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

## SUMMARY

**[0006]** The present disclosure provides 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 example, 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 example in the method described above, the harvest operations comprise a homogenization stage. In another example, the $dO_2$ is maintained at about 30% to about 75% prior to homogenization. In yet another example, the $dO_2$ is maintained at levels greater than 75% prior to homogenization. In still another example, the $dO_2$ is maintained at about 50% after homogenization. In another example, the $dO_2$ is maintained at levels greater than 50% after homogenization. In one example, the $dO_2$ is maintained for a period of greater than or equal to 1.5 hours. In still another example, 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 example, the overlay air is from about 0.4 vvm to about 0.8 vvm. In another example, the overlay air is targeted at 0.6 vvm. In another example, the increased backpressure is between about 1.0 to about 30 psi. In one example, the increased backpressure is targeted at 19 psi. In still another example, the agitation rate is from about 6 Watts/L to about 8 Watts/L. In yet another example, the agitation rate is at least 6 Watts/L. In another example, the agitation rate is targeted at 6 Watts/L.

**[0012]** In an 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, wherein said prokaryotic host cell is *Escherichia coli (E. coli), Enterobacter, Azotobacter, Erwinia, Bacillus, Pseudomonas, Klebsiella, Proteus, Salmonella, Serratia, Shigella, Rhizobia, Vitreoscilla*, or *Paracoccus*. As a further example 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.

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, wherein said prokaryotic host cell is *Escherichia coli (E. coli), Enterobacter, Azotobacter, Erwinia, Bacillus, Pseudomonas, Klebsiella, Proteus, Salmonella, Serratia, Shigella, Rhizobia, Vitreoscilla*, or *Paracoccus*.

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. The method of claim 1 or 2, wherein the fermentation is scale-independent.

4. The method of any one of claims 1-3, wherein said recombinant protein is a recombinant polypeptide or an isolated antibody.

5. The method of claim 4, wherein the isolated antibody is a therapeutic monoclonal antibody.