Summary

In one aspect of the present disclosure, there is provided a method for purifying proteins with a continuous phase separation technology, characterized in that the method comprises following steps:

a) mixing a surfactant and an aqueous protein solution, which contains at least ~~one hydrophobin, a hydrophobin-like protein, or~~ a fusion of ~~any of these~~a hydrophobin with a further molecule of interest, to form an extraction solution,
b) allowing an aqueous phase and a surfactant phase to separate, and
c) continuously removing a stream of the surfactant phase, containing at least ~~one hydrophobin or a hydrophobin-like protein or~~ a fusion of ~~any of these~~a hydrophobin with a further molecule of interest, and a stream of the aqueous phase from the extraction solution.

In another aspect of the present disclosure, there is provided a method for treating antibody solutions with a phase separation technology, characterized in that the method comprises following steps:

a) mixing at least one hydrophobin ~~or a hydrophobin-like protein,~~ fused to at least one antibody-binding protein, into an aqueous antibody-containing solution to form an aqueous protein solution containing at least one hydrophobin-protein-antibody complex,
b) adding a surfactant to form an extraction solution,
c) allowing an aqueous phase and a surfactant phase to separate,
d) removing a stream of the separated surfactant phase, containing at least said complex, and a stream of the aqueous phase from the extraction solution, and
e) adding a low-pH buffer to the separated surfactant phase to release antibody or antibodies from the antibody-binding protein(s) of the formed complex.

In a further aspect of the present disclosure, there is provided a use of a method disclosed herein in enzyme, pharmaceutical, food, fodder or chemical industries for separation and purification of hydrophobins and/or antibodies.

Claims

1. A method for purifying proteins with a continuous phase separation technology, **characterized** in that the method comprises following steps:
    a) mixing a surfactant and an aqueous protein solution, which contains at least ~~one hydrophobin, a hydrophobin-like protein, or~~ a fusion of ~~any of these~~a hydrophobin with a further molecule of interest, to form an extraction solution,
    b) allowing an aqueous phase and a surfactant phase to separate, and
    c) continuously removing a stream of the surfactant phase, containing at least ~~one hydrophobin or a hydrophobin-like protein or~~ a fusion of ~~any of these~~a hydrophobin with a further molecule of interest, and a stream of the aqueous phase from the extraction solution.

2. The method according to claim 1, **characterized** in that the hydrophobin ~~or the hydrophobin-like protein~~ is fused to a molecule of interest to form a hydrophobin fusion, which is able to carry said molecule of interest into the surfactant phase.

3. The method according to claim 1 or 2, **characterized** in that a part of the formed extraction solution from step a) is transferred to a separation vessel with a first flow speed, in which vessel step b) is carried out, followed by carrying out step c) with a second flow speed.

4. The method according to claims 1 to 3, **characterized** in that the surfactant is a non-ionic surfactant, and most suitably selected from the group consisting of ethoxylated sorbitan ester, polyoxyethylene polyoxypropylene tert-C12/C13-alkyl amine, polyethylene glycol tert-octyphenyl ether, polyethylene glycol-co-propylene glycol monobutyl ether, ethoxylated alkyl polyethylene glycol ether, ethoxylated polypropylene oxide, ethylene oxide/propylene oxide -copolymer, ethoxylated natural fatty acid, alkyl polyglycol ether and ethoxylated natural fatty alcohol.

5. The method according to any of the preceding claims, **characterized** in that the surfactant is removed and recovered from the surfactant phase, after separation, with an organic solvent in a back extraction process, the organic solvent preferably being isobutanol.

6. A method for treating antibody solutions with a phase separation technology, **characterized** in that the method comprises following steps:

a) mixing at least one hydrophobin ~~or a hydrophobin-like protein,~~ fused to at least one antibody-binding protein, into an aqueous antibody-containing solution to form an aqueous protein solution containing at least one hydrophobin-protein-antibody complex,

b) adding a surfactant to form an extraction solution,

c) allowing an aqueous phase and a surfactant phase to separate,

d) removing a stream of the separated surfactant phase, containing at least said complex, and a stream of the aqueous phase from the extraction solution, and

e) adding a low-pH buffer to the separated surfactant phase to release antibody or antibodies from the antibody-binding protein(s) of the formed complex.

7. The method according to claim 6, **characterized** in that the antibody-binding protein is originated from a genus *Staphylococcus*, preferably from *Staphylococcus aureus*, more preferably the protein is Protein A, Protein G or Protein S, most suitably Protein A.

8. The method according to claim 6 or 7, **characterized** in that a part of the formed extraction solution from step b) is transferred to a separation vessel with a first flow speed, in which vessel step c) is carried out, followed by carrying out step d) with a second flow speed.

9. The method according to any of claims 6 to 8, **characterized** in that the hydrophobin-protein fusion, from where antibodies have been released, is recycled back to the step a).

10. The method according to claim 3 or 8, **characterized** in that the first flow speed is adjusted together with the second flow speed in a way that the volume of the surfactant phase and the aqueous phase keeps constant in the separation vessel during one separation cycle, preferably the first flow speed and the second flow speed are adjusted to be equal or essentially equal.

11. The method according to any of the preceding claims, **characterized** in that plurality of parallel separation vessels are used that are preferably heat adjustable.