The present invention is therefore based on the object of making available a process for the improved preparation and production of biotechnologically produced products in a biphasic system comprising an aqueous culture medium using a metabolically active cell and a hydrophobic organic solution. In particular, the object is to improve the separation of the hydrophobic organic solution and the extraction of a biotechnologically produced product soluble therein with respect to the rapidity of the process and/or the extent of the separation of the hydrophobic organic solution from the aqueous culture medium in a given time window.

A further object on which the present invention is based consists in making available a biotechnologically usable cell with high resistance against the toxicity to such a cell emanating from hydrophobic solutions.

The present invention is furthermore based on the object of making available a biotechnological process for the production of hydrophobic compounds, in which the oxygen consumption is reduced, and a cell suitable for this purpose.

This and further objects are achieved by the subject of the present application and in particular also by the subject of the attached independent claims, embodiments resulting from the sub-claims.

In an aspect, there is provided an enzymatic two-phase system process, comprising: preparing an aqueous culture medium of a metabolically active cell having a decreased activity; contacting the aqueous culture medium with a hydrophobic organic solution comprising a substrate for biotransformation; conducting a biotransformation of the substrate; and separating the hydrophobic organic solution comprising a biotransformed substrate from the aqueous culture medium; wherein the decreased activity of the metabolically active cell is in comparison to a wild-type of the active cell, the decreased activity is of at least of one enzyme that catalyses one reaction of ß-oxidation of fatty acids, and the metabolically active cell comprises a recombinant alkane hydroxylase of alkB type.

The object on which the invention is based is achieved in a first aspect by a process comprising the steps

a) provision of an aqueous culture medium comprising a metabolically active cell,

b) contacting of the aqueous culture medium with a hydrophobic organic solution,

c) separation of the hydrophobic organic solution from the aqueous culture medium,

wherein the cell has a decreased activity, compared to its wild-type, of at least one enzyme that catalyses one of the reactions of the β-oxidation of fatty acids.

Claims

1. Process comprising the steps

   a) provision of an aqueous culture medium comprising a metabolically active cell,

   b) contacting of the aqueous culture medium with a hydrophobic organic solution,

   c) separation of the hydrophobic organic solution from the aqueous culture medium,

   wherein the cell has a decreased activity, compared to its wild-type, of at least one enzyme that catalyses one of the reactions of the β-oxidation of fatty acids.

2. Process according to Claim 1, the enzyme that catalyses one of the reactions of the β-oxidation of fatty acids being an enzyme from the group that comprises FadA, FadB, FadD, FadE and FadL or their variants, preferably FadE or a variant thereof.

3. Process according to one of Claims 1 or 2, the organic solution comprising at least one solvent selected from the group that comprises room-temperature-liquid substituted and unsubstituted alkanes, cycloalkanes, cycloalkenes, aryls, fatty acids, fatty acid esters, alcohols, heterocycloalkanes, heterocycloalkenes and heteroaryls.

4. Process according to Claim 3, the organic solution comprising a fatty acid having more than 12 carbon atoms or an ester thereof, preferably oleic acid, erucic acid or lauric acid methyl ester.

5. Process according to one of Claims 1 to 4, the cell containing a recombinant alkane hydroxylase.

6. Use of a metabolically active cell that has a decreased activity, compared to its wild-type, of an enzyme that catalyses one of the reactions of the β-oxidation of fatty acids, preferably an enzyme selected from the group comprising FadA, FadB, FadD, FadE and FadL and variants thereof, more preferably FadE, for improving the separation of a hydrophobic organic solution from an aqueous culture medium comprising the metabolically active cell.

7. Use of a knockout of an enzyme that catalyses one of the reactions of the β-oxidation of fatty acids, preferably an enzyme selected from the group comprising FadA, FadB, FadD, FadE and FadL or variants thereof, more preferably FadE, as part of the genetic make-up of a metabolically active cell for improving the separation of a hydrophobic organic solution from an aqueous culture medium comprising the metabolically active cell.

8. Cell that has a decreased activity, compared to its wild-type, of an enzyme that catalyses one of the reactions of the β-oxidation of fatty acids, further comprising a recombinant monooxygenase, more preferably an alkane hydroxylase.

9. Cell according to Claim 8, the enzyme that catalyses one of the reactions of the β-oxidation of fatty acids being an enzyme from the group that comprises FadA, FadB, FadD, FadE and FadL or variants thereof, more preferably FadE or a variant thereof.

10. Reaction mixture comprising an aqueous solution containing a metabolically active cell, and a hydrophobic organic solution,

    wherein the cell has a decreased activity, compared to its wild-type, of an enzyme that catalyses one of the reactions of the β-oxidation of fatty acids.

11. Reaction mixture according to Claim 10, the enzyme that catalyses one of the reactions of the β-oxidation of fatty acids being an enzyme from the group that comprises FadA, FadB, FadD, FadE and FadL or variants thereof, more preferably FadE or a variant thereof.

12. Reaction mixture according to one of Claims 10 to 11, the hydrophobic organic solution comprising at least one solvent selected from the group that comprises room-temperature-liquid substituted and unsubstituted alkanes, cycloalkanes, cycloalkenes, aryls, fatty acids, fatty acid esters, alcohols, heterocycloalkanes, heterocycloalkenes and heteroaryls.

13. Reaction mixture according to one of Claims 10 to 12, the hydrophobic organic solution comprising a fatty acid having more than 12 carbon atoms or an ester thereof, preferably oleic acid, erucic acid or lauric acid methyl ester.

14. Process according to one of Claims 1 to 5, use according to one of Claims 6 to 7 or reaction mixture according to one of Claims 10 to 13, the metabolically active cell being a cell that contains a recombinant alkane hydroxylase and a transaminase, preferably further at least one enzyme from the group comprising alcohol dehydrogenase, alanine dehydrogenase and lactam hydrolase.

15. Process according to one of Claims 1 to 5 and 14, use according to one of Claims 6 to 7 and 14 or reaction mixture according to one of Claims 10 to 14, the cell being a lower eukaryotic or a prokaryotic cell, preferably *E. coli*.

16. Process according to one of Claims 1 to 5 and 14 to 15, use according to one of Claims 6 to 7 and 14 to 15 or reaction mixture according to one of Claims 10 to 15, the cell being a recombinant cell.

17. Process according to one of Claims 1 to 5 and 14 to 16, use according to one of Claims 6 to 7 and 14 to 16 or reaction mixture according to one of Claims 10 to 16, the hydrophobic organic solution making up at least 5, preferably at least 20, per cent of the total volume of hydrophobic organic solution and aqueous culture medium.

18. Process according to one of Claims 1 to 5 and 14 to 17, use according to one of Claims 6 to 7 and 14 to 17 or reaction mixture according to one of Claims 10 to 17, the pH of the aqueous culture medium at the time of contacting being between 5 and 9, more preferably 6 and 8.

Claims

1. An enzymatic two-phase system process, comprising: preparing an aqueous culture medium of a metabolically active cell having a decreased activity; contacting the aqueous culture medium with a hydrophobic organic solution comprising a substrate for biotransformation; conducting a biotransformation of the substrate; and separating the hydrophobic organic solution comprising a biotransformed substrate from the aqueous culture medium; wherein the decreased activity of the metabolically active cell is in comparison to a wild-type of the active cell, the decreased activity is of at least of one enzyme that catalyses one reaction of ß-oxidation of fatty acids, and the metabolically active cell comprises a recombinant alkane hydroxylase of alkB type.

2. The process according to claim 1, wherein the at least one enzyme having decreased activity is selected from the group consisting of FadA, FadB, FadD, FadE, and FadL, and variants thereof.

3. The process according to claim 1, wherein the hydrophobic organic solution comprises at least one solvent which is liquid at room temperature, and which is selected from the group consisting of a substituted alkane, an unsubstituted alkane, a cycloalkane, a cycloalkene, an aryl, a fatty acid, a fatty acid ester, an alcohol, a heterocycloalkane, a heterocycloalkene, and a heteroaryl.

4. The process according to claim 3, wherein the organic solution further comprises a fatty acid having more than 12 carbon atoms or an ester thereof.

5. The process according to claim 4, wherein the organic solution comprises a fatty acid and the fatty acid is oleic acid or erucic acid, or the organic solution comprises an ester and the ester is lauric acid methyl ester.

6. The process according to claim 1, wherein the metabolically active cell further comprises a transaminase.

7. The process according to claim 1, wherein the metabolically active cell is a lower eukaryotic cell or a prokaryotic cell.

8. The process according to claim 1, wherein a content of the hydrophobic organic solution relative to a total volume of hydrophobic organic solution and aqueous culture medium is at least 5 percent of the total volume.

9. The process according to claim 1, wherein a pH of the aqueous culture medium at a time of contacting the hydrophobic organic solution is from 5 to 9.

10. The process according to claim 1, wherein the metabolically active cell is *E. coli*.

11. The process according to claim 1, wherein the recombinant alkane hydroxylase of alkB type has a sequence homology of at least 75% to the sequence of AlkB of *Pseudomonas putida* Gop1 of database code: CAB54050.1.

12. The process according to claim 1, wherein the recombinant alkane hydroxylase of alkB type has a sequence homology of at least 90% to the sequence of AlkB of *Pseudomonas putida* Gop1 of database code: CAB54050.1.

13. The process according to claim 1, wherein the recombinant alkane hydroxylase of alkB type has a sequence homology of at least 99% to the sequence of AlkB of *Pseudomonas putida* Gop1 of database code: CAB54050.1.