It is also an object of the invention to improve the ability of a multi-phase reaction mixture from the production of alkyl monoamines and diamines from fatty acids to be worked up, especially with regard to reusability relating to work-up of used hydrophobic solvent and liquid cation exchanger, and also with regard to phase formation and separation in a biphasic system comprising an aqueous phase in which the conversion of the carboxylic acid or the carboxylic ester proceeds, and an organic phase containing organic solvents and/or liquid cation exchangers.

These and further objects are achieved by the subject matter of the present application and in particular also by the subject matter of the accompanying independent claims, the dependent claims specifying embodiments of the invention.

In a first example, the object underlying the disclosure is achieved 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 which phosphopantetheinylates the fatty acid reductase, and which additionally expresses a transaminase, wherein the phosphopantetheinyl transferase and/or transaminase is preferably recombinant.

In one aspect, the present disclosure refers to a whole-cell catalyst, comprising heterologous expression of: a combination of a recombinant fatty acid reductase and a phosphopantetheinyl transferase capable of phosphopantetheinylating the fatty acid reductase; and a transaminase, wherein the recombinant fatty acid reductase and the phosphopantetheinyl transferase, and the transaminase, are expressed in the whole-cell catalyst, and wherein the whole-cell catalyst is a microorganism.

In one example, the object is achieved by a whole-cell catalyst which additionally expresses an amino acid dehydrogenase which is preferably recombinant.

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

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

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

In another example, the object is achieved by a whole-cell catalyst, wherein the activity of at least one enzyme involved in the β-oxidation is reduced with respect to the wild type of the whole-cell catalyst.

In another example, the object is achieved by a whole-cell catalyst, wherein the activity of BioH or a variant thereof is reduced or elevated with respect to the wild type of the whole-cell catalyst.

In yet another example, the object is achieved by a whole-cell catalyst, wherein the activity of FadL or a variant thereof is elevated with respect to the wild type of the whole-cell catalyst.

In a second example, the object underlying the disclosure is achieved by a process for converting a carboxylic acid or dicarboxylic acid or a monoester thereof to an amine or diamine, comprising the steps of

a) providing a carboxylic acid or dicarboxylic acid or a monoester thereof, in the case of a dicarboxylic acid, preferably by contacting a carboxylic acid with an alkane hydroxylase and/or alcohol dehydrogenase,

b) contacting the carboxylic acid or dicarboxylic acid or the monoester thereof with a phosphopantetheinylated fatty acid reductase or an α-dioxygenase to form an aldehyde product, and

c) contacting the product from step a) with a transaminase.

In a second example, the object underlying the disclosure is achieved by a process, wherein an amino acid dehydrogenase is present in step c).

In one example, the object is achieved 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

the enzymes used from said group, are provided in the form of a whole-cell catalyst the first example of the present disclosure.

In another example, the object is achieved by a process, wherein the carboxylic acid or dicarboxylic acid or the monoester thereof is a compound of the formula (I)

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

where R^1 is selected from the group comprising -H and $COOR^3$,

where R^2 and R^3 are each independently selected from the group comprising H, methyl, ethyl and propyl,

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

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

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

In a third example, the object underlying the disclosure is achieved by using the whole-cell catalyst according to the first example or the process according to the second example for aminating a fatty acid, ω-hydroxy fatty acid, ω-oxo fatty acid or a monoester thereof.

In a fourth example, the object underlying the disclosure is achieved by a reaction mixture comprising the whole-cell catalyst according to the first example in aqueous solution and also a fatty acid, ω-hydroxy fatty acid, ω-oxo fatty acid or a monoester thereof of the formula (I)

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

where R^1 is selected from the group comprising -H, -CHO, -OH and $COOR^3$,

where R^2 and R^3 are each independently selected from the group comprising H, methyl, ethyl and propyl,

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

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

In another aspect, the present disclosure refers to a reaction mixture, comprising: the whole-cell catalyst as disclosed herein in an aqueous solution; and a carboxylic acid, dicarboxylic acid, or a monoester thereof having the formula (I):

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

wherein R^1 is -H or $COOR^3$,

R^2 and R^3 are each independently selected from the group consisting of H, methyl, ethyl and propyl, with the proviso that at least one of the radicals R^2 and R^3 is H, and

A is an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group having at least four carbons.

The present invention is based on the finding by the inventor that a functionally recombinant fatty acid reductase or α-dioxygenase in a whole-cell catalyst which is used to produce amines and diamines from fatty acids and has a corresponding enzyme environment surprisingly increases the yield of amines and diamines.

Furthermore, the present invention is based on the finding by the inventor that a functionally recombinant fatty acid reductase or α-dioxygenase in a whole-cell catalyst which is used to produce amines and diamines from fatty acids and has a corresponding enzyme environment surprisingly reduces the concentration of interfering by-products, more particularly overoxidized fatty acids in the form of dicarboxylic acids and esters thereof, in the product which arises.

Furthermore, the present invention is based on the finding by the inventor that a functionally recombinant fatty acid reductase or α-dioxygenase in a whole-cell catalyst which is used to produce amines and diamines fatty acids and has a corresponding enzyme environment improves the purity and reusability of liquid cation exchangers such as oleic acid, which are used to remove the amine and diamine from a fermentation solution comprising the whole-cell catalyst.

The present invention provides an improved process for converting a carboxylic acid or dicarboxylic acid or a monoester thereof to an amine or diamine, which process is notable for the fact that, besides the enzymes catalysing the transfer of the fatty acid via its various oxidation stages to the amine, a fatty acid reductase or α-dioxygenase is also present,

Claims

1. A whole-cell catalyst, comprising heterologous expression of: a combination of a recombinant fatty acid reductase and a phosphopantetheinyl transferase capable of phosphopantetheinylating the fatty acid reductase; and a transaminase, wherein the recombinant fatty acid reductase and the phosphopantetheinyl transferase, and the transaminase, are expressed in the whole-cell catalyst, and wherein the whole-cell catalyst is a microorganism.

2. The whole-cell catalyst according to claim 1, further comprising: an amino acid dehydrogenase expressed in the whole-cell catalyst.

3. The whole-cell catalyst according to claim 1, further comprising: an alkane hydroxylase expressed in the whole-cell catalyst.

4. The whole-cell catalyst according to claim 1, further comprising: a polypeptide of the AlkL family expressed in the whole-cell catalyst.

5. The whole-cell catalyst according to claim 1, further comprising: an alcohol dehydrogenase expressed in the whole-cell catalyst.

6. The whole-cell catalyst according to claim 1, wherein an activity of at least one enzyme involved in β-oxidation is reduced as compared to an activity of the at least one enzyme in the wild type of the whole-cell catalyst.

7. The whole-cell catalyst according to claim 1, wherein an activity of BioH is reduced or elevated as compared to an activity of BioH in the wild type of the whole-cell catalyst.

8. The whole-cell catalyst according to claim 1, wherein an activity of FadL is elevated as compared to an activity of FadL in the wild type of the whole-cell catalyst.

9. A reaction mixture, comprising: the whole-cell catalyst according to claim 1 in an aqueous solution; and a carboxylic acid, dicarboxylic acid, or a monoester thereof having the formula (I):

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

wherein R^1 is -H or $COOR^3$,

R^2 and R^3 are each independently selected from the group consisting of H, methyl, ethyl and propyl, with the proviso that at least one of the radicals R^2 and R^3 is H, and

A is an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group having at least four carbons.

10. The whole-cell catalyst according to claim 1, wherein at least one of the phosphopantetheinyl transferase and the transaminase is recombinant.

11. The whole-cell catalyst according to claim 1, further comprising: at least one of a recombinant amino acid dehydrogenase, a recombinant alkane hydroxylase, a recombinant polypeptide of the AlkL family, and a recombinant alcohol dehydrogenase, expressed in the whole-cell catalyst.

12. The whole-cell catalyst according to claim 1, wherein the recombinant fatty acid reductase is encoded by a gene having at least 70% sequence identity to the nucleotide sequence of SEQ ID NO: 1, the phosphopantetheinyl transferase is encoded by a gene having at least 70% sequence identity to the nucleotide sequence of SEQ ID NO: 2, and the transaminase is encoded by a gene having at least 70% sequence identity to the nucleotide sequence of SEQ ID NO: 12.

13. The whole-cell catalyst according to claim 1, further comprising: an alanine dehydrogenase expressed in the whole cell catalyst and having at least 95% sequence identity to the nucleotide sequence of SEQ ID NO: 11, wherein the recombinant fatty acid reductase is encoded by a gene having at least 95% sequence identity to the nucleotide sequence of SEQ ID NO: 1. the phosphopantetheinyl transferase is encoded

by a gene having at least 95% sequence identity to the nucleotide sequence of SEQ ID NO: 2, and the transaminase is encoded by a gene having at least 95% sequence identity to the nucleotide sequence of SEQ ID NO: 12.

5