for Runs 1-3. A slight shoulder peak behind the main peak was observed for Runs 2 and 3, while the profile for Run 1 was comparable to the Reference Material.

[0019] Figure 4 shows a 2D LC-MS analysis of intact Runs 1-3, monitored at 280 nm and 310 nm. An expected mass was observed for Run 1, while the expected mass and an additional mass at 157 Da were observed for Runs 2 and 3.

[0020] Figure 5 shows a 2D-LC MS and mass identification by tryptic peptide map with MS detection of a collected fraction of the brown adduct – a minor peak from the IEC assay was collected. From the 2D

WHAT IS CLAIMED IS:

1. A method of producing a recombinant protein comprising (a) fermenting a *menE* gene-deleted prokaryotic host cell wherein said prokaryotic host cell has been transformed with a nucleic acid encoding said recombinant protein, (b) harvesting said recombinant protein; and (c) purifying said recombinant protein to a filtered bulk for storage (FBS), wherein said filtered bulk does not contain detectable 1,4-dihydroxy-2-naphthoate (DHNA)-recombinant protein adduct, as measured by an ion exchange chromatography (IEC) assay at 310 nm.

2. The method of claim 1, wherein the recombinant protein yield is increased by about 20% or greater, by about 30% or greater, by about 40% or greater, by about 50% or greater, by about 60% or greater, as compared to the yield using a control prokaryotic host cell.

3. A method of producing a recombinant protein comprising (a) fermenting a prokaryotic host cell wherein said prokaryotic host cell has been transformed with a nucleic acid encoding said recombinant protein, and (b) harvesting said recombinant protein under conditions where dissolved oxygen (dO_2) levels are greater than 0%, and (c) purifying said recombinant protein to a filtered bulk for storage (FBS), wherein said filtered bulk does not contain detectable 1,4-dihydroxy-2-naphthoate (DHNA)-recombinant protein adduct, as measured by an ion exchange chromatography (IEC) assay at 310 nm.

4. The method of claim 3, wherein said analytical assay is by HPLC, RP HPLC, HIC HPLC, NMR, mass spectrometry, or UV spectroscopy.

5. The method of claim 3, wherein said dO_2 is maintained at levels greater than 0% continuously throughout the harvest operations of step (b).

6. The method of claim 5, wherein the harvest operations comprise a homogenization stage.

7. The method of claim 6, wherein said dO_2 is maintained at about 30% to about 75% prior to homogenization.

8. The method of claim 6, wherein said dO_2 is maintained at levels greater than 75% prior to homogenization.

9. The method of claim 6 or 7, wherein said dO_2 is maintained at about 50% after homogenization.

10. The method of claim 6 or 7, wherein said dO_2 is maintained at levels greater than 50% after homogenization.

11. The method of claim 7, wherein said dO_2 is maintained for a period of greater than or equal to 1.5 hours.

12. The method of claim 6, wherein said dO_2 is maintained for a period of greater than or equal to 2 hours.

13. The method of claim 3, wherein said dO_2 is maintained with overlay or sparged air, with increased back-pressure, or with agitation rate.

14. The method of claim 13, wherein the overlay air is from about 0.4 vvm to about 0.8 vvm.

15. The method of claim 14, wherein the overlay air is targeted at 0.6 vvm.

16. The method of claim 13, wherein the increased backpressure is between about 1.0 to 30 psi.

17. The method of claim 14, wherein the increase backpressure is targeted at 19 psi.

18. The method of claim 13, wherein the agitation rate is from about 6 Watts/L to about 8 Watts/L.

19. The method of claim 18, wherein the agitation rate is targeted at about 6 Watts/L.

20. The method of claims 1-19, wherein the fermentation is scale-independent.

21. The method of claim 1 or 18, wherein said recombinant protein is a recombinant polypeptide or an isolated antibody.

22. The method of claim 1 or 18, wherein said prokaryotic host cell is *Escherichia coli (E. coli), Enterobacter, Azotobacter, Erwinia, Bacillus, Pseudomonas, Klebsiella, Proteus, Salmonella, Serratia, Shigella, Rhizobia, Vitreoscilla*, and *Paracoccus*.