US Patent Application No. 20160333046 also identified a number of detergents that show robust viral clearance, as well as ecological safety.

As such, there is a need for detergents that are effective for inactivation of envelope viruses, particularly detergents that are considered eco-friendly, for use within viral clearance process steps in biomanufacturing. The invention described herein meets this need by identifying effective, viral inactivating detergents that could be incorporated into the biomanufacturing processes for multiple therapeutic modalities.

SUMMARY

The disclosure provides a method for inactivating envelope virus in a fluid known or suspected to contain at least one enveloped virus, comprising obtaining a fluid known or suspected to contain at least one enveloped virus; exposing the fluid to a detergent from Table 1; at a concentration and for a time sufficient to cause viral inactivation. In one aspect, the present disclosure provides a method for inactivating enveloped virus in a fluid known or suspected to contain at least one enveloped virus, comprising obtaining a fluid known or suspected to contain at least one enveloped virus; and exposing the fluid to a detergent having a CAS registry number of CAS 3055-99-0, CAS 9043-30-5, CAS 181135-58-0, CAS 181135-57-9, CAS 250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-12-7, at a concentration and for a time sufficient to cause viral inactivation, wherein the concentration of the detergent is at least 2.5X to at least 10X or higher of its Critical Micelle Concentration (CMC). In another aspect, the present disclosure provides a method for inactivating enveloped viruses during purification of a recombinant protein of interest comprising obtaining a fluid comprising the recombinant protein of interest which is known or suspected to contain at least one virus; subjecting the fluid to at least one detergent, wherein the detergent has a CAS registry number of CAS 3055-99-0, CAS 9043-30-5, CAS 181135-58-0, CAS 181135-57-9, CAS 250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-12-7 at a concentration and for a time sufficient to cause inactivation of enveloped viruses in the fluid; and subjecting the viral inactivated fluid to at least one unit operation which includes at least a filtration step or a chromatography step, wherein the concentration is 5X of its CMC and the time is at least 10 minutes. In another aspect, the present disclosure provides a method for producing an isolated, purified, recombinant protein of interest comprising establishing a cell culture in a bioreactor with a host cell expressing a recombinant protein and culturing the cells to express the recombinant protein of interest; harvesting cell culture fluid containing the recombinant protein of interest; subjecting the harvested fluid containing the recombinant protein of interest to a detergent having a CAS registry number of CAS 3055-99-0, CAS 9043-30-5, CAS 181135-58-0, CAS 181135-57-9, CAS 250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-

12-7, at a detergent concentration and for a time sufficient to cause inactivation of enveloped virus; processing the viral inactivated fluid containing the recombinant protein of interest through at least two additional unit operations; and obtaining an isolated, purified, recombinant protein of interest, wherein the concentration is 5X of its CMC and the time is at least 10 minutes. In another aspect, the present disclosure provides a method for producing an isolated, purified, recombinant protein of interest comprising establishing a cell culture in a bioreactor with a host cell expressing a recombinant protein and culturing the cells to express the recombinant protein of interest; harvesting cell culture fluid containing the recombinant protein of interest; processing the fluid containing the recombinant protein of interest through at least two unit operations, wherein during at least one unit operation there is a step where the fluid containing the recombinant protein of interest is combined with a detergent having a CAS registry number of CAS 3055-99-0, CAS 9043-30-5, CAS 181135-58-0, CAS 181135-57-9, CAS 250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-12-7, at a detergent concentration and for a time sufficient to cause inactivation of enveloped virus; and obtaining an isolated, purified, recombinant protein of interest, wherein the concentration is 5X of its CMC and the time is at least 10 minutes. In one example, the detergent has a CAS registry number of CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, CAS 85618-20-8, CAS 181135-58-0, CAS 181135-57-9, CAS 250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-12-7. In one example the detergent is selected from CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, or CAS 85618-20-8. In one example the fluid is exposed to the detergent for at least 30 seconds to at least 60 minutes or longer. In one example the fluid is exposed to the detergent for at least 10 minutes. In one example the fluid is exposed to the detergent for at least 30 minutes. In one example the fluid is exposed to the detergent for at least 60 minutes or longer. In one example the concentration of the detergent is at least 2.5X to at least 10X or higher of its Critical Micelle Concentration (CMC). In one example the concentration of the detergent is at least 5X of its CMC. In a related example the concentration of the detergent is at least 7.5X of its CMC. In another related example the concentration of the detergent is at least 10X of its CMC. In one example exposure of the fluid to the detergent occurs at a temperature of at least 5º C to 22º C. In a related example exposure of the fluid to the detergent occurs at a temperature of at least 5ºC. In a related example exposure of the fluid to the detergent occurs at a temperature of at least 15ºC. In a related example exposure of the fluid to the detergent occurs at a temperature of at least 20ºC. In another example inactivation is determined using a $TCID_{50}$ assay. In one example the fluid comprises a recombinant protein of interest. In one example the fluid is harvested host cell culture fluid. In one example the fluid is from an effluent stream, eluate, pool, storage or hold from a unit operation comprising a harvest step, a filtration step or a chromatography step. In a related example the fluid is eluate collected from depth filtration, affinity chromatography, ion exchange chromatography, multimodal chromatography, hydrophobic interaction chromatography or hydroxyapatite chromatography. In one related example the fluid is a pool containing harvested cell culture fluid, eluate from depth filtration,

CLAIMS

What is claimed is

1. A method for inactivating enveloped virus in a fluid known or suspected to contain at least one enveloped virus, comprising

obtaining a fluid known or suspected to contain at least one enveloped virus; and

exposing the fluid to a detergent having a CAS registry number of CAS 3055-99-0, CAS 9043-30-5, CAS 181135-58-0, CAS 181135-57-9, CAS 250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-12-7, at a concentration and for a time sufficient to cause viral inactivation, wherein the concentration of the detergent is at least 2.5X to at least 10X or higher of its Critical Micelle Concentration (CMC).

2. The method according to claim 1, wherein the detergent is selected from CAS 3055-99-0 and CAS 9043-30-5.

3. The method according to claim 1 wherein the fluid is exposed to the detergent for at least 30 seconds to at least 60 minutes or longer, preferably for at least 10 minutes.

4. The method according to claim 1, wherein the concentration of the detergent is at least 5X of its CMC.

5. The method according to claim 1, wherein exposure of the fluid to the detergent occurs at a temperature of at least 5º C to 22º C, preferably at least 15ºC.

6. The method according to claim 1, wherein the concentration is 5X of its CMC and the time is at least 10 minutes.

7. The method according to claim 1, wherein the fluid comprises a recombinant protein of interest.

8. The method according to claim 1, wherein the fluid is harvested host cell culture fluid.

9. The method according to claim 1, wherein the fluid is from an effluent stream, eluate, pool, storage or hold from a unit operation comprising a harvest step, a filtration step or a chromatography step, preferably wherein the fluid is

(i) eluate collected from depth filtration, microfiltration, affinity chromatography, ion exchange chromatography, multimodal chromatography, hydrophobic interaction chromatography or hydroxyapatite chromatography, or

(ii) a pool containing harvested cell culture fluid, eluate from depth filtration, eluate from microfiltration, eluate from affinity chromatography, eluate from ion exchange chromatography, eluate

from multimodal chromatography, eluate from hydrophobic interaction chromatography, or eluate from hydroxyapatite chromatography, optionally wherein the affinity chromatography is Protein A, Protein G, Protein A/G, or Protein L chromatography.

10. A method for inactivating enveloped viruses during purification of a recombinant protein of interest comprising

obtaining a fluid comprising the recombinant protein of interest which is known or suspected to contain at least one virus;

subjecting the fluid to at least one detergent, wherein the detergent has a CAS registry number of CAS 3055-99-0, CAS 9043-30-5, CAS 181135-58-0, CAS 181135-57-9, CAS 250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-12-7 at a concentration and for a time sufficient to cause inactivation of enveloped viruses in the fluid; and

subjecting the viral inactivated fluid to at least one unit operation which includes at least a filtration step or a chromatography step, wherein the concentration is 5X of its CMC and the time is at least 10 minutes.

11. The method according to claim 10, wherein the chromatography is selected from affinity chromatography, Protein A chromatography, ion exchange chromatography, anion exchange chromatography, cation exchange chromatography; hydrophobic interaction chromatography; mixed modal or multimodal chromatography, or hydroxyapatite chromatography.

12. The method according to claim 10, wherein:
(i) the fluid is harvested host cell culture fluid and the unit operation includes depth filtration;
(ii) the fluid is harvested host cell culture fluid and the unit operation includes microfiltration;
(iii) the fluid is harvested host cell culture fluid and the unit operation includes Protein A affinity chromatography; or
(iv) the fluid is Protein A eluant and the unit operation includes depth filtration.

13. The method according to claim 10, wherein the unit operation includes depth filtration or microfiltration.

14. A method for producing an isolated, purified, recombinant protein of interest comprising

establishing a cell culture in a bioreactor with a host cell expressing a recombinant protein and culturing the cells to express the recombinant protein of interest;

harvesting cell culture fluid containing the recombinant protein of interest;

subjecting the harvested fluid containing the recombinant protein of interest to a detergent having a CAS registry number of CAS 3055-99-0, CAS 9043-30-5, CAS 181135-58-0, CAS 181135-57-9, CAS 250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-12-7, at a detergent concentration and for a time sufficient to cause inactivation of enveloped virus;

processing the viral inactivated fluid containing the recombinant protein of interest through at least two additional unit operations; and

obtaining an isolated, purified, recombinant protein of interest, wherein the concentration is 5X of its CMC and the time is at least 10 minutes.

15. A method for producing an isolated, purified, recombinant protein of interest comprising

establishing a cell culture in a bioreactor with a host cell expressing a recombinant protein and culturing the cells to express the recombinant protein of interest;

harvesting cell culture fluid containing the recombinant protein of interest;

processing the fluid containing the recombinant protein of interest through at least two unit operations, wherein during at least one unit operation there is a step where the fluid containing the recombinant protein of interest is combined with a detergent having a CAS registry number of CAS 3055-99-0, CAS 9043-30-5, CAS 181135-58-0, CAS 181135-57-9, CAS 250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-12-7, at a detergent concentration and for a time sufficient to cause inactivation of enveloped virus; and

obtaining an isolated, purified, recombinant protein of interest, wherein the concentration is 5X of its CMC and the time is at least 10 minutes.