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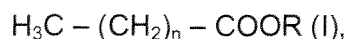
## Enzymatic omega-oxidation and omega-amination of fatty acids

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The invention relates to a process for the oxidation of a fatty acid or of an ester thereof,

comprising the step of oxidising the fatty acid or the ester thereof by contacting with a cytochrome P450 monooxygenase of the CYP153 family in the presence of molecular oxygen and NAD(P)H and a whole-cell catalyst expressing a recombinant cytochrome P450 monooxygenase of the CYP153 family, a recombinant alcohol dehydrogenase, a recombinant transaminase and optionally one or more than one recombinant enzyme from the group comprising alanine dehydrogenase, ferredoxin and ferredoxin reductase, and to the use of this whole-cell catalyst for the oxidation of a fatty acid or of an ester thereof,

wherein the fatty acid or the ester thereof preferably has the formula (I)



wherein R is selected from the group comprising H, methyl, ethyl, propyl and butyl,

wherein n is 0 to 30, preferably 6 to 24.

Polyamides are a class of polymers which are characterized by repeating amide groups. In contrast to the chemically related proteins, the term "polyamide" usually relates to synthetic, commercially available, thermoplastic polymers. Polyamides are derived from primary amines or from secondary amines which are conventionally obtained during the cracking of hydrocarbons. However, it is also possible to use derivatives, more precisely aminocarboxylic acid, lactams and diamines, for producing the polymer. Also of interest as starting materials are short-chain, gaseous alkanes which can be obtained proceeding from renewable raw materials using biotechnological processes.

Many commercial polyamides in high demand are produced starting from lactams. For example, "Polyamide 6" can be obtained by polymerization of  $\epsilon$ -caprolactam and "Polyamide 12" can be obtained by polymerization of lauro lactam. Further commercially interesting products include copolymers of lactam, e.g. copolymers of  $\epsilon$ -caprolactam and lauro lactam.

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The conventional chemical-technical production of amines is dependent on the supply of fossil raw materials, inefficient, and in the process large amounts of undesired by-products are produced, in some steps of the synthesis up to 80%. One example of such a process is the production of lauro lactam, which is conventionally obtained by trimerization of butadiene. The  
10 trimerization product cyclododecatriene is hydrogenated and the cyclododecane resulting therefrom is oxidized to cyclododecanone, which is then reacted with hydroxylamine to give cyclododecane oxime, which is finally converted to lauro lactam via a Beckmann rearrangement.

In view of the said disadvantages, processes have been developed in order to obtain amines  
15 using biocatalysts starting from renewable raw materials. Suitable renewable raw materials are in particular sources of fatty acids which can be obtained in the form of rapeseed oil, globe thistle oil, palm kernel oil, coconut oil, sunflower kernel oil and similar natural products from a large number of biological sources, in particular from plants.

20 PCT/EP 2008/067447 describes a biological system for producing chemically related products, more precisely  $\omega$ -aminocarboxylic acids, using a cell which has a series of suitable enzymatic activities and is able to convert carboxylic acids to corresponding  $\omega$ -aminocarboxylic acid.

A known disadvantage of the AlkBGT oxidase system from *Pseudomonas putida* GPO1 used  
25 therein, however, is that it is not able to achieve selective oxidation of aliphatic alkanes to primary alcohols. Rather, a multitude of oxidation products arise; in particular, the fraction of more highly oxidized products, such as the corresponding aldehyde, ketone or the corresponding carboxylic acid, increases with increasing reaction time (C. Grant, J. M. Woodley and F. Baganz (2011), *Enzyme and Microbial Technology* 48, 480-486), which correspondingly  
30 reduces the yield of desired amine.

The problem of the relatively unselective oxidation is exacerbated by the fact that the corresponding oxidation products are structurally very similar. This means that it is very difficult

to separate them off from the desired oxidation products efficiently and without a significant loss in yield.

There is therefore a need for processes in which the enzymatically catalysed reactions proceed  
5 more selectively and the formation of irreversibly produced by-products is minimized.

Against this background, the problem addressed by the invention consists in providing an improved process for the oxidation and amination of fatty acids using biocatalysts.

10 A further object addressed by the invention consists in improving the process such that the yield, based on the amount of fatty acid substrate or other substrates, increases the amount of the carbon substrate for cells used for biotechnological synthesis and/or the concentration of by-products or the ratio of by-products to desired product is reduced.

15 A further problem addressed by the invention consists in improving the process such that the selectivity of the biocatalysts used, in particular of fatty acid oxidases, is increased and/or extended, either at the start of the reaction, i.e. before reaching a plateau during the product concentration over time, or after adjusting the equilibrium, i.e. after reaching the plateau.

20 A further object addressed by the invention consists in improving the ability to be worked up of the reaction mixture formed during the biotechnological oxidation and/or amination of fatty acids, in particular with regard to the efficiency and rate of the phase separation of hydrophilic and hydrophobic substances.

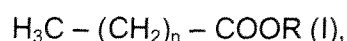
25 These and other objects are achieved by the subject matter of the present application and particularly also by the subject matter of the accompanying independent claims, with embodiments arising from the dependent claims.

The object underlying the invention is achieved in a first aspect by a process for the oxidation of  
30 a fatty acid or of an ester thereof

comprising the step of:

- a) oxidising the fatty acid or the ester thereof by contacting with a cytochrome P450 monooxygenase of the CYP153 family in the presence of molecular oxygen and NAD(P)H,

5 wherein the fatty acid or the ester thereof preferably has the formula (I)



wherein R is selected from the group comprising H, methyl, ethyl, propyl and butyl,

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wherein n is 0 to 30, preferably 6 to 24.

In a first embodiment of the first aspect, the object is achieved by a process further comprising the steps of:

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- b) further oxidising of the oxidized fatty acid or of the ester thereof from step a) by contacting with an alcohol dehydrogenase,

- c) amination of the further oxidized fatty acid or of the ester thereof from step b) by  
20 contacting with a transaminase in the presence of an amine donor, preferably alanine,

wherein step c) optionally takes place in the presence of an alanine dehydrogenase, ammonium and NAD(P)H.

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In a second embodiment, which is also an embodiment of the first embodiment, the object is achieved by a process wherein the cytochrome P450 monooxygenase of the CYP153 family has the peptide sequence LL(I/L)(V/I)GGNDTTRN and is preferably the cytochrome P450 monooxygenase of the CYP153 family from *Alcanivorax borkumensis* SK2 (database code YP\_691921) or a variant thereof.

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This and all other database codes used in this document originate from the Genbank Protein database of the NCBI in the release available online on 9 March 2012.

In a third embodiment, which is also an embodiment of the first to second embodiment, the object is achieved by a process wherein the alcohol dehydrogenase is a NAD(P)<sup>+</sup>-dependent alcohol dehydrogenase, preferably the NAD-dependent alcohol dehydrogenase from *Escherichia coli* MS 187-1 (database code ZP\_07145023) or a variant thereof or the alcohol dehydrogenase from *Bacillus stearothermophilus* (database code P42328) or a variant thereof, or an oxidoreductase of the glucose-methanol-choline-oxidoreductase family, preferably that from *Pseudomonas putida* (database code CAB54054.1) or a variant thereof, or a flavin-containing alcohol dehydrogenase, preferably the flavin-containing alcohol dehydrogenase from *Candida tropicalis* (database code AAS46878.1) or a variant thereof.

In a fourth embodiment, which is also an embodiment of the first to third embodiment, the object is achieved by a process wherein, in step a), additionally a ferredoxin reductase, preferably the ferredoxin reductase from *Alcanivorax borkumensis* SK2 (database code YP\_691923) or a variant thereof, and/or a ferredoxin, preferably the ferredoxin from *Alcanivorax borkumensis* SK2 (database code YP\_691920) or a variant thereof, is present.

In a fifth embodiment, which is also an embodiment of the first to fourth embodiment, the object is achieved by a process, wherein step c) takes place in the presence of an alanine dehydrogenase, ammonium and NADH and the alanine dehydrogenase is the alanine dehydrogenase from *Bacillus subtilis subsp. subtilis* str. 168 (database code NP\_391071) or a variant thereof.

In a sixth embodiment, which is also an embodiment of the first to fifth embodiment, the object is achieved by a process, wherein at least one enzyme from the group comprising cytochrome P450 monooxygenase of the CYP153 family, alcohol dehydrogenase, transaminase, alanine dehydrogenase, ferredoxin and ferredoxin reductase is provided recombinantly in the form of a whole-cell catalyst.

In a seventh embodiment, which is also an embodiment of the sixth embodiment, the object is achieved by a process, wherein all of the enzymes from the group comprising cytochrome P450 monooxygenase of the CYP153 family, alcohol dehydrogenase, transaminase, alanine dehydrogenase, ferredoxin and ferredoxin reductase present or contacted in at least one of the steps a), b) or c) with the fatty acid or the ester thereof, the further oxidized fatty acid or the

ester thereof from step b) or the aminated further oxidized fatty acid or the ester thereof from step c) are provided recombinantly in the form of one or more than one whole-cell catalyst.

In an eighth embodiment, which is also an embodiment of the sixth to seventh embodiment, the object is achieved by a process wherein the whole-cell catalyst additionally expresses a polypeptide of the AlkL family, preferably an AlkL from the group comprising AlkL from *Pseudomonas putida* (database code CAB69081), *Marinobacter aquaeolei* VT8 (database code YP\_957722), *Oceanicaulis alexandrii* HTCC2633 (database code ZP\_00953584), *Marinobacter manganoxydans* Mnl7-9 (database code ZP\_09158756), *Caulobacter* sp. K31 (database code YP\_001672217), *Pseudomonas oleovorans* (database code Q00595) or a variant thereof.

In a ninth embodiment, which is also an embodiment of the first to eighth embodiment, the object is achieved by a process wherein the whole-cell catalyst is a cell which has an activity, reduced compared to its wildtype, of at least one enzyme which catalyses one of the reactions of the  $\beta$ -oxidation of fatty acids, wherein the enzyme is preferably selected from the group which comprises fatty acid importer, fatty acid-CoA ligase, acyl-CoA dehydrogenase, 2,4-dienoyl-CoA reductase, enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase.

In a tenth embodiment, which is also an embodiment of the first to ninth embodiment, the object is achieved by a process wherein the alanine dehydrogenase in step c) is selected such that it reduces the redox cofactor oxidized by the alcohol dehydrogenase in step b), preferably  $\text{NAD}^+$  or  $\text{NADP}^+$ .

In a second aspect, the object is achieved by a whole-cell catalyst expressing a recombinant cytochrome P450 monooxygenase of the CYP153 family, a recombinant alcohol dehydrogenase, a recombinant transaminase and optionally one or more than one recombinant enzyme from the group comprising alanine dehydrogenase, ferredoxin and ferredoxin reductase.

In a first embodiment of the first aspect, the object is achieved by a whole-cell catalyst, wherein the whole-cell catalyst additionally expresses a polypeptide of the AlkL family, preferably an AlkL from the group comprising AlkL from *Pseudomonas putida* (database code CAB69081), *Marinobacter aquaeolei* VT8 (database code YP\_957722), *Oceanicaulis alexandrii* HTCC2633 (database code ZP\_00953584), *Marinobacter manganoxydans* Mnl7-9 (database code

ZP\_09158756), *Caulobacter* sp. K31 (database code YP\_001672217), *Pseudomonas oleovorans* (database code Q00595) or a variant thereof.

In a second embodiment, which is also an embodiment of the first embodiment, the object is achieved by a whole-cell catalyst wherein the whole-cell catalyst is a cell which has an activity, reduced compared to its wildtype, of at least one enzyme which catalyses one of the reactions of the  $\beta$ -oxidation of fatty acids, wherein the enzyme is preferably selected from the group which comprises fatty acid importer, fatty acid-CoA ligase, acyl-CoA dehydrogenase, 2,4-dienoyl-CoA reductase, enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase.

In a third embodiment, which is also an embodiment of the first to second embodiment, the object is achieved by a whole-cell catalyst, wherein the whole-cell catalyst expresses a ferredoxin reductase and a ferredoxin.

In a fourth embodiment, which is also an embodiment of the first to third embodiment, the object is achieved by a whole-cell catalyst, wherein the whole-cell catalyst expresses an alanine dehydrogenase, and wherein the alanine dehydrogenase is from *Bacillus subtilis subsp. subtilis* str. 168 (database code NP\_391071) or a variant thereof.

In a fifth embodiment, which is also an embodiment of the first to fourth embodiment, the object is achieved by a whole-cell catalyst, wherein the cytochrome P450 monooxygenase of the CYP153 family has the peptide sequence LL(I/L)(V/I)GGNDTTRN and/or it is the cytochrome P450 monooxygenase of the CYP153 family from *Alcanivorax borkumensis* SK2 (database code YP\_691921) or a variant thereof, and the ferredoxin reductase is the ferredoxin reductase from *Alcanivorax borkumensis* SK2 (database code YP\_691923) or a variant thereof and the ferredoxin is the ferredoxin from *Alcanivorax borkumensis* SK2 (database code YP\_691920) or a variant thereof.

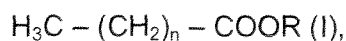
In a sixth embodiment, which is also an embodiment of the first to fifth embodiment, the object is achieved by a whole-cell catalyst, wherein the alcohol dehydrogenase is a NAD(P)<sup>+</sup>-dependent alcohol dehydrogenase, an oxidoreductase of the glucose-methanol-choline-oxidoreductase family or a flavin-containing alcohol oxidase, preferably a NAD(P)<sup>+</sup>-dependent alcohol dehydrogenase, most preferably the NAD-dependent alcohol dehydrogenase from *Escherichia coli* MS 187-1 (database code ZP\_07145023) or a variant thereof.

In a seventh embodiment, which is also a further embodiment of the first or second aspect and embodiments thereof, the object is achieved by a whole-cell catalyst according to the second aspect or an embodiment of the second aspect or processes according to the first aspect or of an embodiment of the first aspect, wherein the transaminase is the transaminase from *Pseudomonas putida* GB-1 (database code YP\_001668026.1) or a variant thereof.

In an eighth embodiment, which is also a further embodiment of the first or second aspect, the fatty acid or the ester thereof is an unsaturated or branched fatty acid or ester thereof.

In a ninth embodiment, which is also a further embodiment of the first or second aspect, the object is achieved by a process wherein at least one enzyme from the group comprising cytochrome P450 monooxygenase of the CYP153 family, alcohol dehydrogenase, transaminase, alanine dehydrogenase, ferredoxin and ferredoxin reductase is provided recombinantly in the form of a whole-cell catalyst, or the object is achieved by a whole-cell catalyst, wherein the whole-cell catalyst is a cell which has an activity, reduced relative to the wildtype of the cell, of at least one endogenous aldehyde dehydrogenase.

In a third aspect, the object underlying the invention is achieved by the use of the whole-cell catalyst according to the second aspect of the invention or one of its embodiments for the oxidation and/or amination of a fatty acid or ester thereof, wherein the fatty acid or the ester thereof preferably has the formula (I)



wherein R is selected from the group comprising H, methyl, ethyl, propyl and butyl,

wherein n is 0 to 30, preferably 6 to 24.

In a first embodiment of the third aspect, the problem is solved through a use, wherein the oxidation produces a mixture of oxidation products which, based on the quantitative amount of the reacted fatty acid or of the ester thereof, comprises at least 90% of the corresponding

alcohol, less than 1% of the corresponding aldehyde and less than 10% of the corresponding acid.

5 In a second embodiment of the third aspect, which is also a further embodiment of the embodiments of the third aspect, the fatty acid is an unsaturated or branched fatty acid or ester thereof.

10 The present invention is based on the discovery by the inventors that the use of specific monooxygenases or of whole-cell catalysts expressing such monooxygenases, more precisely cytochrome P450 monooxygenase of the CYP153 family, surprisingly leads to the oxidation and/or amination of fatty acids for the formation of the desired products with higher selectivity and better relative yield.

15 Without wishing to be bound to any theory, the inventors assume that the nature of the active catalytic centre of these monooxygenases is such that already hydroxylated products are bonded and further oxidized not only to the aldehyde or even products oxidized to the acid with lower affinity than is the case for comparable monooxygenases known from the prior art.

20 The process according to the invention envisages, in step a), the oxidation of fatty acids by a cytochrome P450 monooxygenase of the CYP153 family. In one embodiment, the term "fatty acid or ester thereof" is a compound of the formula  $\text{H}_3\text{C}-(\text{CH}_2)_x-\text{COOR}$ , where x 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 and R is hydrogen, methyl, ethyl or propyl, preferably hydrogen. In a particularly preferred embodiment, x is 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 and R is hydrogen. In a most preferred embodiment, it is lauric acid or methyl laurate. In a preferred embodiment, it is an unsaturated fatty acid from the group comprising myristoleic acid, palmitoleic acid, petroselinic acid, oleic acid, elaidic acid, vaccenic acid, gadoleic acid, eicosenoic acid or erucic acid. Likewise possible are mixtures of different fatty acids, for example mixtures of fatty acids or fatty acid esters obtained by hydrolysis and optional esterification of globe thistle oil, coconut oil, cuphea oil or palm kernel oil. Since not all fatty acids are soluble to a noteworthy extent at room temperature, it may be necessary to resort to other measures, such as, for example, increasing the temperature or, preferably, adding an organic solvent, in order to make them accessible to the aqueous phase. In a particularly preferred embodiment, a fatty acid or an ester thereof, most preferably methyl laurate or oleic acid, is used as such a further solvent.

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As in the case of all of the compounds specified in this application, fatty acids include not only the protonated form of the fatty acid, but also all forms, formulations or salts dissociated in aqueous solution. For example, the term lauric acid also includes laurate or sodium laurate. As  
5 a further example, the amino acid alanine includes the forms deprotonated or protonated on the carboxyl group in water and the forms deprotonated or protonated on the amino group.

The process according to the invention provides not only the oxidation for the hydroxylated fatty acid, but permits the efficient reaction of fatty acids to the corresponding  $\omega$ -aminocarboxylic  
10 acid by means of an enzyme system comprising a cytochrome P450 monooxygenase of the CYP153 family, an alcohol dehydrogenase, a transaminase and optionally an amino acid dehydrogenase. The use of such enzymes takes place under conditions which are compatible with their enzymatic activity. These include firstly the selection of a suitable aqueous buffer system comprising at least one pH-stabilizing buffer, for example sodium phosphate, optionally  
15 additionally at least one salt, for example sodium chloride, at a suitable pH. In a most preferred embodiment, the pH is 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9 or 10, preferably 2.5 to 7.5, particularly preferably 5.5 to 7.5. The temperature must also be matched to the activity of the enzyme or enzymes to be used. In a preferred embodiment, the temperature is 1 to 45, more preferably 20 to 45, most preferably 28 to 42°C. The selection of a suitable buffer system and the stabilization  
20 of the activity can be performed by a person skilled in the art using standard processes, see for example A Cornish-Bowden (1995), *Fundamentals of Enzyme Kinetics*, Portland Press Limited, 1995. The activity of the cytochrome P450 monooxygenase of the CYP153 family, without or in combination with ferredoxin and ferredoxin reductase, can be determined by means of the assay described by Scheps, D., Malca, H., Hoffmann, B., Nestl, B. M, and Hauer, B. (2011) *Org.*  
25 *Biomol. Chem.*, 9, 6727. Whether ferredoxin and/or ferredoxin reductase are active is evident to the person skilled in the art from a considerable increase in activity of the monooxygenase in the presence of the two active enzymes compared with their absence. An enzyme test for the activity of transaminases is sold by Cayman Chemical Company, Ann Arbor, MI, ("Alanine Transaminase Activity Assay Kit, Item No. 700260"). The activity of an amino acid  
30 dehydrogenase can be determined according to Germano, H. J., and Anderson, K. E. (1968), *J. Bact.* 96 (1), pages 55-60.

The use of enzymes further requires the presence of all necessary substrates. Thus, besides the fatty acid to be reacted according to the invention or the ester thereof, the presence of

oxygen and of an electron donor is necessary for the activity of the cytochrome P450 monooxygenase of the CYP153 family. Preferably, oxygen is made available by contacting the reaction mixture comprising enzyme(s) or cells and substrates with atmospheric air, alternatively with pure oxygen or with oxygen-enriched atmospheric air, particularly preferably by stirring the reaction mixture while it is in contact with atmospheric air, alternatively with pure oxygen or with oxygen-enriched atmospheric air.

For the optimal supply of the cytochrome P450 monooxygenase of the CYP153 family with electrons from the reducing agent, preferably NADH, it is preferred that the monooxygenase is used together with ferredoxin reductase that interacts functionally with it and ferredoxin that interacts functionally with it. These may be isolated polypeptides or, in the case of using a whole-cell catalyst, coexpressed polypeptides or polypeptides fused on the N- or C-terminus with the cytochrome P450 monooxygenase of the CYP153 family. Whether a ferredoxin reductase or a ferredoxin with a given cytochrome P450 monooxygenase of the CYP153 family interact functionally with one another can be readily established by a person skilled in the art by whether the reducing agent is oxidized in the presence of an alkane substrate and the three polypeptides. Alternatively, it is possible to use the enzyme test described by Scheps, D., Malca, H., Hoffmann, B., Nestl, B. M, and Hauer, B. (2011) *Org. Biomol. Chem.*, 9, 6727 which, in the case of functionally interacting polypeptides, exhibits a considerable increase in the reaction rate. In a particularly preferred embodiment, the cytochrome P450 monooxygenase of the CYP153 family, the ferredoxin and the ferredoxin reductase originate from the same organism. In a particularly preferred embodiment, it is the ferredoxin reductase from *Alcanivorax borkumensis* SK2 (database code YP\_691923) or a variant thereof, the ferredoxin from *Alcanivorax borkumensis* SK2 (database code YP\_691920) or a variant thereof and the cytochrome P450 monooxygenase of the CYP153 family from *Alcanivorax borkumensis* SK2 (database code YP\_691921) or a variant thereof.

For all of the enzymes used according to the invention, they may be cells comprising corresponding enzymatically active polypeptides or lysates thereof or preparations of the polypeptides in all purification states, from the crude lysate ranging to the pure polypeptide, or whole-cell catalysts. Numerous methods are known to those experts in the field, by which enzymatically active polypeptides may be overexpressed in suitable cells and may be purified or isolated. To express the polypeptides, all expression systems available to the person skilled in the art can be used. For the purification, chromatographic processes are suitable, for example

the affinity-chromatographic purification of a recombinant protein provided with a tag using an immobilized ligand, for example a nickel ion in the case of a histidine tag, of immobilized glutathione in the case of a glutathione S-transferase fused to the target protein or of immobilized maltose in the case of a tag comprising maltose-binding protein. For numerous  
5 biotechnologically important types of cells, e.g. *E. coli*, suitable processes and vectors are known which can be used for the expression or overexpression of a nucleic acid molecule, for example the vectors of the pET or pGEX type and cells suitable for their expression (B A Moffatt, and F W Studier (1986) *J. Mol. Biol.* **189**, 113–130, A H Rosenberg, B N Lade, D Chui, S Lin, J J Dunn, and F W Studier (1987) *Gene* **56**, 125–135 and F W Studier, A H Rosenberg, J  
10 J Dunn, and J W Dubendorff (1990) *Meth. Enzymol.* **185**, 60–89.

The purified enzymes can be used either in soluble form or immobilized. Suitable processes are known to the person skilled in the art with which polypeptides can be covalently or noncovalently immobilized on organic or inorganic solid phases, for example by sulphhydryl  
15 coupling chemistry (e.g. kits from Pierce). Cell-membrane-associated or cell-membrane-embedded enzymes can be used in the form of membrane preparations or solubilized.

In the case of the use of at least one whole-cell catalyst, in the case of a prolonged reaction time it must be ensured that the conditions are compatible with the viability of the at least one  
20 cell used as whole-cell catalyst. The person skilled in the art can refer to standard works, for example Fuchs/Schlegel (2007) *Allgemeine Mikrobiologie*, 2008, Georg Thieme Verlag, for conditions and solutions which permit the retention of such cells in a viable state.

In a preferred embodiment, the term “whole-cell catalyst”, as used herein, is understood as  
25 meaning an intact, viable and metabolically active cell which provides a desired enzymatic activity. The whole-cell catalyst can transport the substrate to be metabolized, in the case of the present invention the alcohol or the oxidation product resulting therefrom, either into the inside of the cell, where it is metabolized by cytosolic enzymes, or it can present the enzyme of interest on its surface, where it is exposed directly to substrates in the medium. The person  
30 skilled in the art is aware of numerous systems for producing whole-cell catalysts, for example from DE 60216245.

When using a whole-cell catalyst, the problem can arise that a substrate has to be brought into contact with an intracellularly localized enzyme so that it results in the desired reaction. In the

case of long-chain alkanes and derivatives thereof, it is preferred that the whole-cell catalyst has a polypeptide of the AlkL family. In a preferred embodiment, a "polypeptide of the AlkL family", as used herein, is a polypeptide which, over a length of 230 successive amino acids, has an at least 80, preferably 90, more preferred 90% sequence identity to AlkL from *Pseudomonas putida* (database code CAB69081) and preferably the ability to assist the import of long-chain alkanes into the inside of a cell. In a further embodiment, a "polypeptide of the AlkL family", as used herein, is a polypeptide located in the outer membrane of a Gram-negative bacterium which has the sequence motif DXWAPAXQ(V/A)GXR, where X is a proteinogenic amino acid, and preferably is additionally AlkL from *Pseudomonas putida* (database code CAB69081) or a variant thereof. Examples of members of the AlkL family include AlkL from *Pseudomonas putida* (database code CAB69081), *Marinobacter aquaeolei* VT8 (database code YP\_957722), *Oceanicaulis alexandrii* HTCC2633 (database code ZP\_00953584), *Marinobacter manganoxydans* Mnl7-9 (database code ZP\_09158756), *Caulobacter* sp. K31 (database code YP\_001672217), *Pseudomonas oleovorans* (database code Q00595) and variants thereof.

The use of isolated enzymes is recommended for a series of applications. In a preferred embodiment, the term "isolated", as used herein, means that the enzyme is in a more pure and/or concentrated form than in its natural source. In a preferred embodiment, the enzyme is classed as being isolated if it is a polypeptide enzyme and constitutes more than 60, 70, 80, 90 or preferably 95% of the mass protein fraction of the corresponding preparation. The person skilled in the art is aware of numerous processes for measuring the mass of a protein in a solution, for example the visual estimation with reference to the thickness of corresponding protein bands on SDS-polyacrylamide gels, NMR spectroscopy or mass spectrometry-based processes.

The enzymes used according to the invention are preferably recombinant enzymes. In a preferred embodiment, the term "recombinant", as used herein, is understood as meaning that the corresponding nucleic acid molecule does not occur in nature and/or it has been produced using genetic engineering methods. In a preferred embodiment, the term recombinant protein is used if the corresponding polypeptide is encoded by a recombinant nucleic acid. In a preferred embodiment, a recombinant cell, as used herein, is understood as meaning a cell which has at least one recombinant nucleic acid or a recombinant polypeptide. Processes suitable for producing recombinant molecules or cells are known to the person skilled in the art, for example

those described in Sambrook/Fritsch/Maniatis (1989): Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 2nd edition.

In a preferred embodiment, the cell used as whole-cell catalyst or as expression system is a  
 5 prokaryotic, preferably a bacterial, cell. It is preferred according to the invention that, on account  
 of the good genetic accessibility, microorganisms are used; selected from the group of bacteria,  
 particularly from the group comprising, preferably consisting of *Magnetococcus*, *Mariprofundus*,  
*Acetobacter*, *Acidiphilium*, *Afipia*, *Ahrensia*, *Asticcacaulis*, *Aurantimonas*, *Azorhizobium*,  
*Azospirillum*, *Bacillus*, *Bartonella*, *tribocorum*, *Beijerinckia*, *Bradyrhizobium*, *Brevundimonas*,  
 10 *subvibrioides*, *Brucella*, *Caulobacter*, *Chelativorans*, *Citricella*, *Citromicrobium*,  
*Corynebacterium*, *Dinoroseobacter*, *Erythrobacter*, *Fulvimarina*, *Gluconacetobacter*,  
*Granulibacter*, *Hirschia*, *Hoeflea*, *Hyphomicrobium*, *Hyphomonas*, *Ketogulonicigenium*,  
*Labrenzia*, *Loktanella*, *Magnetospirillum*, *Maricaulis*, *Maritimibacter*, *Mesorhizobium*,  
*Methylobacterium*, *Methylocystis*, *Methylosinus*, *Nitrobacter*, *Novosphingobium*, *Oceanibulbus*,  
 15 *Oceanicaulis*, *Oceanicola*, *Ochrobactrum*, *Octadecabacter*, *Oligotropha*, *Paracoccus*,  
*Parvibaculum*, *Parvularcula*, *Pelagibaca*, *Phaeobacter*, *Phenylobacterium*, *Polymorphum*,  
*Pseudovibrio*, *Rhodobacter*, *Rhodomicrobium*, *Rhodopseudomonas*, *Rhodospirillum*,  
*Roseibium*, *Roseobacter*, *Roseomonas*, *Roseovarius*, *Ruegeria*, *Sagittula*, *Silicibacter*,  
*Sphingobium*, *Sphingomonas*, *Sphingopyxis*, *Starkeya*, *Sulfitobacter*, *Thalassiobium*,  
 20 *Xanthobacter*, *Zymomonas*, *Agrobacterium*, *Rhizobium*, *Sinorhizobium*, *Anaplasma*, *Ehrlichia*,  
*Neorickettsia*, *Orientia*, *Rickettsia*, *Wolbachia*, *Bordetella*, *Burkholderia*, *Cupriavidus*,  
*taiwanensis*, *Lautropia*, *Limnobacter*, *Polynucleobacter*, *Ralstonia*, *Chromobacterium*, *Eikenella*,  
*corrodens*, *Basfia*, *Kingella*, *Laribacter*, *Lutiella*, *Neisseria*, *Simonsiella*, *Achromobacter*,  
*Acidovorax*, *Alicyciphilus*, *Aromatoleum*, *Azoarcus*, *Comamonas*, *Dechloromonas*, *Delftia*,  
 25 *Gallionella*, *Herbaspirillum*, *Hermiimonas*, *Hylemonella*, *Janthinobacterium*, *Leptothrix*,  
*Methylibium*, *Methylobacillus*, *Methylophilales*, *Methyloversatilis*, *Methylovorus*, *Nitrosomonas*,  
*Nitrospira*, *Oxalobacter*, *Parasutterella*, *Polaromonas*, *Polaromonas*, *Pusillimonas*,  
*Rhodoferax*, *Rubrivivax*, *Sideroxydans*, *Sutterella*, *wadsworthensis*, *Taylorella*, *Thauera*,  
*Thiobacillus*, *Thiomonas*, *Variovorax*, *Verminephrobacter*, *Anaeromyxobacter*, *Bdellovibrio*,  
 30 *bacteriovorus*, *Bilophila*, *Desulfarculus*, *Desulfatibacillum*, *Desulfobacca*, *Desulfobacterium*,  
*Desulfobulbus*, *Desulfococcus*, *Desulfohalobium*, *Desulfitobacterium*, *Desulfomicrobium*,  
*Desulfonatronospira*, *Desulfotalea*, *Desulfovibrio*, *Desulfuromonas*, *Geobacter*, *Haliangium*,  
*Hippea*, *Lawsonia*, *Myxococcus*, *Pelobacter*, *Plesiocystis*, *Sorangium*, *Stigmatella*,  
*Syntrophobacter*, *Syntrophus*, *Arcobacter*, *Caminibacter*, *Campylobacter*, *Helicobacter*,

*Nitratifactor*, *Nitratiruptor*, *Sulfuricurvum*, *Sulfurimonas*, *Sulfurospirillum*, *Sulfurovum*, *Wolinella*, *Buchnera*, *Blochmannia*, *Hamiltonella*, *Regiella*, *Riesia*, *Citrobacter*, *Cronobacter*, *Dickeya*, *Edwardsiella*, *Enterobacter*, *Erwinia*, *Escherichia*, *Klebsiella*, *Pantoea*, *Pectobacterium*, *Proteus*, *Providencia*, *Rahnella*, *Salmonella*, *Serratia*, *Shigella*, *Sodalis*, *Wigglesworthia*, *Glossina*,  
5 *Xenorhabdus*, *Yersinia*, *Acidithiobacillus*, *Acinetobacter*, *Aeromonas*, *Alcanivorax*, *Alkalilimnicola*, *Allochromatium*, *Alteromonadales*, *Alteromonas*, *Baumannia*, *Beggiatoa*, *Bermanella*, *Carsonella*, *Ruthia*, *Vesicomysocius*, *Cardiobacterium*, *Chromohalobacter*, *Colwellia*, *Congregibacter*, *Coxiella*, *Dichelobacter*, *Endoriftia*, *Enhydrobacter*, *Ferrimonas*, *Francisella*, *Glaciecola*, *Hahella*, *Halomonas*, *Halorhodospira*, *Halothiobacillus*, *Idiomarina*,  
10 *Kangiella*, *Legionella*, *Marinobacter*, *Marinomonas*, *Methylobacter*, *Methylococcus*, *Methylomicrobium*, *Methylophaga*, *Moraxella*, *Moritella*, *Neptuniibacter*, *Nitrococcus*, *Pseudoalteromonas*, *Psychrobacter*, *Psychromonas*, *Reinekea*, *Rickettsiella*, *Saccharophagus*, *Shewanella*, *Succinatimonas*, *Teredinibacter*, *Thioalkalimicrobium*, *Thioalkalivibrio*, *Thiomicrospira*, *Tolumonas*, *Vibrionales*, *Actinobacillus*, *Aggregatibacter*, *Gallibacterium*,  
15 *Haemophilus*, *Histophilus*, *Mannheimia*, *Pasteurella*, *Azotobacter*, *Cellvibrio*, *Pseudomonas*, *Aliivibrio*, *Grimontia*, *Photobacterium*, *Photobacterium*, *Vibrio*, *Pseudoxanthomonas*, *Stenotrophomonas*, *Xanthomonas*, *Xylella*, *Borrelia*, *Brachyspira*, *Leptospira*, *Spirochaeta*, *Treponema*, *Hodgkinia*, *Puniceispirillum*, *Liberibacter*, *Pelagibacter*, *Odyssella*, *Accumulibacter*,  
in particular *B. subtilis*, *B. megaterium*, *C. glutamicum*, *E. coli*, *Pseudomonas* sp.,  
20 *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas stutzeri*, *Acinetobacter* sp., *Burkholderia* sp., *Burkholderia thailandensis*, *Cyanobakterien*, *Klebsiella* sp., *Klebsiella oxytoca*, *Salmonella* sp., *Rhizobium* sp. and *Rhizobium meliloti*, with *E. coli* being particularly preferred.

In a preferred embodiment, the term “cytochrome P450 monooxygenase of the CYP153 family”  
25 is understood as meaning a cytosolic oxidase which is part of a 3-component system which further comprises a ferredoxin and a ferredoxin reductase, with an alkane binding site and the ability to hydroxylate alkanes. In a particularly preferred embodiment, it is an enzyme which has to at least 80, preferably 90, most preferably 95 or 99%, sequence identity to the cytochrome P450 monooxygenase of the CYP153 family from *Alcanivorax borkumensis* SK2 (database  
30 code YP\_691921) or an enzyme which comprises a polypeptide sequence which has at least 80, preferably 90, most preferably 95 or 99%, sequence identity to the cytochrome P450 monooxygenase of the CYP153 family from *Alcanivorax borkumensis* SK2 (database code YP\_691921) and moreover has alkane hydroxylase activity. In a preferred embodiment, the term “alkane hydroxylase activity”, as used herein, is to be understood as meaning the ability to

catalyse the hydroxylation of alkanes or unsubstituted linear alkyl radicals comprising at least five, preferably twelve, carbon atoms. In a further preferred embodiment, the term "cytochrome P450 monooxygenase of the CYP153 family" is understood as meaning a non-membrane-bonded oxidase which includes a binding site for alkanes, unsubstituted linear alkyl radicals comprising at least five, preferably twelve, carbon atoms or monohydroxylated alkanes and the polypeptide chain of which the motif LL(I/L)(V/I)GGNDTTRN. In a preferred embodiment, a "cytochrome P450 monooxygenase of the CYP153 family", as used herein, is a cytochrome P450 monooxygenase of the CYP153 family from *Alcanivorax borkumensis* SK2 (database code YP\_691921) or a variant which preferably has alkane hydroxylase activity.

The use of cytochrome P450 monooxygenases of the CYP153 family for the hydroxylation of alkanes is described in the prior art, as are enzyme tests for determining the enzyme activity and processes for the expression and purification (Scheps, D., Malca, H., Hoffmann, B., Nestl, B. M, and Hauer, B. (2011) *Org. Biomol. Chem.*, 9, 6727). Besides an alkane to be oxidized or unsubstituted linear alkyl radical comprising at least five, preferably twelve, carbon atoms, the substrates involved in the reaction of the enzyme comprise oxygen and electrons which are transferred in the form of NADH preferably via the other two components, ferredoxin and a ferredoxin reductase, to the oxidase. Scheps *et al.* (2011) and Roome, P. W., Jr., Philley, J. C., and Peterson (1983) *J. Biol. Chem.* 258, 2593, Roome, P.W., and Peterson, J. A. (1988), *Arch. Biochem. Biophys.*, 266, 41 and Peterson, J. A., Lorence, M. C., and Amarneh, B. (1990) *J. Biol. Chem.*, 265, 6066 also disclose processes for obtaining ferredoxin and ferredoxin reductase in functional form.

According to the invention, in step b), an alcohol dehydrogenase is used for the oxidation of the fatty acid alcohol resulting in step a). Alcohol dehydrogenases have for decades been a greatly observed and biotechnologically highly relevant enzyme class in biochemistry in connection with brewing fermentation processes, said class including various groups of isoforms. Thus, membrane-bonded, flavin-dependent alcohol dehydrogenases of *Pseudomonas putida* GPO1 AlkJ type exist which use flavocofactors instead of NAD<sup>+</sup>. A further group includes iron-containing alcohol dehydrogenases that are sensitive towards oxygen and which are found in bacteria and in inactive form in yeast. Another group includes NAD<sup>+</sup>-dependent alcohol dehydrogenases, among them zinc-containing alcohol dehydrogenases in which the active centre has a cysteine-coordinated zinc atom which fixes the alcohol substrate. In a preferred embodiment, the term "alcohol dehydrogenase", as used herein, is understood as meaning an

enzyme which oxidizes an aldehyde or ketone to the corresponding primary or secondary alcohol, respectively. Preferably, the alcohol dehydrogenase in the process according to the invention is a NAD<sup>+</sup>-dependent alcohol dehydrogenase, i.e. an alcohol dehydrogenase which uses NAD<sup>+</sup> as cofactor for the oxidation of the alcohol or NADH for reducing the corresponding aldehyde or ketone. In the most preferred embodiment, the alcohol dehydrogenase is a NAD<sup>+</sup>-dependent zinc-containing alcohol dehydrogenase.

In a further preferred embodiment, the alcohol dehydrogenase is an alcohol dehydrogenase oxidoreductase of the glucose-methanol-choline-oxidoreductase family. In a preferred embodiment, the term "oxidoreductase of the glucose-methanol-choline-oxidoreductase family", as used herein, is understood as meaning an alcohol dehydrogenase which comprises FAD as cofactor and is preferably the enzyme from *Pseudomonas putida* (database code CAB69081) or a variant thereof. Examples include SEQ ID No. 26, 27, 28 and 29, and also the enzyme from *Pseudomonas putida* (database code CAB69081).

In a further preferred embodiment, the alcohol dehydrogenase is an alcohol dehydrogenase of the family of the flavin-containing alcohol dehydrogenases. In preferred embodiment, the term "family of flavin-containing alcohol dehydrogenases", as used herein, is understood as meaning the group of alcohol oxidases which constitute a haem protein of the c type and contain FAD as cofactor and preferably additionally belong to the group of the enzyme with the database code AAS46878.1 and variants thereof. Examples of flavin-containing alcohol dehydrogenases include the enzymes with the database codes AAS46878.1, AAS46880.1 from *Candida tropicalis* and the enzyme with the database code CAB75351.1 from *Candida cloacae*.

According to the invention, a transaminase is used in step c). In a preferred embodiment, the term "transaminase", as used herein, is understood as meaning an enzyme which catalyses the transfer of  $\alpha$ -amino groups from a donor, preferably an amino acid, to an acceptor molecule, preferably an  $\alpha$ -ketocarboxylic acid. In a preferred embodiment, the term "amine donor", as used herein, is understood as meaning an L-amino acid, the amino group of which can be transferred from the transaminase to the  $\alpha$ -ketocarboxylic acid with the formation of an amino acid. In a particularly preferred embodiment, the amine donor is L-alanine. In a preferred embodiment, the transaminase is selected from the group of transaminases and variants thereof which are characterized in that it has, at the position of the amino acid sequence which corresponds to Val224 from the transaminase of *Pseudomonas putida* GB-1 (database code

YP\_001668026.1), an amino acid selected from the group comprising isoleucine, valine, phenylalanine, methionine and leucine, and at the position of the amino acid sequence which corresponds to Gly230 from the transaminase from *Pseudomonas putida* GB-1 (database code YP\_001668026.1), an amino acid other than threonine and preferably an amino acid from the group comprising serine, cysteine, glycine and alanine. In a particularly preferred embodiment, the transaminase is selected from the group which comprises the  $\omega$ -transaminase from *Chromobacterium violaceum* DSM30191, transaminases from *Pseudomonas putida* GB-1, *Pseudomonas putida* W619, from *Pseudomonas aeruginosa* PA01, *Streptomyces coelicolor* A3(2), *Pseudomonas putida* (database code YP\_001668026), *Pseudomonas putida* (database code YP\_001668026.1 or YP\_001671460); *Rhodobacter sphaeroides* (strain ATCC 17023; database code YP\_353455) and *Streptomyces avermitilis* MA 4680, and variants thereof.

In a preferred embodiment, the term "alanine dehydrogenase", as used herein, is understood as meaning an enzyme which catalyses the conversion of L-alanine with consumption of water and NAD(P)<sup>+</sup> to pyruvate, ammonia and NAD(P)H. Preferably, the alanine dehydrogenase is an intracellular alanine dehydrogenase, even more preferably a recombinant intracellular alanine dehydrogenase of a bacterial whole-cell catalyst. Examples include the enzymes from *Rhizobium leguminosarum* (database code YP\_002975437), *Bacillus megaterium* (database code YP\_003565624), *Rhodobacter capsulatus* (database code ADE84249.1) and *Bacillus subtilis* (database code NP\_391071).

While the alcohol dehydrogenase in step b), if it is a NAD(P)-dependent one, consumes one molecule of the redox cofactor NAD(P)H per reacted substrate molecule, the amino dehydrogenase oxidizes NAD(P)H. Of particular advantage is therefore the use of a system in which alcohol dehydrogenase and amino acid dehydrogenase convert the same redox cofactor. NADP-dependent alcohol dehydrogenases include the enzyme from *E. coli* (YjgB, database code ZP\_07117674) and a further enzyme from *E. coli* (YahK, database code BAE76108.1). NAD-dependent alcohol dehydrogenases include an enzyme from *E. coli* (AdhP, database code ZP\_07145023), the enzyme from *Bacillus subtilis* (database code NP\_391071), the enzyme from *Bacillus stearothermophilus* (database code P42328.1) and the enzyme from *Rhizobium leguminosarum* (database code YP\_002975437). NADP-dependent alanine dehydrogenases include the enzyme from *Rhodobacter capsulatus* (database code ADE84249.1). NAD-dependent alanine dehydrogenases include the alanine dehydrogenase from *Bacillus subtilis* subsp. *subtilis* str. 168 (database code NP\_391071).

The teaching of the present invention can not only be carried out or applied using the, or on the, exact amino acid or nucleic acid sequences of the biological macromolecules described herein, but also using or on variants of those macromolecules which can be obtained by deleting, adding or substituting one or more than one amino acid or nucleic acid. In a preferred embodiment, the term "variant" of a nucleic acid sequence or amino acid sequence, which is used hereinbelow synonymously and exchangeably with the term "homologon", as used herein, means another nucleic acid or amino acid sequence which, with regard to the corresponding original wildtype nucleic acid or amino acid sequence, has a homology, used synonymously here with identity, of 70, 75, 80, 85, 90, 92, 94, 96, 98, 99% or more per cent, where preferably amino acids other than those forming the catalytically active centre or amino acids essential for the structure or folding are deleted or substituted or such are merely conservatively substituted, for example a glutamate instead of an aspartate or a leucine instead of a valine. The prior art describes algorithms, which may be used to calculate the degree of homology of two sequences, e.g. Arthur Lesk (2008), Introduction to Bioinformatics, 3rd edition. In a further more preferred embodiment of the present invention, the variant of an amino acid or nucleic acid sequence, preferably in addition to the sequence homology mentioned above, has essentially the same enzymatic activity of the wildtype molecule and of the original molecule. For example, a variant of an enzymatically active polypeptide protease has the same, or essentially the same, proteolytic activity as the polypeptide enzyme, i.e. the capability to catalyse the hydrolysis of a peptide bond. In a particular embodiment, the term "essentially the same enzymatic activity" means an activity, with respect to the substrates of the wildtype polypeptide, which clearly lies above the background activity or/and differs from the  $K_M$  and/or  $k_{cat}$  values by less than 3, preferably 2, more preferably one order of magnitude, which the wildtype polypeptide exhibits with respect to the same substrates. In a further preferred embodiment, the term "variant" of a nucleic acid or amino acid sequence includes at least one active part/or fragment of the nucleic acid or amino acid sequence. In a further preferred embodiment, the term "active part", as used herein, means an amino acid sequence or a nucleic acid sequence which has a smaller than the full length of the amino acid sequence or codes for a smaller than the full length of the amino acid sequence, where the amino acid sequence or the coded amino acid sequence with the smaller length than the wildtype amino acid sequence has essentially the same enzymatic activity as the wildtype polypeptide or a variant thereof, for example as protease. In a particular embodiment, the term "variant" of a nucleic acid comprises a nucleic acid whose complementary strand, preferably under stringent conditions, binds to the wildtype nucleic acid.

The stringency of the hybridization reaction is readily determinable by those skilled in the art and depends in general on the length of the probe, the washing temperatures and the salt concentration. Generally, longer probes require higher temperatures for the hybridization, whereas shorter probes work at lower temperatures. Whether hybridization takes place depends in general on the capability of the denatured DNA to anneal to complementary strands which are present in its environment and below the melting temperature. The stringency of hybridization reaction and corresponding conditions are described in detail in F M Ausubel (1995), *Current Protocols in Molecular Biology*. John Wiley & Sons, Inc. Instructions for identifying DNA sequences by means of hybridization can be found by the person skilled in the art inter alia in the handbook "The DIG System Users Guide for Filter Hybridization" from Boehringer Mannheim GmbH (Mannheim, Germany, 1993) and in Liebl *et al.* (*International Journal of Systematic Bacteriology* 41: 255-260 (1991)). The hybridization takes place in a preferred embodiment under stringent conditions, i.e. only hybrids are formed in which probe and target sequence, i.e. the polynucleotides treated with the probe, are at least 70% identical. It is known that the stringency of the hybridization including the washing steps is influenced and/or determined by varying the buffer composition, the temperature and the salt concentration. The hybridization reaction is generally carried out at a relatively lower stringency compared to the washing steps (Hybaid Hybridisation Guide, Hybaid Limited, Teddington, UK, 1996). For the hybridization reaction, for example, a buffer corresponding to 5x SSC buffer can be used at a temperature of about 50°C - 68°C. In this connection, probes can also hybridize with polynucleotides which have less than 70% identity to the sequence of the probe. Such hybrids are less stable and are removed by washing under stringent conditions. This can be achieved for example by lowering the salt concentration to 2x SSC and optionally subsequently 0.5x SSC (The DIG System User's Guide for Filter Hybridisation, Boehringer Mannheim, Mannheim, Germany, 1995), in which case a temperature of, increasing in order of preference, about 50°C - 68°C, about 52°C - 68°C, about 54°C - 68°C, about 56°C - 68°C, about 58°C - 68°C, about 60°C - 68°C, about 62°C - 68°C, about 64°C - 68°C, about 66°C - 68°C is established. Temperature ranges from about 64°C - 68°C or about 66°C - 68°C are preferred. It is optionally possible to reduce the salt concentration down to a concentration corresponding to 0.2 x SSC or 0.1 x SSC. By means of a stepwise increase in the hybridization temperature in steps of about 1 - 2°C from 50°C to 68°C, polynucleotide fragments can be isolated which, for example in the order of increasing preference, at least 70% or at least 80% or at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to the sequence of the nucleic acid molecule used. Further

instructions relating to the hybridization are commercially available in the form of so-called kits (e.g. DIG Easy Hyb from Roche Diagnostics GmbH, Mannheim, Germany, Catalogue No. 1603558). In a preferred embodiment, the term "variant" of a nucleic acid, as used here, comprises any nucleic acid sequence which codes for the same amino acid sequence as the original nucleic acid or a variant of this amino acid sequence in terms of the degeneracy of the genetic code.

If a whole-cell catalyst is used according to the invention, then it is preferred if this is a cell which has an activity, reduced compared to its wildtype, of at least one enzyme which catalyses one of the reactions of the  $\beta$ -oxidation of fatty acids, with the enzyme preferably being selected from the group which includes fatty acid importer, fatty acid-CoA ligase, acyl-CoA dehydrogenase, 2,4-dienoyl-CoA reductase, enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase. The  $\beta$ -oxidation of fatty acids is a widespread metabolic route which equally permits prokaryotic and eukaryotic organisms to oxidize fatty acids and to make available the chemical energy present therein to the metabolism (Y Fujita, H Matsuoka, and K Hirooka (2007) *Mol. Microbiology* 66(4), 829-839). In the further sense, it starts with the uptake of a fatty acid into the cell, in the case of *E. coli* by means of the transporter FadL (P N Black (1991) *J. Bacteriol.* 173, 435-442), which channels it through the outer or inner membrane of the Gram-negative bacteria cell and the FadD gene product (P N Black, C C DiRusso, A K Metzger, and T L Heimert (1992) *J. Biol. Chem.* 267, 25513-25520), which releases the fatty acid in the form of the CoA ester into the cytosol. There, if the conditions require it, the fatty acid is firstly oxidized at the  $\beta$  position of the CoA fatty acid ester by an acyl-CoA dehydrogenase, in the case of *E. coli* FadE (J. W. Campbell & J. E. Cronan (200) *J. Bacteriol.* 184, 3759-3764). A similar molecule can alternatively also be formed from a double-unsaturated fatty acid by reduction by means of a 2,4-dienoyl-CoA reductase, in the case of *E. coli* FadH. A multifunctional enzyme, the enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase, in the case of *E. coli* FadB, then catalyses the hydration with the formation of the secondary alcohol and its subsequent oxidation to the ketone. In the last step, a 3-ketoacyl-CoA thiolase, in the case of *E. coli* FadA, catalyses the cleavage of the ketoacyl-CoA, with the result that acetyl-CoA and a CoA ester of the fatty acid that is two carbon atoms shorter compared to the starting molecule are released. If it is not likewise acetyl-CoA, the latter can be fed again into the  $\beta$ -oxidation cycle and be shortened via oxidation. Also involved in the regulation of the  $\beta$ -oxidation of fatty acids is FadR, a regulator of the Fad operon, which includes the genes required for the degradation of fatty acids, without FadR appearing to catalyse a reaction of the  $\beta$ -oxidation. In a preferred embodiment, the term

“enzyme which catalyses one of the reactions of the  $\beta$ -oxidation of fatty acids” is understood as meaning any enzyme which interacts directly with the fatty acid substrate or a molecule formed therefrom on the route to the acetyl-CoA, preferably recognizes it as substrate, and catalyses its conversion to a metabolic product lying closer on this degradation route to the acetyl-CoA, preferably including the fatty acid importer, which effects the uptake of the fatty acid into the cell. For example, according to the preceding definition, these enzymes include acyl-CoA dehydrogenase since it interacts with the fatty acid-CoA ester and catalyses its conversion to the enoyl-CoA, which lies closer to the acetyl-CoA on the metabolic route of the  $\beta$ -oxidation than the fatty acid-CoA ester. In a particularly preferred embodiment, the term “enzyme which catalyses one of the reactions of the  $\beta$ -oxidation of fatty acids”, as used herein, is understood as meaning any enzyme from the group which comprises the gene products FadA, FadB, FadD, FadL and FadE from *E. coli* and/or their variants or homologues from other organisms. The gene products FadA, FadB, FadD, FadL and FadE from *E. coli* as well as variants and homologues from numerous other biotechnologically useful organisms and their nucleic acid and polypeptide sequences are described in the prior art, for example FadA under access number AP009048.1, FadB under access number BAE77457.1, FadD under access number BAA15609.1, FadE under access number BAA77891.2 and FadL under access number BAA16205.1

With the development of modern genetic, microbiological and molecular biological methods, numerous tools are available to the person skilled in the art with which he is able to routinely measure and influence the activity of enzymes present in living cells. To determine the activity of an enzyme which is present in the form of a suspension, a pellet or can be removed in processed form from a cell culture, enzymatic standard tests can be used and evaluated, as described in textbooks, for example A Cornish-Bowden (1995), *Fundamentals of Enzyme Kinetics*, Portland Press Limited. The prior art discloses numerous tests which are suitable specifically for measuring the activity of enzymes which catalyse one of the reactions of the  $\beta$ -oxidation of fatty acids, for example in K Kameda & W D Nunn (1981) *J. Biol. Chem.* 256, 5702-5707, Hi Marrakchi, W E DeWolf, C Quinn, J West, B J Polizzi, C Y So et al. (2003) *Biochem. J.* 370, 1055-1062, S Lobo, G Florova, and K A Reynolds (2001) *Biochemistry* 40 (39), 11955-64 and X Yu, T Liu, F Zhu, and C Khosla (2011) *PNAS, electronic publication before printing*. Routinely applicable processes for reducing the activity of an enzyme in a cell, for example by undirected mutagenesis of cells by exposure to radioactive radiation followed by enrichment or screening of the mutants, by site-directed insertion of point mutations or by the knock-out of a

gene coding for an active enzyme integrated chromosomally into a cell are also described in the prior art, for example in Sambrook/Fritsch/Maniatis (1989): Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 2nd edition or in Fuchs/Schlegel (2007) Allgemeine Mikrobiologie, 2008, Georg Thieme Verlag. In the particular case of the Fad gene product, the overexpression of a transcriptional repressor, for example of FadR (Y Fujita, H Matsuoka, and K Hirooka (2007) *Mol. Microbiology* 66(4), 829-839) is also appropriate for reducing the activity. A reduction in activity based on RNA interference (T Tuschl (2001) *ChemBioChem* 2: 239-145) or using specific inhibitors is also possible. In a preferred embodiment, the wording "where the cell has an activity reduced compared to its wildtype" of an enzyme, as used herein, means that the activity of the enzyme in the modified cell is reduced compared to the activity of the same enzyme in a wildtype cell. In a preferred embodiment, the relative reduction is, in order of increasing preference, 5, 10, 20, 40, 50, 75, 90, 95, 99 or more per cent of the activity. In a particularly preferred embodiment, activity of the enzyme compared to the background can no longer be detected.

If a whole-cell catalyst is used according to the invention, then it is furthermore advantageous if the whole-cell catalyst is a cell which is an activity, reduced relative to the wildtype of the cell, of at least one endogenous aldehyde dehydrogenase. In a preferred embodiment, the term "endogenous aldehyde dehydrogenase", as used herein, is understood as meaning an enzyme which is able to catalyse the oxidation of an aldehyde to the corresponding carboxylic acid, and which is naturally present in the genome of the wildtype of the cell used. One example of an alcohol dehydrogenase endogenous for *E. coli* is the enzyme with the database code BAA15032.1 (AldA) and variants thereof.

The present application encompasses a sequence protocol with the following polypeptide (Polyp)- and nucleotide (DNA) sequences:

| SEQ ID NO | Type  | Description                                                    |
|-----------|-------|----------------------------------------------------------------|
| 1         | Polyp | AlkL from <i>Pseudomonas oleovorans</i> (database code Q00595) |
| 2         | DNA   | AlkL from <i>Pseudomonas oleovorans</i> (database code Q00595) |
| 3         | Polyp | AlkL from <i>Pseudomonas putida</i> (database code CAB69081)   |
| 4         | DNA   | AlkL from <i>Pseudomonas putida</i> (database code CAB69081)   |
| 5         | Polyp | AlkL from <i>Marinobacter aquaeolei</i> VT8 (database code     |

|    |       |                                                                                                        |
|----|-------|--------------------------------------------------------------------------------------------------------|
|    |       | YP_957722)                                                                                             |
| 6  | DNA   | AlkL from <i>Marinobacter aquaeolei</i> VT8 (database code YP_957722)                                  |
| 7  | Polyp | AlkL from <i>Oceanicaulis alexandrii</i> HTCC2633 (database code ZP_00953584)                          |
| 8  | DNA   | AlkL from <i>Oceanicaulis alexandrii</i> HTCC2633 (database code ZP_00953584)                          |
| 9  | Polyp | AlkL from <i>Marinobacter manganoxydans</i> Mnl7-9 (database code ZP_09158756)                         |
| 10 | DNA   | AlkL from <i>Marinobacter manganoxydans</i> Mnl7-9 (database code ZP_09158756)                         |
| 11 | Polyp | AlkL from <i>Caulobacter</i> sp. K31 (database code YP_001672217)                                      |
| 12 | DNA   | AlkL from <i>Caulobacter</i> sp. K31 (database code YP_001672217)                                      |
| 13 | Polyp | Ferredoxin reductase from <i>Alcanivorax borkumensis</i> SK2 (database code YP_691923)                 |
| 14 | DNA   | Ferredoxin reductase from <i>Alcanivorax borkumensis</i> SK2 (database code YP_691923)                 |
| 15 | Polyp | Ferredoxin from <i>Alcanivorax borkumensis</i> SK2 (database code YP_691920)                           |
| 16 | DNA   | Ferredoxin from <i>Alcanivorax borkumensis</i> SK2 (database code YP_691920)                           |
| 17 | Polyp | NAD-dependent alcohol dehydrogenase from <i>Escherichia coli</i> MS 187-1 (database code ZP_07145023)  |
| 18 | DNA   | NAD-dependent alcohol dehydrogenase from <i>Escherichia coli</i> MS 187-1 (database code ZP_07145023)  |
| 19 | Polyp | CYP153 family from <i>Alcanivorax borkumensis</i> SK2 (database code YP_691921)                        |
| 20 | DNA   | CYP153 family from <i>Alcanivorax borkumensis</i> SK2 (database code YP_691921)                        |
| 21 | Polyp | LL(I/L)(V/I)GGNDTTRN                                                                                   |
| 22 | Polyp | Alanine dehydrogenase from <i>Bacillus subtilis subsp. subtilis</i> str. 168 (database code NP_391071) |
| 23 | DNA   | Alanine dehydrogenase from <i>Bacillus subtilis subsp. subtilis</i> str. 168                           |

|    |       |                                                                                                                                 |
|----|-------|---------------------------------------------------------------------------------------------------------------------------------|
|    |       | (database code NP_391071)                                                                                                       |
| 24 | Polyp | Transaminase from <i>Chromobacterium violaceum</i> ATCC 12472 (database code NP_901695)                                         |
| 25 | DNA   | Transaminase from <i>Chromobacterium violaceum</i> ATCC 12472 (database code NP_901695)                                         |
| 26 | Polyp | Oxidoreductase of the glucose-methanol-choline-oxidoreductase family (Deletion of AlkJ Q00593)                                  |
| 27 | DNA   | Oxidoreductase of the glucose-methanol-choline-oxidoreductase family (Deletion of AlkJ Q00593)                                  |
| 28 | Polyp | Oxidoreductase of the glucose-methanol-choline-oxidoreductase family YP_694430 (Ab_AlkJ)                                        |
| 29 | DNA   | Oxidoreductase of the glucose-methanol-choline-oxidoreductase family YP_694430 (Ab_AlkJ)                                        |
| 30 | Polyp | Ferredoxin reductase (YP_957889(Maqu_FdOR))                                                                                     |
| 31 | DNA   | Ferredoxin reductase (YP_957889(Maqu_FdOR))                                                                                     |
| 32 | Polyp | Ferredoxin reductase (BAE78453 (Ac_FdOR))                                                                                       |
| 33 | DNA   | Ferredoxin reductase (BAE78453 (Ac_FdOR))                                                                                       |
| 34 | Polyp | Ferredoxin YP_957887 (Maqu_Fd)                                                                                                  |
| 35 | DNA   | Ferredoxin YP_957887 (Maqu_Fd)                                                                                                  |
| 36 | Polyp | Ferredoxin BAE78451 (Ac_Fd)                                                                                                     |
| 37 | DNA   | Ferredoxin BAE78451 (Ac_Fd)                                                                                                     |
| 38 | Polyp | Oxidoreductase of the glucose-methanol-choline-oxidoreductase family from <i>Caulobacter</i> sp. K31 (database code ABZ74557.1) |
| 39 | DNA   | Oxidoreductase of the glucose-methanol-choline-oxidoreductase family from <i>Caulobacter</i> sp. K31 (database code ABZ74557.1) |
| 40 | Polyp | Flavin-containing alcohol dehydrogenase from <i>Candida tropicalis</i> (database code AAS46878.1)                               |
| 41 | DNA   | Flavin-containing alcohol dehydrogenase from <i>Candida tropicalis</i> (database code AAS46878.1)                               |
| 42 | Polyp | CYP153 family from <i>Marinobacter aquaeolei</i> VT8 (database code YP_YP_957888)                                               |
| 43 | DNA   | CYP153 family from <i>Marinobacter aquaeolei</i> VT8 (database code YP_YP_957888)                                               |

|    |       |                                                                               |
|----|-------|-------------------------------------------------------------------------------|
| 44 | Polyp | CYP153 family from <i>Acinetobacter</i> sp. OC4 (database code YP_YP_957888)  |
| 45 | DNA   | CYP153 family from <i>Acinetobacter</i> sp. OC4 (database code YP_YP_957888)  |
| 46 | Polyp | Alcohol dehydrogenase from <i>Pseudomonas putida</i> (database code CAB54054) |
| 47 | DNA   | Alcohol dehydrogenase from <i>Pseudomonas putida</i> (database code CAB54054) |
| 48 | Polyp | Aminotransferase from <i>Pseudomonase putida</i> (database code YP_001668026) |
| 49 | DNA   | Aminotransferase from <i>Pseudomonase putida</i> (database code YP_001668026) |
| 50 | DNA   | AlkB promoter                                                                 |
| 51 | DNA   | Primer Fd_CYP153                                                              |
| 52 | DNA   | Primer pHg-LL-08                                                              |
| 53 | DNA   | Primer pHg-LL-09                                                              |
| 54 | DNA   | Primer pHg-LL-10                                                              |
| 55 | DNA   | Primer pHg-LL-11                                                              |
| 56 | DNA   | Primer pHg-LL-06                                                              |
| 57 | DNA   | Vector pCOM10                                                                 |
| 58 | DNA   | Expression vector pCOM[Ab_Fd/CYP153-2/FdOR/alkL]                              |
| 59 | Polyp | CYP52 family from <i>Candida tropicalis</i> (database code AAO73952)          |
| 60 | DNA   | CYP52A                                                                        |
| 61 | Polyp | CYP52 family from <i>Candida tropicalis</i> (database code AAO73958)          |
| 62 | DNA   | CYP52 family from <i>Candida tropicalis</i> (database code AAO73958)          |
| 63 | Polyp | NADPH-dependent cytochrome P450 oxidoreductase (database code P37201)         |
| 64 | DNA   | NADPH-dependent cytochrome P450 oxidoreductase (database code P37201)         |
| 65 | DNA   | pCOM10-Ct CYP52A12_co plus OR_co                                              |
| 66 | DNA   | pCOM10-Ct CYP52A17_co plus OR_co                                              |

The present invention is further illustrated by the following Figures and non-limiting examples, from which further features, embodiments, aspects and advantages of the present invention may be taken.

5    Example 1

**Preparation of expression vectors for the genes *CYP153*, *Fd* and *FdOR* from *Alcanivorax borkumensis* *SK2* and *alkL* from *Pseudomonas oleovorans***

To prepare an *E. coli* expression vector for the genes *CYP153* (SEQ ID No. 20), *Fd* (SEQ ID No. 16) and *FdOR* (SEQ ID No. 14) from *Alcanivorax borkumensis*, as well as the gene *alkL* (SEQ ID No. 2) from *Pseudomonas oleovorans*, the genes were cloned under the control of the *alkB* promoter (SEQ ID No. 50) in the plasmid pCOM10. The different DNA fragments were amplified by inserting homologous regions for recombination cloning. The template used was the respective chromosomal DNA.

15    The following oligonucleotides were used for the amplification of the respective fragments:

*Fd\_CYP153*

pHg-LL-07: 5'-TTAATAAAAATTGGAGTACAGACTTTTGGTAGGAGAATGC-3' (SEQ ID No. 51)

pHg-LL-08: 5'-CCTTGGGCTTATTTTTTAGCCGTCAACTTAAC-3' (SEQ ID No. 52)

5 *FdOR*

pHg-LL-09: 5'-AAAAATAAGCCCAAGGCACAGATAAAGAGAGA-3' (SEQ ID No. 53)

pHg-LL-10: 5'-TAGATCCTTCAGATCAAAGACTTTAATTCAAC-3' (SEQ ID No. 54)

*alkL*

10 pHg-LL-11: 5'-TGATCTGAAGGATCTAGGAACCAAGGAGAGTG-3' (SEQ ID No. 55)

pHg-LL-06: 5'-CTTGGCTGCAGGTCGATTAGAAAACATATGACGCACCAAG-3' (SEQ ID No. 56)

The following parameters were used for the PCR:

15

*Fd-CYP153:*

Denaturation: 98°C 30s

Denaturation: 98°C 10s 35x

Annealing: 62°C 20s 35x

20 Elongation: 72°C 1:10 min 35x

Final elongation: 72°C 10 min

*FdOR*

Denaturation: 98 °C 30s

25 Denaturation: 98°C 10s 35x

Annealing: 53°C 20s 35x

Elongation: 72°C 55s 35x

Final elongation: 72°C 10 min

30 *alkL*

Denaturation: 98°C 30s

Denaturation: 98°C 10s 25x

Annealing: 65°C 20s 25x

Elongation: 72°C 18s 25x

Final elongation: 72°C 10 min

For the amplification, the Phusion™ High-Fidelity Master Mix from New England Biolabs (Frankfurt) was used according to the manufacturer's recommendations. In each case, 50 µl of the PCR reactions were then separated on a 1% strength TAE agarose gel. The implementation of the PCR, of the agarose gel electrophoresis, of the ethidium bromide staining of the DNA and determination of the PCR fragment sizes was performed in the manner known to the person skilled in the art. In all cases, PCR fragments of the expected size could be amplified (*Fd-CYP153*: 1800 bp; *FdOR*: 1276 bp; *alkL*: 745 bp). For isolating and purifying the DNA, the PCR products were cut out of a preparative gel using a scalpel and purified using the *QiaQuick Gel extraction Kit* in accordance with the manufacturer's instructions (Qiagen, Hilden). The purified PCR products were cloned using the Geneart® Seamless Cloning and Assembly Kit in accordance with the manufacturer's instructions (Life Technologies, Carlsbad, CA, USA) into a pCOM10 vector cleaved with *EcoRI*-HF and *SaI* (SEQ ID No. 57) behind the *alkB* promoter (SEQ ID No. 50). The transformation of chemically competent *E. coli* 10 beta cells (New England Biolabs, Frankfurt) was carried out in the manner known to the person skilled in the art. The correct insertion of the target genes was checked by restriction analysis and the authenticity of the inserted genes confirmed by DNA sequencing. The resulting expression vector was referred to as pCOM[Ab\_Fd / CYP153-2 / FdOR / *alkL*] (SEQ ID No. 58).

## Example 2

### **Preparation of expression vectors for the genes *CYP52A12* and *OR* from *Candida tropicalis*, and also *alkL* from *Pseudomonas oleovorans***

To prepare an *E. coli* expression vector for the genes *CYP52A12* (SEQ ID No. 60) and *OR* (SEQ ID No. 64) from *Candida tropicalis*, and also the gene *alkL* (SEQ ID No. 2) from *Pseudomonas oleovorans*, the genes *CYP52A12* and *OR* were codon-optimized for the expression in *Escherichia coli in silico* and synthesized together with the gene *alkL* as operon. During the synthesis, cleavage sites for *Ascl* and *SaI* were inserted upstream of the *CYP52A12* gene and downstream of the *alkL* gene. The synthesized DNA fragment *CYP52A12\_OR\_alkL* was digested with the restriction endonucleases *Ascl* and *SaI*, ligated into the correspondingly cleaved vector pCOM10 and the product was transformed into chemically competent *E. coli* 10 beta cells (New England Biolabs, Frankfurt). The finished vector was referred to as pCOM10-Ct *CYP52A12\_co* plus *OR\_co* (SEQ ID No. 65).

Example 3**Preparation of expression vectors for the genes *CYP52A17* and *OR* from *Candida tropicalis*, and *alkL* from *Pseudomonas oleovorans***

To prepare an *E. coli* expression vector for the genes *CYP52A17* (SEQ ID No. 62), and *OR* (SEQ ID No. 64) from *Candida tropicalis*, and also the gene *alkL* (SEQ ID No. 2) from *Pseudomonas oleovorans*, the genes *CYP52A17* and *OR* were codon-optimized for the expression in *Escherichia coli in silico* and synthesized together with the gene *alkL* as operon. During the synthesis, cleavage sites for *Ascl* and *Sall* were inserted upstream of the *CYP52A17* gene and downstream of the *alkL*- gene. The synthesized DNA fragment *CYP52A12\_OR\_alkL* was digested with the restriction endonucleases *Ascl* and *Sall*, ligated into the correspondingly cleaved vector pCOM10 and the product was transformed into chemically competent *E. coli* beta cells (New England Biolabs, Frankfurt). The finished vector was referred to as pCOM10-Ct *CYP52A17\_co* plus *OR\_co* (SEQ ID No. 66).

Example 4**Production of methyl hydroxylaurate by an *E. coli* strain with expression vectors for the genes *CYP153*, *Fd* and *FdOR* from *Alcanivorax borkumensis SK2* and *alkL* from *Pseudomonas oleovorans*, or for the genes *CYP52A17* and *OR* from *Candida tropicalis* and *alkL* from *Pseudomonas oleovorans***

To produce an *E. coli* strain with the expression vector pCOM[Ab\_Fd / *CYP153*-2 / *FdOR* / *alkL*] or pCOM10-Ct *CYP52A17\_co* plus *OR\_co*, electrocompetent cells of *E. coli* W3110 were prepared. This was carried out in a manner known to the person skilled in the art. *E. coli* W3110 was transformed in each case with one of the two listed plasmids and plated out onto LB-agar plates with kanamycin (50 µg/ml). Transformants were checked as regards the presence of the correct plasmids by plasmid preparation and analytical restriction analysis. The following strains were constructed in this way:

- *E. coli* W3110 pCOM[Ab\_Fd / *CYP153*-2 / *FdOR* / *alkL*]

*E. coli* W3110 pCOM10-Ct *CYP52A17\_co* plus *OR\_co*

The strains were subjected to a *fed-batch* fermentation in order to investigate their ability to produce methyl hydroxylaurate, methyl oxolaurate and methyl carboxylaurate from methyl laurate. This was carried out in an 8-fold parallel fermentation system from DASGIP.

For the fermentation, 1 l reactors were used which were equipped with overhead stirrers and impeller turbines. To monitor the process, pH and  $pO_2$  were measured online. OTR/CTR measurements served inter alia for estimating the metabolic activity and fitness of the cells.

5 The pH probes were calibrated by means of a two-point calibration with measurement solutions of pH 4.0 and pH 7.0 according to technical reference of DASGIP. The reactors were provided according to technical reference with the required sensors and connections and the stirrer shaft was installed. The reactors were then filled with 300 ml of water and autoclaved for 20 min at 121°C in order to ensure sterility. The  $pO_2$  probes were polarized overnight (at least 10 6 h) following connection to the measurement amplifier. The water was then removed under the clean bench and replaced by high-cell-density medium consisting of  $(NH_4)_2SO_4$  1.76 g/l,  $K_2HPO_4$  19.08 g/l,  $KH_2PO_4$  12.5 g/l, yeast extracts 6.66 g/l, trisodium citrate dihydrate 11.2 g/l, 17 ml/l of a filter-sterilized 1% strength ammonium iron citrate solution, and 5 ml/l of a filter-sterilized trace element stock solution (consisting of HCl (37%) 36.50 g/l,  $MnCl_2 \cdot 4H_2O$  1.91 g/l, 15  $ZnSO_4 \cdot 7H_2O$  1.87 g/l, ethylenediaminetetraacetic acid dihydrate 0.84 g/l,  $H_3BO_3$  0.30 g/l,  $Na_2MoO_4 \cdot 2H_2O$  0.25 g/l,  $CaCl_2 \cdot 2H_2O$  4.70 g/l,  $FeSO_4 \cdot 7H_2O$  17.80 g/l,  $CuCl_2 \cdot 2H_2O$  0.15 g/l) with 15 g/l glucose as carbon source (added by metered addition of 30 ml/l of a sterile feed solution consisting of 500 g/l glucose, 1% (w/v)  $MgSO_4 \cdot 7H_2O$  and 2.2% (w/v)  $NH_4Cl$ ) with 50 mg/l kanamycin.

20 Subsequently, the  $pO_2$  probes were calibrated using a single-point calibration (stirrer: 600 rpm/gassing: 10 sL/h air) to 100% and the feed, correction agent and induction agent stretches were cleaned by means of cleaning-in-place according to technical reference. For this, the tubes were firstly flushed with 70% ethanol, then with 1 M NaOH, then with sterile 25 demineralized water and finally filled with the respective media.

All of the aforementioned *E. coli* strains were cultured firstly from a cryoculture in LB medium (25 ml in a 100 ml chicane flask) with 50 mg/l kanamycin overnight at 37°C and 200 rpm for about 18 h. Then, 2 ml of this culture were transferred for a second preculture stage into 25 ml 30 of high-cell-density medium consisting of  $(NH_4)_2SO_4$  1.76 g/L,  $K_2HPO_4$  19.08 g/l,  $KH_2PO_4$  12.5 g/l, yeast extract 6.66 g/l, trisodium citrate dihydrate 11.2 g/l, 17 ml/l of a filter-sterilized 1% strength ammonium iron citrate solution, and 5 ml/l of a filter-sterilized trace element stock solution (consisting of HCl (37%) 36.50 g/l,  $MnCl_2 \cdot 4H_2O$  1.91 g/l,  $ZnSO_4 \cdot 7H_2O$  1.87 g/l, ethylenediaminetetraacetic acid dihydrate 0.84 g/l,  $H_3BO_3$  0.30 g/l,  $Na_2MoO_4 \cdot 2H_2O$  0.25 g/l,

CaCl<sub>2</sub>\*2H<sub>2</sub>O 4.70 g/l, FeSO<sub>4</sub>\*7H<sub>2</sub>O 17.80 g/l, CuCl<sub>2</sub>\*2H<sub>2</sub>O 0.15 g/l) with 15 g/l glucose as carbon source (added by metered addition of 30 ml/l of a sterile feed solution consisting of 500 g/l glucose, 1% (w/v) MgSO<sub>4</sub>\*7H<sub>2</sub>O and 2.2% (w/v) NH<sub>4</sub>Cl) with the already described antibiotics in a 100 ml shake flask and incubated at 37°C/200 rpm for a further 6 h.

5

In order to inoculate the reactors with an optical density of 0.1, the OD<sub>600</sub> of the second preculture stage was measured and the amount of culture required for the inoculation was calculated. The required amount of culture was added with the help of a 5 ml syringe through a septum into the heat-treated and aerated reactor.

10

The following standard program was used:

| DO regulator |       | pH regulator |         |
|--------------|-------|--------------|---------|
| Preset       | 0%    | Preset       | 0 ml/h  |
| P            | 0.1   | P            | 5       |
| Ti           | 300 s | Ti           | 200 s   |
| min          | 0%    | min          | 0 ml/h  |
| max          | 100%  | max          | 40 ml/h |

| N<br>(Rotation)                     | from    | to       | XO <sub>2</sub> (gas<br>mixture)    | from | to   | F (gas flow<br>rate)                | from   | to         |
|-------------------------------------|---------|----------|-------------------------------------|------|------|-------------------------------------|--------|------------|
| growth and<br>biotransfor<br>mation | 0%      | 30%      | growth and<br>biotransfor<br>mation | 0%   | 100% | growth and<br>biotransfor<br>mation | 15%    | 80%        |
|                                     | 400 rpm | 1500 rpm |                                     | 21%  | 21%  |                                     | 6 sL/h | 72<br>sL/h |

|                   |                          |
|-------------------|--------------------------|
| Script            |                          |
| Trigger<br>sharp  | 31% DO (1/60h)           |
| Induction<br>DCPK | 10 h after feed<br>start |
| Feed trigger      | 50% DO                   |
| Feed rate         | 3 [ml/h]                 |

15 The pH was regulated to pH 6.8 on one side with 12.5% strength ammonia solution. During cultivation and biotransformation, the dissolved oxygen (pO<sub>2</sub> or DO) in the culture was regulated to at least 30% by means of stirrer feed and gassing rate. Following inoculation, the DO dropped from 100% to this 30%, where it was kept stable for the remainder of the fermentation.

20 The fermentation was carried out as fed-batch, where the feed start was triggered as delivery to the feed phase with 5 g/l\*h glucose feed, consisting of 500 g/l glucose, 1% (w/v) MgSO<sub>4</sub>\*7H<sub>2</sub>O

and 2.2% (w/v)  $\text{NH}_4\text{Cl}$ , via the DO peak inducing the end of the batch phase. With feed start, the temperature of 37°C was lowered to 30°C. 10 h after feed start, the expression of the oxidation genes was induced with 0.025% (v/v) DCPK. The start of the methyl hydroxylaurate production (= start of the biotransformation) was carried out 14 h after feed start. For this purpose, 150 ml of a mixture of methyl laurate and oleic acid (technical-grade 90%) were added as batch to the fermentation broth.

To quantify LSME and HLS in fermentation samples, samples were taken 1/2/4/20/22 h after the start of biotransformation. These samples were prepared for analysis. (see LC-ESI/MS<sup>2</sup>-based quantification of products).

#### LC-ESI/MS<sup>2</sup>-based quantification of products

The quantification of LSME and HCL in fermentation samples was carried out by means of LC-ESI/MS<sup>2</sup> by reference to an external calibration for all analytes (0.1 – 50 mg/l) and using the internal standard aminoundecanoic acid (AUD for HLSME), and d3-LSME (for LSME).

The following instruments were used here:

- HPLC system 1260 (Agilent; Böblingen) with autosampler (G1367E), binary pump (G1312B) and column oven (G1316A)
- Mass spectrometer TripleQuad 6410 (Agilent; Böblingen) with ESI source
- HPLC column: Kinetex C18, 100 x 2.1 mm, particle size: 2.6  $\mu\text{m}$ , pore size 100 Å (Phenomenex; Aschaffenburg)
- Precolumn: KrudKatcher Ultra HPLC In-Line Filter; 0.5  $\mu\text{m}$  filter depth and 0.004 mm internal diameter (Phenomenex; Aschaffenburg)

The samples were prepared by pipetting 1900  $\mu\text{l}$  of solvent (80% (v/v) acetonitrile, 20% double-distilled  $\text{H}_2\text{O}$  (v/v), + 0.1% formic acid) and 100  $\mu\text{l}$  sample in a 2-ml reaction vessel. The mixture was vortexed for about 10 seconds and then centrifuged at about 13 000 rpm for 5 min. The clear supernatant was removed using a pipette and, after appropriate dilution, analysed with diluents (80% (v/v) ACN, 20% double-distilled.  $\text{H}_2\text{O}$  (v/v), + 0.1% formic acid). 100  $\mu\text{L}$  of ISTD were pipetted into each 900  $\mu\text{L}$  sample (10  $\mu\text{L}$  for a sample volume of 90  $\mu\text{L}$ ).

The HPLC separation was carried out with the aforementioned column and precolumn. The injection volume was 0.7  $\mu\text{L}$ , the column temperature 50°C, the flow rate 0.6 mL/min. The

mobile phase consisted of eluent A (0.1% strength (v/v) aqueous formic acid) and eluent B (acetonitrile with 0.1% (v/v) formic acid). The following gradient profile was used:

| Time [min] | Eluent A [%] | Eluent B [%] |
|------------|--------------|--------------|
| 0          | 77           | 23           |
| 0.3        | 77           | 23           |
| 0.4        | 40           | 60           |
| 2.5        | 40           | 60           |
| 2.6        | 2            | 98           |
| 5.5        | 2            | 98           |
| 5.6        | 77           | 23           |
| 9          | 77           | 23           |

5 The ESI-MS<sup>2</sup> analysis was carried out in the positive mode with the following parameters of the ESI source:

- Gas temperature 280°C
- Gas flow rate 11 L/min
- 10 • Nebulizing pressure 50 psi
- Capillary voltage 4000 V

The detection and quantification of the compounds DDS, DDSME, HLS, HLSME, OLS, OLSME was carried out with the following MRM parameters, with in each case a product ion being used as *qualifier* and one as *quantifier*.

| Analyte | Precursor ion<br>[m/z] | Product ion<br>[m/z] | Residence<br>time [ms] | Collision energy [eV] |
|---------|------------------------|----------------------|------------------------|-----------------------|
| HLSME   | 231.3                  | 181.2                | 15                     | 2                     |
| HLSME   | 231.3                  | 163.2                | 25                     | 5                     |

The analyte LSME was detected in the SIM mode (*m/z* 201 and 215).

20 It was able to be shown that the strain *E. coli* W3110 pCOM[Ab\_Fd / CYP153-2 / FdOR / alkL] is able to form methyl  $\omega$ -hydroxylaurate from methyl laurate. The strain *E. coli* W3110 pCOM10-Ct CYP52A17\_co plus OR\_co was able to convert methyl laurate to methyl  $\omega$ -hydroxylaurate or further oxidation products only to a considerably lesser extent.

25 The concentrations of methyl laurate and methyl  $\omega$ -hydroxylaurate are given after a fermentation time of 22 hours.

| Strain                                                    | C (Lauric acid)<br>methylester<br>[g/L] | C (ω-Hydroxyacid<br>methylester)<br>[g/L] |
|-----------------------------------------------------------|-----------------------------------------|-------------------------------------------|
| <i>E. coli</i> W3110 pCOM[Ab_Fd / CYP153-2 / FdOR / alkL] | 88.1                                    | 4.35                                      |
| <i>E. coli</i> W3110 pCOM10-Ct CYP52A17_co plus OR_co     | 106.9                                   | <0.1                                      |

#### Example 5 (prophetic)

**Production of methyl hydroxylaurate by an *E. coli* strain with expression vectors for the genes *CYP153*, *Fd* and *FdOR* from *Alcanivorax borkumensis* *SK2* and *alkL* from *Pseudomonas oleovorans* or for the genes *CYP52A12* and *OR* from *Candida tropicalis* and *alkL* from *Pseudomonas oleovorans***

To produce an *E. coli* strain with the expression vector pCOM[Ab\_Fd / CYP153-2 / FdOR / alkL] or pCOM10-Ct CYP52A12\_co plus OR\_co, electrocompetent cells of *E. coli* W3110 are prepared. This is carried out in a manner known to the person skilled in the art. *E. coli* W3110 is transformed in each case with one of the two listed plasmids and plated out onto LB-agar plates with kanamycin (50 µg/ml). Transformants are tested as regards the presence of the correct plasmids by plasmid preparation and analytical restriction analysis. The following strains are constructed in this way:

- *E. coli* W3110 pCOM[Ab\_Fd / CYP153-2 / FdOR / alkL]
- *E. coli* W3110 pCOM10-Ct CYP52A12\_co plus OR\_co

The strains are subjected to a *fed-batch* fermentation in order to investigate their ability to produce HLSME. This is carried out in an 8-fold parallel fermentation system from DASGIP.

1L reactors equipped with overhead stirrers and impeller turbines are used for the fermentation. pH and  $pO_2$  are measured online for monitoring the process. OTR/CTR measurements serve inter alia to estimate the metabolic activity and fitness of the cells.

5 The pH probes are calibrated by means of a two-point calibration with measurement solutions of pH 4.0 and pH 7.0 according to technical reference from DASGIP. The reactors are provided according to technical reference with the required sensors and connections and the stirrer shaft is installed. Then, the reactors are filled with 300 mL of water and autoclaved for 20 min at 121°C in order to ensure sterility. The  $pO_2$  probes are polarized overnight (at least 6 h) following  
 10 connection to the measurement amplifier. The water is then removed under the clean bench and replaced by high-cell-density medium consisting of  $(NH_4)_2SO_4$  1.76 g/L,  $K_2HPO_4$  19.08 g/L,  $KH_2PO_4$  12.5 g/L, yeast extract 6.66 g/L, trisodium citrate dihydrate 11.2 g/L, 17 mL/L of a filter-sterilized 1% strength ammonium iron citrate solution, and 5 mL/L of a filter-sterilized trace element strain solution (consisting of HCl (37%) 36.50 g/L,  $MnCl_2 \cdot 4H_2O$  1.91 g/L,  $ZnSO_4 \cdot 7H_2O$   
 15 1.87 g/L, ethylenediaminetetraacetic acid dihydrate 0.84 g/L,  $H_3BO_3$  0.30 g/L,  $Na_2MoO_4 \cdot 2H_2O$  0.25 g/L,  $CaCl_2 \cdot 2H_2O$  4.70 g/L,  $FeSO_4 \cdot 7H_2O$  17.80 g/L,  $CuCl_2 \cdot 2H_2O$  0.15 g/L) with 15 g/L glucose as carbon source (added by metered addition of 30 mL/L of a sterile feed solution consisting of 500 g/L glucose, 1% (w/v)  $MgSO_4 \cdot 7H_2O$  and 2.2% (w/v)  $NH_4Cl$ ) with 50 mg/L kanamycin.

20

Subsequently, the  $pO_2$  probes are calibrated with a single-point calibration (stirrer: 600 rpm / gassing: 10 sL/h air) to 100%, and the feed, correcting agent and induction agent stretches are cleaned by means of cleaning-in-place according to technical reference. For this, the tubes are first flushed with 70% ethanol, then with 1 M NaOH, then with sterile demineralized water, and  
 25 finally filled with the respective media.

All of the aforementioned *E. coli* strains are first cultivated from a cryoculture in LB medium (25 mL in a 100 mL shake flask) with 50 mg/L kanamycin overnight at 37°C and 200 rpm for about 18 h. Then, 2 mL of this culture are transferred for a second preculture stage in 25 mL of high-cell-density medium consisting of  $(NH_4)_2SO_4$  1.76 g/L,  $K_2HPO_4$  19.08 g/L,  $KH_2PO_4$  12.5 g/L,  
 30 yeast extract 6.66 g/L, trisodium citrate dihydrate 11.2 g/L, 17 mL/L of a filter-sterilized 1% strength ammonium iron citrate solution, and 5 mL/L of a filter-sterilized trace element strain solution (consisting of HCl (37%) 36.50 g/L,  $MnCl_2 \cdot 4H_2O$  1.91 g/L,  $ZnSO_4 \cdot 7H_2O$  1.87 g/L, ethylenediaminetetraacetic acid dihydrate 0.84 g/L,  $H_3BO_3$  0.30 g/L,  $Na_2MoO_4 \cdot 2H_2O$  0.25 g/L,

CaCl<sub>2</sub>\*2H<sub>2</sub>O 4.70 g/L, FeSO<sub>4</sub>\*7H<sub>2</sub>O 17.80 g/L, CuCl<sub>2</sub>\*2H<sub>2</sub>O 0.15 g/L) with 15 g/L glucose as carbon source (added by metered addition of 30 mL/L of a sterile feed solution consisting of 500 g/L glucose, 1% (w/v) MgSO<sub>4</sub>\*7H<sub>2</sub>O and 2.2% (w/v) NH<sub>4</sub>Cl) with the already described antibiotics in a 100 mL shake flask and incubated at 37°C / 200 rpm for a further 6 h.

5

In order to inoculate the reactors with an optical density of 0.1, the OD<sub>600</sub> of the second preculture stage is measured and the amount of culture required for the inoculation is calculated. The amount of culture required is added with the help of a 5 mL syringe through a septum into the heat-treated and aerated reactor.

10

The following standard program is used:

| DO regulator |       | pH regulator |         |
|--------------|-------|--------------|---------|
| Preset       | 0%    | Preset       | 0 ml/h  |
| P            | 0.1   | P            | 5       |
| Ti           | 300 s | Ti           | 200 s   |
| min          | 0%    | min          | 0 mL/h  |
| max          | 100%  | max          | 40 mL/h |

| N (rotation)                 | from    | to       | XO <sub>2</sub> (gas mixture) | from | to   | F (gas flow rate)            | from   | to      |
|------------------------------|---------|----------|-------------------------------|------|------|------------------------------|--------|---------|
|                              | 0%      | 30%      |                               | 0%   | 100% |                              | 15%    | 80%     |
| Growth and biotransformation | 400 rpm | 1500 rpm | Growth and biotransformation  | 21%  | 21%  | Growth and biotransformation | 6 sL/h | 72 sL/h |

| Script         |                       |
|----------------|-----------------------|
| Trigger sharp  | 31% DO (1/60 h)       |
| Induction DCPK | 10 h after feed start |
| Feed trigger   | 50% DO                |
| Feed rate      | 3 [mL/h]              |

15 The pH is regulated to pH 6.8 on one side with 12.5% strength ammonia solution. During cultivation and biotransformation, the dissolved oxygen (pO<sub>2</sub> or DO) in the culture is regulated to at least 30% via stirrer speed and gassing rate. Following inoculation, the DO drops from 100% to this 30%, where it is kept stable for the remainder of the fermentation.

20 The fermentation is carried out as fed batch, where the feed start is triggered as entry to the feed phase with 5 g/L\*h glucose feed, consisting of 500 g/L glucose, 1% (w/v) MgSO<sub>4</sub>\*7H<sub>2</sub>O and

2.2% (w/v)  $\text{NH}_4\text{Cl}$ , via the DO peak indicating the end of the batch phase. With feed start, the temperature is lowered from 37°C to 30°C. 10 h after feed start, the expression of the oxidation genes is induced with 0.025% (v/v) DCPK. The start of the methyl hydroxylaurate production (= start of the biotransformation) takes place 14 h after feed start. For this, 150 mL of a mixture of  
 5 methyl laurate and oleic acid (technical-grade 90%) were added as batch to the fermentation broth.

For quantification of LSME and HLSME, fermentation samples are taken 1/2/4/20/22 h after the start of biotransformation. These samples are prepared for analysis. (See LC-ESI/MS<sup>2</sup>-based  
 10 quantification of products).

### **LC-ESI/MS<sup>2</sup>-based quantification of products.**

The quantification of LSME and HLSME in fermentation samples takes place by means of LC-ESI/MS<sup>2</sup> by reference to an external calibration for all analytes (0.1-50 mg/L) and using the  
 15 internal standard aminoundecanoic acid (AUD for HLSME) and d3-LSME (for LSME).

The following equipment is used here:

- HPLC system 1260 (Agilent; Böblingen) with autosampler (G1367E), binary pump  
 20 (G1312B) and column oven (G1316A)
- Mass spectrometer TripleQuad 6410 (Agilent; Böblingen) with ESI source
- HPLC column: Kinetex C18, 100 x 2.1 mm, particle size: 2.6  $\mu\text{m}$ , pore size 100 Å (Phenomenex; Aschaffenburg)
- Precolumn: KrudKatcher Ultra HPLC In-Line Filter; 0.5  $\mu\text{m}$  filter depth and 0.004 mm  
 25 internal diameter (Phenomenex; Aschaffenburg)

The samples are prepared by pipetting 1900  $\mu\text{L}$  of solvent (80% (v/v) of acetonitrile, 20% double-distilled  $\text{H}_2\text{O}$  (v/v), + 0.1% formic acid) and 100  $\mu\text{L}$  of sample in a 2-mL reaction vessel. The mixture is vortexed for about 10 seconds and then centrifuged at about 13 000 rpm for 5  
 30 min. The clear supernatant is removed using a pipette and analysed following appropriate dilution with diluent (80% (v/v) ACN, 20% double-distilled  $\text{H}_2\text{O}$  (v/v), + 0.1% formic acid). 100  $\mu\text{L}$  of ISTD are pipetted in for each 900  $\mu\text{L}$  of sample (10  $\mu\text{L}$  for a sample volume of 90  $\mu\text{L}$ ).

The HPLC separation takes place with the aforementioned column or precolumn. The injection volume is 0.7  $\mu\text{L}$ , the column temperature is 50°C, and the flow rate is 0.6 mL/min. The mobile phase consists of eluent A (0.1% strength (v/v) aqueous formic acid) and eluent B (acetonitrile with 0.1% (v/v) formic acid). The following gradient profile is used:

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| Time [min] | Eluent A [%] | Eluent B [%] |
|------------|--------------|--------------|
| 0          | 77           | 23           |
| 0.3        | 77           | 23           |
| 0.4        | 40           | 60           |
| 2.5        | 40           | 60           |
| 2.6        | 2            | 98           |
| 5.5        | 2            | 98           |
| 5.6        | 77           | 23           |
| 9          | 77           | 23           |

The ESI-MS<sup>2</sup> analysis takes place in the positive mode with the following parameters of the ESI source:

- Gas temperature 280°C
- Gas flow rate 11 L/min
- Nebulizer pressure 50 psi
- Capillary voltage 4000 V

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The detection and quantification of the compound HLSME takes place with the following MRM parameters, with in each case one product ion being used as *qualifier* and one as *quantifier*.

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| Analyte | Precursor ion<br>[m/z] | Product ion<br>[m/z] | Residence<br>time [ms] | Collision energy [eV] |
|---------|------------------------|----------------------|------------------------|-----------------------|
| HLSME   | 231.3                  | 181.2                | 15                     | 2                     |
| HLSME   | 231.3                  | 163.2                | 25                     | 5                     |

The analyte is detected in the SIM mode ( $m/z$  201 and 215).

It is found that the strain *E. coli* W3110 pCOM[Ab\_Fd / CYP153-2 / FdOR / alkL] is able to form methyl  $\omega$ -hydroxylaurate from methyl laurate. The strain *E. coli* W3110 pCOM10-Ct CYP52A12\_co plus OR\_co can convert methyl laurate to methyl  $\omega$ -hydroxylaurate or other oxidation products only to a lesser extent.

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The features of the invention disclosed in the preceding description, the claims and the examples may be essential both individually and also in any desired combination for realizing the invention in its various embodiments.

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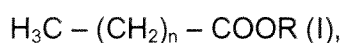
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## Claims

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1. Process for the oxidation of a fatty acid or of an ester thereof of the formula (I)

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wherein R is selected from the group comprising H, methyl, ethyl, propyl and butyl,

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wherein n is 0 to 30, preferably 6 to 24,

comprising the step of:

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- a) oxidising the fatty acid or the ester thereof by contacting with a cytochrome P450 monooxygenase of the CYP153 family in the presence of molecular oxygen and NAD(P)H.

2. Process according to Claim 1, further comprising the steps of:

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- b) further oxidising of the oxidized fatty acid or of the ester thereof from step a) by contacting with an alcohol dehydrogenase,

- c) amination of the further oxidized fatty acid or of the ester thereof from step b) by contacting with a transaminase in the presence of an amine donor, preferably alanine,

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wherein step c) optionally takes place in the presence of an alanine dehydrogenase, ammonium and NAD(P)H.

3. Process according to one of Claims 1 or 2, wherein the cytochrome P450 monooxygenase of the CYP153 family has the peptide sequence LL(I/L)(V/I)GGNDTTRN and is preferably the cytochrome P450 monooxygenase of the CYP153 family from *Alcanivorax borkumensis* SK2 (database code YP\_691921) or a variant thereof.

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4. Process according to one of Claims 1 to 3, wherein the alcohol dehydrogenase is a NAD(P)<sup>+</sup>-dependent alcohol dehydrogenase, preferably the NAD-dependent alcohol dehydrogenase from *Escherichia coli* MS 187-1 (database code ZP\_07145023) or a variant thereof, or an oxidoreductase of the glucose-methanol-choline-oxidoreductase family, preferably that from *Pseudomonas putida* GB-1 (database code CAB54054.1) or a variant thereof, or a flavin-containing alcohol dehydrogenase, preferably the flavin-containing alcohol dehydrogenase from *Candida tropicalis* (database code AAS46878.1) or a variant thereof.
5. Process according to one of Claims 1 to 4, wherein, in step a), additionally a ferredoxin reductase, preferably the Ferredoxin reductase from *Alcanivorax borkumensis* SK2 (database code YP\_691923) or a variant thereof, and/or a ferredoxin, preferably the ferredoxin from *Alcanivorax borkumensis* SK2 (database code YP\_691920) or a variant thereof, is present.
6. Process according to one of Claims 1 to 5, wherein step c) takes place in the presence of an alanine dehydrogenase, ammonium and NADH and the alanine dehydrogenase is the alanine dehydrogenase from *Bacillus subtilis subsp. subtilis* str. 168 (database code NP\_391071) or a variant thereof.
7. Process according to one of Claims 1 to 6, wherein at least one enzyme from the group comprising cytochrome P450 monooxygenase of the CYP153 family, alcohol dehydrogenase, transaminase, alanine dehydrogenase, ferredoxin and ferredoxin reductase is provided recombinantly in the form of a whole-cell catalyst.
8. Process according to Claim 7, wherein all of the enzymes from the group comprising cytochrome P450 monooxygenase of the CYP153 family, alcohol dehydrogenase, transaminase, alanine dehydrogenase, ferredoxin and ferredoxin reductase present or contacted in at least one of steps a), b) or c) with the fatty acid or the ester thereof, the further oxidized fatty acid or the ester thereof from step b) or the aminated further oxidized fatty acid or the ester thereof from step c) are provided recombinantly in the form of one or more than one whole-cell catalyst.

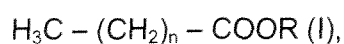
9. Process according to one of Claims 7 to 8, wherein the whole-cell catalyst additionally expresses a polypeptide of the AlkL family, preferably an AlkL from the group comprising AlkL from *Pseudomonas putida* (database code CAB69081), *Marinobacter aquaeolei* VT8 (database code YP\_957722), *Oceanicaulis alexandrii* HTCC2633 (database code ZP\_00953584), *Marinobacter manganoxydans* Mnl7-9 (database code ZP\_09158756), *Caulobacter* sp. K31 (database code YP\_001672217), *Pseudomonas oleovorans* (database code Q00595) or a variant thereof.
10. Process according to one of Claims 7 to 9, wherein the whole-cell catalyst is a cell which has an activity, reduced compared to its wildtype, of at least one enzyme which catalyses one of the reactions of the  $\beta$ -oxidation of fatty acids, wherein the enzyme is preferably selected from the group which comprises fatty acid importer, fatty acid-CoA ligase, acyl-CoA dehydrogenase, 2,4-dienoyl-CoA reductase, enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase.
11. Process according to one of Claims 6 to 10, wherein the alanine dehydrogenase in step c) is selected such that it reduces the redox cofactor, preferably  $\text{NAD}^+$  or  $\text{NADP}^+$ , oxidized by the alcohol dehydrogenase in step b).
12. Whole-cell catalyst expressing a recombinant cytochrome P450 monooxygenase of the CYP153 family, a recombinant alcohol dehydrogenase, a recombinant transaminase and optionally one or more than one recombinant enzyme from the group comprising alanine dehydrogenase, ferredoxin and ferredoxin reductase.
13. Whole-cell catalyst according to Claim 12, wherein the whole-cell catalyst additionally expresses a polypeptide of the AlkL family, preferably an AlkL from the group comprising AlkL from *Pseudomonas putida* (database code CAB69081), *Marinobacter aquaeolei* VT8 (database code YP\_957722), *Oceanicaulis alexandrii* HTCC2633 (database code ZP\_00953584), *Marinobacter manganoxydans* Mnl7-9 (database code ZP\_09158756), *Caulobacter* sp. K31 (database code YP\_001672217), *Pseudomonas oleovorans* (database code Q00595) or a variant thereof.
14. Whole-cell catalyst according to one of Claims 12 to 13, wherein the whole-cell catalyst is a cell which has an activity, reduced compared to its wildtype, of at least one enzyme

which catalyses one of the reactions of the  $\beta$ -oxidation of fatty acids, wherein the enzyme is preferably selected from the group which comprises fatty acid importer, fatty acid-CoA ligase, acyl-CoA dehydrogenase, 2,4-dienoyl-CoA reductase, enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase.

- 5 15. Whole-cell catalyst according to one of Claims 12 to 14, wherein the whole-cell catalyst expresses a ferredoxin reductase and a ferredoxin.
- 10 16. Whole-cell catalyst according to one of Claims 12 to 15, wherein the whole-cell catalyst expresses an alanine dehydrogenase, and wherein the alanine dehydrogenase is from *Bacillus subtilis subsp. subtilis* str. 168 (database code NP\_391071) or a variant thereof.
- 15 17. Whole-cell catalyst according to one of Claims 12 to 16, wherein the cytochrome P450 monooxygenase of the CYP153 family has the peptide sequence LL(I/L)(V/I)GGNDTTRN and/or it is the cytochrome P450 monooxygenase of the CYP153 family from *Alcanivorax borkumensis* SK2 (database code YP\_691921) or a variant thereof, and the ferredoxin reductase is the ferredoxin reductase from *Alcanivorax borkumensis* SK2 (database code YP\_691923) or a variant thereof and the ferredoxin is the ferredoxin from *Alcanivorax borkumensis* SK2 (database code YP\_691920) or a variant thereof.
- 20 18. Whole-cell catalyst or process according to one of Claims 1 to 17, wherein the alcohol dehydrogenase is a NAD(P)<sup>+</sup>-dependent alcohol dehydrogenase, an oxidoreductase of the glucose-methanol-choline-oxidoreductase family or a flavin-containing alcohol oxidase, preferably a NAD(P)<sup>+</sup>-dependent alcohol dehydrogenase, most preferably the NAD-dependent alcohol dehydrogenase from *Escherichia coli* MS 187-1 (database code ZP\_07145023) or a variant thereof.
- 25 19. Whole-cell catalyst or process according to one of Claims 1 to 18, wherein the transaminase is the transaminase from *Pseudomonas putida* GB-1 (database code YP\_001668026.1) or a variant thereof.
- 30 20. Whole-cell catalyst or process according to one of Claims 1 to 19, wherein the fatty acid is an unsaturated or branched fatty acid.

21. Whole-cell catalyst or process according to one of Claims 1 to 20, wherein at least one enzyme from the group comprising cytochrome P450 monooxygenase of the CYP153 family, alcohol dehydrogenase, transaminase, alanine dehydrogenase, ferredoxin and ferredoxin reductase is provided recombinantly in the form of a whole-cell catalyst, or the problem is solved by a whole-cell catalyst, and wherein the whole-cell catalyst is a cell which is an activity, reduced relative to the wildtype of the cell, of at least one endogenous aldehyde dehydrogenase.

22. Use of the whole-cell catalyst according to one of Claims 12 to 21 for the oxidation and/or amination of a fatty acid or of an ester thereof, wherein the fatty acid or the ester thereof preferably has the formula (I)



wherein R is selected from the group comprising H, methyl, ethyl, propyl and butyl,

wherein n is 0 to 30, preferably 6 to 24.

23. Use of the whole-cell catalyst according to Claim 22, wherein the oxidation produces a mixture of oxidation products which, based on the quantitative amount of the reacted fatty acid or of the ester thereof, comprises at least 90% of the corresponding alcohol, less than 1% of the corresponding aldehyde and less than 10% of the corresponding acid.

24. Use of the whole-cell catalyst according to one of Claims 22 to 23, wherein the fatty acid is an unsaturated or branched fatty acid.