in the desired end product, instead of for example being converted to products other than that desired.

A further problem on which the invention is based consists in improving the processability of a multiphase reaction mixture from the conversion of a carboxylic acid ester, particularly with regard to the reusability of the hydrophobic solvents and liquid cation exchangers used for the processing, and with regard to the phase formation and separation in a biphasic system comprising an aqueous phase, in which the conversion of the carboxylic acid ester proceeds, and an organic phase with organic solvents and/or liquid cation exchangers.

These and other problems are solved by the subject matter of the present application and in particular by the subject matter of the appended independent claims, wherein embodiments follow from the subclaims.

Summary

In one aspect, the present disclosure refers to a whole cell catalyst which comprises a transaminase and one selected from the group consisting of

1) an α-dioxygenase and

2) a combination of 2a) a recombinant fatty acid reductase and 2b) a phosphopantetheinyl transferase,

wherein:

the phosphopantetheinyl transferase phospho-pantetheinylates the fatty acid reductase, thereby forming a phosphopantetheinylated fatty acid reductase,

the fatty acid reductase catalyzes the conversion of an ω-carboxy acid to a corresponding ω-oxo fatty acid,

at least one of the phosphopantetheinyl transferase and transaminase is recombinant, when the catalyst comprises the transaminase and the α-dioxygenase, the transaminase is overexpressed, and when the catalyst comprises the transaminase and the combination of

2a) a recombinant fatty acid reductase and 2b) a phosphopantetheinyl transferase, the transaminase and/or the phosphopantetheinyl transferase is overexpressed.

In one example, the problem on which the disclosure is based is solved by a whole cell catalyst which expresses a recombinant α-dioxygenase or the combination of a recombinant fatty acid reductase and of a phosphopantetheinyl transferase phosphopantetheinylating the fatty acid reductase, and which additionally expresses a transaminase, wherein the phosphopantetheinyl transferase and/or transaminase is preferably recombinant.

In another example, the problem is solved by a whole cell catalyst which additionally expresses an amino acid dehydrogenase which is preferably recombinant.

In another example, the problem is solved by a whole cell catalyst which additionally expresses an alkane hydroxylase which is preferably recombinant.

In another example, the problem is solved by a whole cell catalyst which additionally expresses a polypeptide of the AlkL family which is preferably recombinant.

In another example, the problem is solved by a whole cell catalyst which additionally expresses an alcohol dehydrogenase which is preferably recombinant.

In another example, the problem is solved by a whole cell catalyst wherein the activity of at least one enzyme involved in β-oxidation is decreased compared to the wild type of the whole cell catalyst.

In another example, the problem is solved by a whole cell catalyst wherein the activity of BioH or a variant thereof is decreased compared to the wild type of the whole cell catalyst.
In another example, the problem is solved by a whole cell catalyst wherein the activity of FadL or a variant thereof is increased compared to the wild type of the whole cell catalyst.

In another aspect, the present disclosure refers to a method for preparing an amine, the method comprising:

a) oxidizing a starting material comprising a fatty acid, ω-hydroxy fatty acid, ω-oxo fatty acid or monoester thereof by contacting the starting material with an alkane hydroxylase, an alcohol dehydrogenase, or a mixture thereof to obtain an oxidation product;

b) contacting the oxidation product with a phosphopantetheinylated fatty acid reductase or an α-dioxygenase to obtain an aldehyde; and

c) contacting the aldehyde with a transaminase to obtain the amine,

wherein the phosphopantetheinylated fatty acid reductase or the α-dioxygenase in addition to the transaminase is provided in the form of the whole cell catalyst as disclosed herein.

In another example, the problem on which the disclosure is based is solved by a process for the conversion of a fatty acid, ω-hydroxy fatty acid, ω-oxo fatty acid or a monoester thereof to an amine, comprising the steps

a) oxidation of the fatty acid, ω-hydroxy fatty acid, ω-oxo fatty acid or the monoester thereof to an oxidation product by contacting with an alkane hydroxylase and/or alcohol dehydrogenase,

b) contacting the oxidation product with a phosphopantetheinylated fatty acid reductase or an α-dioxygenase to give an aldehyde, and

c) contacting the aldehyde with a transaminase.

In another example, the problem on which the disclosure is based is solved by a process wherein in step c) an amino acid dehydrogenase is present.

In another example, the problem is solved by a process wherein at least one enzyme from the group comprising phosphopantetheinylated fatty acid reductase,

α-dioxygenase, transaminase, amino acid dehydrogenase and alkane hydroxylase, preferably all enzymes used from this group, are provided in the form of a whole cell catalyst according to an example of the disclosure.

In another example, the problem is solved by a process wherein the fatty acid, ω-hydroxy fatty acid, ω-oxo fatty acid or the monoester thereof is a compound of the formula (I)

$$R^1 - A - COOR^2 \quad (I)$$

wherein R^1 is selected from the group which comprises -H, -CHO, -OH and $COOR^3$,

wherein R^2 and R^3 each and independently of one another are selected from the group which comprises H, methyl, ethyl and propyl,

with the proviso that at least one of the residues R^2 and R^3 is H, and

wherein A represents an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group with at least four carbon atoms.

In another example, the problem is solved by a process wherein A has the formula - $(CH_2)_n$ -, wherein n is at least 4, preferably at least 10.

In another aspect, the present disclosure refers to a method for preparing an amine, the method comprising:

a) oxidizing a starting material comprising a fatty acid, ω-hydroxy fatty acid, ω-oxo fatty acid or monoester thereof by contacting the starting material with an alkane hydroxylase, an alcohol dehydrogenase, or a mixture thereof to obtain an oxidation product;

b) contacting the oxidation product with a phosphopantetheinylated fatty acid reductase or an α-dioxygenase to obtain an aldehyde; and

c) contacting the aldehyde with a transaminase to obtain the amine;

wherein at least one enzyme of a), b) and c) is present in the form of the whole cell catalyst as disclosed herein.

In another example, the problem on which the disclosure is based is solved by use of the whole cell catalyst according to an example or of the process according to an example for the amination of a fatty acid, ω-hydroxy fatty acid, ω-oxo fatty acid or a monoester thereof.

In another example, the problem on which the disclosure is based is solved by a reaction mixture comprising the whole cell catalyst according to an example in aqueous solution and a fatty acid, ω-hydroxy fatty acid, ω-oxo fatty acid or a monoester thereof of the formula (I)

$$R^1 - A - COOR^2 \text{ (I)},$$

wherein R^1 is selected from the group which comprises -H, -CHO, -OH and $COOR^3$,

wherein R^2 and R^3 each and independently of one another are selected from the group which comprises H, methyl, ethyl and propyl,

with the proviso that at least one of the residues R^2 and R^3 is H, and

wherein A represents an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group with at least four carbon atoms, preferably the formula - $(CH_2)_n$ -, wherein n is at least 4, particularly preferably at least 10.

In another aspect, the present disclosure refers to a reaction mixture comprising:

water;

the whole cell catalyst as disclosed herein; and

a fatty acid, ω-hydroxy fatty acid, ω-oxo fatty acid or a monoester thereof of the formula (I)

$$R^1 - A - COOR^2 \text{ (I)},$$

wherein

R^1 is -H, -CHO, -OH and $COOR^3$,

R^2 and R^3 are each independently H, methyl, ethyl or propyl, with the proviso that at least one of the residues R^2 and R^3 is H, and

A is an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group with at least four carbon atoms.

The present disclosure is based on the discovery of the inventors that a functionally coexpressed recombinant fatty acid reductase or α-dioxygenase in a whole cell catalyst which is

used for the production of ω-amino fatty acids from fatty acids and has an appropriate enzyme composition, surprisingly increases the yield of ω-amino fatty acids.

Furthermore, the present disclosure is based on the discovery of the inventors that a functionally coexpressed recombinant fatty acid reductase or α-dioxygenase in a whole cell catalyst which is used for the production of ω-amino fatty acids from fatty acids and has an appropriate enzyme composition, surprisingly decreases the concentration of interfering by-products, in particular of overoxidized fatty acids in the form of dicarboxylic acids and esters thereof, in the product obtained.

Furthermore, the present disclosure is based on the discovery of the inventors that a functionally coexpressed recombinant fatty acid reductase or α-dioxygenase in a whole cell catalyst which is used for the production of ω-amino fatty acids from fatty acids and has an appropriate enzyme composition, improves the purity and reusability of liquid cation exchangers such as oleic acid, which are used for the removal of an ω-amino fatty acid from a fermentation solution containing the whole cell catalyst.

The present invention relates to an improved process for the conversion of a fatty acid, ω-hydroxy fatty acid, ω-oxo fatty acid or a monoester thereof to an amine, which is distinguished

the pathway to acetyl-CoA, preferably recognizes it as substrate, and catalyses its conversion to a metabolic product lying closer to acetyl-CoA on this degradation pathway, preferably including the fatty acid importer which effects the uptake of the fatty acid into the cell. For example, acyl-CoA dehydrogenase is among these enzymes according to the foregoing definition, since it interacts with the fatty acid CoA ester and catalyses its conversion to the enoyl-CoA, which lies closer to acetyl-CoA on the metabolic pathway of the β oxidation than the fatty acid CoA ester. In a particularly preferred embodiment, the term "enzyme which catalyses one of the reactions of the β oxidation of fatty acids", as used herein, is understood to mean any enzyme from the group which comprises the gene products FadA, FadB, FadD, FadL and FadE from *E. coli* and/or variants or homologues thereof from other organisms. The gene products FadA, FadB, FadD, FadL and FadE from *E. coli*, like also variants and homologues of many other biotechnologically usable organisms and their nucleic acid and polypeptide sequences, are described in the state of the art, for example FadA under access number AP009048.1, FadB under access number BAE77457.1, FadD under access number BAA15609.1, and FadE under access number BAA77891.2. The state of the art discloses many tests which are especially suitable for the measurement of the activity of enzymes which catalyse one of the reactions of the β oxidation of fatty acids, for example in K Kameda & W D Nunn (1981) *J. Biol. Chem.* 256, 5702-5707, H Marrakchi, W E DeWolf, C Quinn, J West, B J Polizzi, C Y So et al. (2003) Biochem. J. 370, 1055-1062, Lobo *et al.* (2001) and X Yu, T Liu, F Zhu, and C Khosla (2011) PNAS, *electronic publication before printing.*

For the effectiveness of the whole cell catalyst according to the invention it is advantageous if the substrate to be converted, preferably the fatty acid, ω-hydroxy- or ω-oxo fatty acid, can easily come into contact with the enzymes necessary according to the invention, which are located in the interior of the whole cell catalyst. Hence it is critical that the substrate can reach the interior of the cell. In order to facilitate this, it is preferable that the whole cell catalyst expresses a fatty acid importer, in the case of a bacterial, in particular Gram-negative whole cell catalyst, particularly preferably the fatty acid importer FadL (database code: BAA16205.1, SEQ ID NO: 31) or a variant, preferably in a concentration and with an activity which is increased compared to the activity of the wild type of the corresponding whole cell catalyst. The raising of the activity of a polypeptide compared to the wild type of the cell can be achieved via various routes routinely accessible to those skilled in the art, for example the incorporation of additional copies of the nucleotide sequence coding for the polypeptide functionally linked with a promoter, or the exchange of the natural promoter for a stronger one.

It has been found that the ω-amino fatty acids are produced according to the invention in higher yield and purity when the background of enzymes endogenously expressed in the whole cell catalyst is optimized such that the activity of endogenous enzymes which degrade the educts, intermediate products or products of the method according to the invention or with use of the cell according to the invention, preferably ω-amino fatty acids, on metabolic pathways or modify them in other ways, which lead away from the formation of the desired product, is decreased or switched off. Against this background, it is advantageous if the whole cell catalyst according to the invention is about one cell, which has decreased activity of the esterase BioH [Database code YP_492020.1, SEQ ID NO: 30] or a variant thereof compared to its wild type. Such cells with decreased BioH activity, their production and tests for activity determination are described in the European patent application EP12007663.3.

The whole cell catalyst according to the invention can be used in a method for the conversion of a fatty acid, ω-hydroxy- or ω-oxo fatty acid to the corresponding amine, preferably an ω-amino fatty acid, wherein the fatty acid, ω-hydroxy fatty acid, ω-oxo fatty acid or the monoester thereof is a compound of the formula (I)

$$R^1 - A - COOR^2 \quad (I)$$

wherein R^1 is selected from the group which comprises -H, -CHO, -OH and $COOR^3$, wherein R^2 and R^3 each and independently of one another are selected from the group which comprises H, methyl, ethyl and propyl, with the proviso that at least one of the residues R^2 and R^3 is H, and wherein A represents an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group with at least four carbon atoms. In a preferred embodiment, A is a structure of the formula $-(CH_2)_n-$, wherein n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30. In a most preferred embodiment, the fatty acid, ω-hydroxy or ω-oxo fatty acid is lauric acid, ω-hydroxy- or ω-oxolauric acid. In a further most preferred embodiment, the fatty acid, ω-hydroxy or ω-oxo fatty acid is hexanoic acid, ω-hydroxy- or ω-oxohexanoic acid. In a further most preferred embodiment the fatty acid, ω-hydroxy or ω-oxo fatty acid is decanoic acid, ω-hydroxy- or ω-oxodecanoic acid.

With regard to the fatty acid as to every chemical compound described in this application, the respective formula stated includes all salts, protonated or deprotonated, of the respective

Claims

1. A whole cell catalyst which comprises a transaminase and one selected from the group consisting of

   1) an α-dioxygenase and

   2) a combination of 2a) a recombinant fatty acid reductase and 2b) a phosphopantetheinyl transferase,

   wherein:

   the phosphopantetheinyl transferase phospho-pantetheinylates the fatty acid reductase, thereby forming a phosphopantetheinylated fatty acid reductase,

   the fatty acid reductase catalyzes the conversion of an ω-carboxy acid to a corresponding ω-oxo fatty acid,

   at least one of the phosphopantetheinyl transferase and transaminase is recombinant,

   when the catalyst comprises the transaminase and the α-dioxygenase, the transaminase is overexpressed, and when the catalyst comprises the transaminase and the combination of 2a) a recombinant fatty acid reductase and 2b) a phosphopantetheinyl transferase, the transaminase and/or the phosphopantetheinyl transferase is overexpressed.

2. The whole cell catalyst according to claim 1, wherein the whole cell catalyst additionally comprises a recombinant amino acid dehydrogenase.

3. The whole cell catalyst according to claim 1, wherein the whole cell catalyst additionally comprises an alkane hydroxylase.

4. The whole cell catalyst according to claim 1, wherein the whole cell catalyst additionally comprises a polypeptide of the AlkL family.

5. The whole cell catalyst according to claim 1, wherein the whole cell catalyst additionally comprises an alcohol dehydrogenase.

6. The whole cell catalyst according to claim 1, wherein an activity of at least one enzyme of the cell involved in β-oxidation, selected from the group consisting of a fatty acid-CoA ligase, an acyl-CoA dehydrogenase, a 2,4-dienoyl-CoA reductase, an enoyl-CoA

hydratase, a 3-ketoacyl-CoA thiolase, and a fatty acid importer, is decreased compared to the wild type of the whole cell of the catalyst.

7. The whole cell catalyst according to claim 1, wherein an esterase activity of BioH, or a variant of BioH having at least 70% amino acid sequence identity to SEQ ID NO: 30 and essentially the same esterase activity as BioH, in the whole cell is decreased compared to a wild type of the whole cell of the catalyst.

8. The whole cell catalyst according to claim 1, wherein a fatty acid importing activity of FadL, or a variant of FadL having at least 70% amino acid sequence identity to SEQ ID NO: 31 and essentially the same fatty acid importing activity as FadL, is increased in the whole cell of the catalyst compared to a wild type of the whole cell of the catalyst.

9. A method for preparing an amine, the method comprising:

a) oxidizing a starting material comprising a fatty acid, ω-hydroxy fatty acid, ω-oxo fatty acid or monoester thereof by contacting the starting material with an alkane hydroxylase, an alcohol dehydrogenase, or a mixture thereof to obtain an oxidation product;

b) contacting the oxidation product with a phosphopantetheinylated fatty acid reductase or an α-dioxygenase to obtain an aldehyde; and

c) contacting the aldehyde with a transaminase to obtain the amine,

wherein the phosphopantetheinylated fatty acid reductase or the α-dioxygenase in addition to the transaminase is provided in the form of the whole cell catalyst according to claim 1.

10. The method according to claim 9, wherein in c) an amino acid acts as an amine donor, and an amino acid dehydrogenase regenerates the amino acid after a transamination reaction.

11. The method according to claim 9, wherein the fatty acid, ω-hydroxy fatty acid, ω-oxo fatty acid or the monoester thereof is a compound of the formula (I)

$$R^1 - A - COOR^2 \quad (I)$$

wherein

R^1 is -H, -CHO, -OH and $COOR^3$,

R^2 and R^3 are each independently H, methyl, ethyl or propyl,

with the proviso that at least one of the residues R^2 and R^3 is H, and

A is an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group with at least four carbon atoms.

12. The method according to claim 11, wherein A is $-(CH_2)_n-$, and n is at least 4.

13. A method for preparing an amine, the method comprising:

a) oxidizing a starting material comprising a fatty acid, ω-hydroxy fatty acid, ω-oxo fatty acid or monoester thereof by contacting the starting material with an alkane hydroxylase, an alcohol dehydrogenase, or a mixture thereof to obtain an oxidation product;

b) contacting the oxidation product with a phosphopantetheinylated fatty acid reductase or an α-dioxygenase to obtain an aldehyde; and

c) contacting the aldehyde with a transaminase to obtain the amine;

wherein at least one enzyme of a), b) and c) is present in the form of the whole cell catalyst according to claim 1.

14. The method according to claim 13, wherein in c) an amino acid acts as an amine donor, and an amino acid dehydrogenase regenerates the amino acid after a transamination reaction.

15. The method according to claim 13, wherein the fatty acid, ω-hydroxy fatty acid, ω-oxo fatty acid or the monoester thereof is a compound of formula (I)

$$R^1 - A - COOR^2 \text{ (I)},$$

wherein
R^1 is -H, -CHO, -OH and $COOR^3$,

R^2 and R^3 are each independently H, methyl, ethyl and propyl,

with the proviso that at least one of the residues R^2 and R^3 is H, and

A is an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group with at least four carbon atoms.

16. The method according to claim 15, wherein A is - $(CH_2)_n$ - , and n is at least 4.

17. A reaction mixture comprising:
water;
the whole cell catalyst according to claim 1; and
a fatty acid, ω-hydroxy fatty acid, ω-oxo fatty acid or a monoester thereof of the formula (I)

$$R^1 - A - COOR^2 \text{ (I)},$$

wherein
R^1 is -H, -CHO, -OH and $COOR^3$,

R^2 and R^3 are each independently H, methyl, ethyl or propyl, with the proviso that at least one of the residues R^2 and R^3 is H, and

A is an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group with at least four carbon atoms.

18. The whole cell catalyst according to claim 1, comprising the transaminase and 1) the α-dioxygenase.

19. The whole cell catalyst according to claim 1, comprising the transaminase and 2) the combination of 2a) a recombinant fatty acid reductase and 2b) a phosphopatetheinyl transferase.