



EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent:
30.05.2018 Bulletin 2018/22

(51) Int Cl.:
C12N 15/53 ^(2006.01) **C12N 15/63** ^(2006.01)
C12N 1/21 ^(2006.01) **C12P 7/06** ^(2006.01)
C07C 59/01 ^(2006.01)

(21) Application number: **13771758.3**

(86) International application number:
PCT/KR2013/002885

(22) Date of filing: **05.04.2013**

(87) International publication number:
WO 2013/151393 (10.10.2013 Gazette 2013/41)

(54) **Use of BVMO (Baeyer-Villiger monooxygenases) and fatty acids**

Verwendung von BVMO (Baeyer-Villiger Monooxygenasen) und Fettsäuren
Utilisation du BVMO (monooxygénases de Baeyer-Villiger) et d'acides gras

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

(30) Priority: **06.04.2012 KR 20120036372**
25.05.2012 KR 20120056029
18.01.2013 KR 20130005814

(43) Date of publication of application:
11.02.2015 Bulletin 2015/07

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Description

[Technical Field]

5 **[0001]** The present invention relates to a method for producing medium-chain ω -hydroxy fatty acids, α,ω -dicarboxylic acids, and ω -amino fatty acids from long-chain fatty acids by biotransformation. More particularly, the present invention relates to a transformant which is transformed to express Baeyer-Villiger monooxygenase (BVMO), a method for producing C5-C14 medium-chain ω -hydroxy fatty acids, α,ω -dicarboxylic acids, ω -amino fatty acids, or alcohols from C16-C20 long-chain fatty acids by biotransformation using the transformant, a method for producing a fatty acid derivative having an ester group which is introduced into the chain thereof from keto fatty acid using the BVMO, and novel ω -hydroxy fatty acids which are prepared by the method.

[Background Art]

15 **[0002]** ω -hydroxy fatty acid is a fatty acid ($\text{HOCH}_2(\text{CH}_2)_n\text{COOH}$) with one hydroxyl group at the end of the fatty acid. It has been used as a monomer in the production of polyethylene-based plastics, and is widely used in the production of emulsifiers, adhesives, or coatings and in the preparation of cosmetics or medicines. ω -hydroxy fatty acid can be also used as a precursor in the synthesis of long-chain dicarboxylic acids which are widely used in the production of polyamide, polyester-based plastics, cosmetics and household items.

20 **[0003]** Medium-chain α,ω -dicarboxylic acid ($\text{HOOC}(\text{CH}_2)_n\text{COOH}$) and ω -amino fatty acid ($\text{H}_2\text{NCH}_2(\text{CH}_2)_n\text{COOH}$) have been used as monomers in the production of plastics of polyamide, polyester, etc., and also used in the production of emulsifiers, antifreezes, paints, and coatings. Further, they have a variety of physiological activities such as antibacterial activity, etc., and thus are widely used in the production of cosmetics, foods, and household items. For example, annual production capacity of a C10 medium-chain dicarboxylic acid, sebacic acid ($((\text{HOOC})(\text{CH}_2)_8(\text{COOH}))$) is over 50,000 MT, and it is used in the production of plastics, candles, cosmetics, emulsifiers, antifreezes, and corrosion inhibitors. Further, sebacic acid is used in the production of acne treatments or cosmetics, and household items owing to its antibacterial activity.

25 **[0004]** Such medium-chain ω -hydroxy fatty acid, α,ω -dicarboxylic acid, and ω -amino fatty acid are rarely found in nature, and thus are industrially produced by chemical synthesis. Chemical synthesis has problems of requiring high temperature and high pressure, strong acids and/or toxic oxides causing severe environmental problems (US Patent Nos. 5,952,517, 6,392,074, 5,420,316, and 20110105774). For example, sebacic acid is produced from ricinoleic acid by chemical cleavage (US Patent Nos. 5,952,517 and 6,392,074). However, chemical cleavage of ricinoleic acid requires a high temperature process at 200-300°C or higher and use of strong acids such as sulfuric acid and use of toxic substances such as a heavy metal ion catalyst, an organic solvent, etc. Problematically, this process is dangerous and produces a large amount of environmental pollutants after production.

30 **[0005]** Azelaic acid, a C9 medium-chain dicarboxylic acid, is produced by ozonolysis of oleic acid (US Patent No. 5,420,316). However, the production of azelaic acid by the above technique requires the use of a strong oxidizer ozone, which produces various by-products. Therefore, a separation/purification process using a heavy metal catalyst is essential to remove the by-products produced thereof. Due to many other problems including the complex separation/purification process, environmental pollution, and excessive energy use, there has been a growing need to study solutions of the problems. Accordingly, there has been an active study focused on a simple and environmentally friendly production method, and the use of a biocatalytic process in the method. For example, a method of producing long-chain ω -hydroxy fatty acids from long-chain fatty acids using enzymes and a production method of medium-chain dicarboxylic acids from a petroleum compound hydrocarbon have been developed. However, a method of producing medium-chain ω -hydroxy fatty acids, α,ω -dicarboxylic acids, ω -amino fatty acids, or alcohols from renewable long-chain fatty acids using enzymes has not been developed yet.

35 **[0006]** The present inventors newly demonstrated that BVMO is able to convert keto fatty acids derived from C16 to C20 long-chain fatty acids into fatty acid derivatives having an ester group introduced into the chain thereof, which can be cleaved by ester hydrolase, and they found that medium-chain ω -hydroxy fatty acids, α,ω -dicarboxylic acids, ω -amino fatty acids, alcohols, etc. can be produced from long-chain fatty acids using a transformed microorganism introduced with the BVMO gene, thereby completing the present invention.

[insert page 4a]

55 [Technical Solution]

[0007] The present description relates to a transformant capable of expressing BVMO.

[0008] An objective of the present invention is to provide a method for producing various degradation products from

long-chain fatty acids by biotransformation using the transformant.

[0009] Still another objective of the present invention is to provide ω -hydroxy fatty acid represented by Chemical Formula 1, which is prepared by the above method.

[0010] BVMO has been described in the prior art by Park et al. ("Microbial formation of esters", Applied Microbiology and biotechnology, vol.85, 2009, pages 13-25) as an enzyme able to react with ketones which are used as carbon source by some microorganisms. This reaction allows obtaining esters and subsequent hydrolysis of the esters to release an organic acid and an alcohol.

[0011] Moreover, Kirschner et al. (Applied Microbiology and biotechnology, March 2007, vol.75, no. 5, pp. 1095-1101) discloses the activity of BVMO for converting ketones into esters by using secondary alcohols as substrate. The obtained final products are primary alcohols and alkylacetates.

[0012] These references do not describe the ability of BVMO or of a transformant expressing BVMO encoding gene to convert long chain fatty acids into fatty acids derivatives as demonstrated in the present invention.

[0013] It appears that this ability of BVMO has a strong industrial potential since after the priority date of the present application further studies were carried out (Ji et al., 2013; Sing et al., 2013; Park et al., 2013) in order to attempt to better understand the part of BVMO in biotransformation processes that directly use a plant oil and to improve them. These references do not describe introducing an amino-transferase encoding enzyme nor all degradation products obtained by the method for producing degradation products from long-chain fatty acid according to the present invention.

[Advantageous Effect]

[0014] Degradation products such as C5 to C14 ω -hydroxy fatty acids, α,ω -dicarboxylic acids, ω -amino fatty acids, alcohols can be produced in a large amount from C16 to C20 long-chain fatty acids contained in a medium by biotransformation using a transformant capable of expressing BVMO of the present invention. Therefore, it can be widely used to produce ω -hydroxy fatty acids, α,ω -dicarboxylic acids, ω -amino fatty acids or alcohols in a more safe and economic manner.

[Description of Figures]

[0015]

FIG. 1a is a schematic diagram showing the sequential steps of reactions of producing medium-chain ω -hydroxy fatty acid (C9 ω -hydroxynonanoic acid) from long-chain fatty acid (C18 oleic acid) using hydratase, alcohol dehydrogenase, BVMO and ester hydrolase;

FIG. 1b is a schematic diagram showing the sequential steps of reactions of producing medium-chain ω -hydroxy fatty acid (C11 ω -hydroxyundec-9-enoic acid) from long-chain fatty acid (C18 ricinoleic acid) using alcohol dehydrogenase, BVMO and ester hydrolase;

FIG. 1c is a schematic diagram showing the sequential steps of reactions of producing ω -hydroxy fatty acid (C13 ω -hydroxytridec-11-enoic acid) from long-chain fatty acid (C20 lesquerolic acid) using alcohol dehydrogenase, BVMO and ester hydrolase;

FIG. 1d is a schematic diagram showing the sequential steps of reactions of producing α,ω -dicarboxylic acid (C10 α,ω -decanedioic acid) and octanol from long-chain fatty acid (C18 oleic acid) using hydratase, alcohol dehydrogenase, BVMO and ester hydrolase;

FIG. 1e is a schematic diagram showing the sequential steps of reactions of producing α,ω -dicarboxylic acid (C9 α,ω -nonanedioic acid) from long-chain fatty acid (C18 oleic acid) using hydratase, *Micrococcus luteus*-derived alcohol dehydrogenase, BVMO, ester hydrolase and *Pseudomonas putida*-derived alcohol dehydrogenase;

FIG. 1f is a schematic diagram showing the sequential steps of reactions of producing ω -amino fatty acid (C9 ω -aminononanoic acid) from long-chain fatty acid (C18 oleic acid) using hydratase, *Micrococcus luteus*-derived alcohol dehydrogenase, BVMO, ester hydrolase, *Pseudomonas putida*-derived alcohol dehydrogenase, and aminotransferase;

FIG. 2 shows the production change over time of fatty acid having an ester group introduced into the chain thereof, which is produced from oleic acid using a transformant expressing oleic acid hydratase, alcohol dehydrogenase and BVMO of the present invention, and a graph showing the results of GC/MS analysis of hydrolysis products of ester; FIG. 3 shows the production change over time of fatty acid having an ester group introduced into the chain thereof, which is produced from ricinoleic acid using a transformant expressing alcohol dehydrogenase and BVMO of the present invention, and a graph showing the results of GC/MS analysis of hydrolysis products of ester;

FIG. 4 shows the results of GC/MS analysis of products, resulting from the reaction of ester hydrolase with fatty acid having an ester group introduced into the chain thereof, which is produced from lesquerolic acid using a transformant expressing alcohol dehydrogenase and BVMO of the present invention;

FIG. 5 shows the production change over time of fatty acid having an ester group introduced into the chain thereof, which is produced from oleic acid using a transformant expressing oleic acid hydratase, alcohol dehydrogenase and BVMO of the present invention, and a graph showing the results of GC/MS analysis of hydrolysis products (sebacic acid) of ester;

FIG. 6 is a graph showing the production amount over time of reaction products, resulting from addition of a transformant expressing ester hydrolase and *Pseudomonas putida*-derived alcohol dehydrogenase to fatty acid having an ester group introduced into the chain thereof, which is produced from oleic acid using a transformant expressing oleic acid hydratase, *Micrococcus luteus*-derived alcohol dehydrogenase and BVMO of the present invention;

FIG. 7 is a graph showing the production change over time of α,ω -undec-2-enedioic acid (*cis*-2-undecene-1,11-dioic acid), which is produced from ricinoleic acid using a transformant expressing oleic acid hydratase, *Micrococcus luteus*-derived alcohol dehydrogenase, BVMO and ester hydrolase, *Pseudomonas putida*-derived alcohol dehydrogenase; and

FIG. 8 is a graph showing the production amount over time of reaction products, resulting from addition of a transformant expressing ester hydrolase, *Pseudomonas putida*-derived alcohol dehydrogenase, and aminotransferase to fatty acid having an ester group introduced into the chain thereof, which is produced from oleic acid using a transformant expressing oleic acid hydratase, *Micrococcus luteus*-derived alcohol dehydrogenase and BVMO of the present invention.

[Best Mode]

[0016] The present description relates to a transformant which is introduced with BVMO (Baeyer-Villiger monooxygenase) gene.

[0017] The term "BVMO (Baeyer-Villiger monooxygenase)", as used herein, refers to a monooxygenase which catalyzes various oxidation reactions, including a Baeyer-Villiger oxidation of producing a lactone or an ester compound by oxidation of a ketone. With respect to the objects of the present invention, as long as BVMO is expressed in the transformant and is capable of catalyzing the reaction of producing a fatty acid derivative having an ester group introduced into the chain thereof from keto fatty acid (e.g., 10-(octyloxy)-10-oxodecanoic acid from 10-ketostearic acid), the BVMO may be, but is not particularly limited to, preferably BVMO derived from a microorganism such as *Pseudomonas* sp. strain, *Rhodococcus* sp. strain, *Brevibacterium* sp. strain, *Comamonas* sp. strain, *Acinetobacter* sp. strain, *Arthrobacter* sp. strain, *Brachymonas* sp. strain, etc. The BVMO is defined in claims 1 and 15, respectively, and in claim 1, the BVMO is derived from *Pseudomonas putida*, *Pseudomonas fluorescens*, *Pseudomonas veronii*, *Rhodococcus jostii*, or *Pseudomonas* sp. strain HI-70, or a sequence having 80% or more homology thereto which maintains BVMO activity. Most preferably, BVMO derived from *Pseudomonas putida*. The nucleotide sequence of the BVMO-encoding gene can be obtained from the known database such as GenBank at NCBI, and exemplified by a gene represented by GenBank Accession No. CAFK01000010, a gene obtained from an expression vector pJOE-KT2440BVMO (Biotechnol. Lett., 29:1393-1398, 2007) which was prepared for BVMO gene expression, etc., and preferably, a polynucleotide sequence capable of encoding an amino acid sequence of SEQ ID NO. 9, etc.

[0018] It is described herein that the substitution, deletion, insertion, addition or inversion of one amino acid or several amino acids (may vary depending on positions of amino acid residues in the three-dimensional structure of the protein and types of the amino acid residues, specifically 2 to 20, preferably 2 to 10, more preferably 2 to 5 amino acids) may be included at one or more positions of amino acid sequence of BVMO, as long as the sequence is able to express BVMO having the ability to catalyze the reaction of producing a fatty acid derivative having an ester group introduced into the chain thereof from keto fatty acid in the transformant. According to the method of the invention, as long as it is able to maintain or enhance the BVMO activity, an amino acid sequence having 80% or more, preferably 90% or more, more preferably 95% or more, even more preferably 97% or more homology with the amino acid sequence of BVMO may be included. Since the amino acid sequence of the enzyme showing the activity of the polypeptide may differ depending on the species or the strain of the microorganism, the substitution, deletion, insertion, addition or inversion of the amino acid also includes a naturally occurring mutated sequence or an artificially mutated sequence if it is based on the difference between individuals or between different species of the microorganism having the BVMO activity. The term "homology", as used herein, refers to identity between two different amino acid sequences or two different nucleotide sequences, and can be determined by a method well known to those skilled in the art, for example, BLAST 2.0, which calculates parameters such as score, identity, and similarity, but is not particularly limited thereto.

[0019] The term "transformant", as used herein, refers to a cell or a microorganism which is mutated to express a desired protein after introduction of a polynucleotide encoding the desired protein into a host by a vector. In this regard, the polynucleotide to be introduced into the host cell may have any form, as long as it can be introduced into the host cell and expressed therein.

[0020] The transformant provided for implementing the present invention may be prepared by introducing an expression vector harboring the known BVMO-encoding polynucleotide sequence or a known expression vector *pJOE*-

KT2440BVMO (Biotechnol. Lett., 29:1393-1398, 2007) into a host cell. In this regard, the host cell to be used is not particularly limited, as long as it is introduced with the polynucleotide sequence encoding the BVMO of the present invention, thereby expressing the BVMO. Preferably, the host cell may be a culturable unicellular prokaryotic or eukaryotic cell which is suitable for a biotransformation process, and more preferably, *E. coli*, yeast, or the like, and most preferably, *E. coli* BL21(DE3) cell.

[0021] The transformant provided for implementing the present invention may be used for the production of C5 to C14 medium-chain ω -hydroxy fatty acids, α,ω -dicarboxylic acids, and ω -amino fatty acids by cleavage of C16 to C20 long-chain fatty acids contained in a medium. To this end, in addition to the gene encoding BVMO, a gene encoding hydratase or lipoxigenase, a gene encoding alcohol dehydrogenase, a gene encoding ester hydrolase (esterase), and a gene encoding aminotransferase may be further introduced.

[0022] The term "hydratase", as used herein, refers to an enzyme that reversibly produces a hydroxyl compound by adding water to a carbon double bond, and also called dehydratase because it catalyzes removal of water from the hydroxyl compound by a reverse reaction. The hydratase may be preferably derived from a strain such as *Stenotrophomonas maltophilia*, *Lysinibacillus fusiformis*, *Micrococcus caseolyticus*, *Propionibacterium acnes*, etc., but it is not particularly limited thereto. With respect to the objects of the present invention, the hydratase may be used for the purpose of producing a hydroxy fatty acid by adding a hydroxyl group to a C16 to C20 long-chain fatty acid.

[0023] The term "lipoxigenase", as used herein, refers to an oxygenase that adds molecular oxygen to unsaturated fatty acids, and an enzyme that produces hydroperoxide by recognizing a *cis,cis*-1,4-pentadiene structure of unsaturated fatty acid for stereospecific abstraction of a hydrogen atom from methylene and antarafacial oxygen insertion. With respect to the objectives of the present invention, the lipoxigenase may be used for the purpose of producing a hydroxy fatty acid by adding a hydroxyl group to a C16 to C20 long-chain fatty acid, like the above described hydratase.

[0024] The term "alcohol dehydrogenase", as used herein, means an enzyme that catalyzes a reaction of removing hydrogen from alcohol to produce aldehyde, ketone or carboxylic acid. The alcohol dehydrogenase may be preferably derived from *Micrococcus luteus*, *Pseudomonas* sp. strain, but it is not particularly limited thereto. With respect to the objects of the present invention, the alcohol dehydrogenase can be used for the purpose of producing keto fatty acid by removing hydrogens from hydroxy fatty acid which is produced by hydratase or lipoxigenase, or producing ω -oxofatty acid or α,ω -dicarboxylic acid from ω -hydroxy fatty acid which is produced by ester hydrolase.

[0025] The term "ester hydrolase (esterase)", as used herein, refers to an enzyme that hydrolyzes an ester bond of an ester compound. With respect to the objects of the present invention, the ester hydrolase may be used for the purpose of cleaving an ester group of a fatty acid derivative having the ester group introduced into the chain thereof, which is produced by BVMO.

[0026] The term "aminotransferase", as used herein, refers to an enzyme that converts an oxo group of ω -oxofatty acid into an amino group. The aminotransferase may be derived from a microorganism, but it is not particularly limited to, preferably, a strain such as *Chromobacterium violaceum*, *Silicibacter pomeroyi*, *Rhodococcus sphaeroides*, *Mesorhizobium lotimaffi*, *Silicibacter* sp., etc. With respect to the objectives of the present invention, the aminotransferase can be used for the purpose of producing ω -amino fatty acid from ω -oxofatty acid produced by alcohol dehydrogenase, and preferably, used for the purpose of producing ω -amino fatty acid from C9, C11, C12 and C13 ω -oxofatty acids.

[0027] According to one embodiment of the present invention, a pACYC/oleic acid hydratase/alcohol dehydrogenase-expressing vector including the oleic acid hydratase gene derived from *Stenotrophomonas maltophilia* and the alcohol dehydrogenase gene derived from *Micrococcus luteus* was introduced into *E. coli* BL21(DE3) strain to prepare a primary transformant expressing hydratase and alcohol dehydrogenase, and pJOE-KT2440BVMO which is a vector expressing the BVMO gene derived from *Pseudomonas putida* was introduced into the primary transformant to prepare a secondary transformant capable of expressing oleic acid hydratase, alcohol dehydrogenase and BVMO at the same time (Example 1).

[0028] As indicated above, the main objective of the present invention is providing a method for producing various degradation products from long-chain fatty acids by biotransformation using the transformant.

[0029] According to the invention, the method for producing degradation products from long-chain fatty acids of the present invention includes the steps of (a) reacting long-chain fatty acid with the transformant which is introduced with the BVMO-encoding gene to obtain reactants; and (b) recovering degradation products from the reactants. In this regard, the degradation products may be medium-chain ω -hydroxy fatty acid, α,ω -dicarboxylic acid, ω -amino fatty acid or alcohol. Preferably, the degradation products may be C5 to C14 medium-chain ω -hydroxy fatty acid, α,ω -dicarboxylic acid, ω -amino fatty acid or C2 to C14 normal alcohol, more preferably, ω -hydroxynonanoic acid, ω -hydroxyundec-9-enoic acid, ω -hydroxytridec-11-enoic acid, α,ω -nonanedioic acid (azelaic acid), α,ω -decanedioic acid (sebacic acid), ω -aminononanoic acid, heptanoic acid, nonanoic acid, ω -hydroxyundecanoic acid, ω -hydroxytridecanoic acid, α,ω -undec-2-enedioic acid (cis-2-undecene-1,11-dioic acid), ω -aminoundec-9-enoic acid, α,ω -tridec-2-enedioic acid, normal octanol, etc.

[0030] In this regard, the long-chain fatty acid is C16 to C20 straight-chain fatty acid, more preferably, oleic acid, ricinoleic acid, 12-hydroxystearic acid, linoleic acid, palmitoleic acid, lesquerolic acid, etc.

[0031] As indicated above, the transformant for use in the method of the invention is a transformant which is introduced

with Baeyer-Villiger monooxygenase (BVMO)-encoding gene, and has ability to produce degradation products from long-chain fatty acids; wherein the long-chain fatty acid is C16 to C20 straight-chain fatty acid and wherein the BVMO-encoding gene encodes an amino acid sequence of BVMO derived from *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas veronii*, *Rhodococcus jostii* or *Pseudomonas sp.* strain HI-70, or a sequence having 80% or more homology thereto which maintains BVMO activity.

[0032] Further, the above reaction means an enzymatic reaction which catalyzes cleavage and degradation of the long-chain fatty acid or transfer of an amino group by reacting the long-chain fatty acid used as a substrate with various enzymes produced by the transformant, such as hydratase, lipoyxygenase, alcohol dehydrogenase, ester hydrolase, aminotransferase. A series of the enzymes produced by the transformant are produced within the transformant, and the produced enzymes are secreted out from the transformant. Therefore, the reaction may occur within or outside the transformant. For example, while the transformant is cultured in a medium containing a carbon source, long-chain fatty acids are added to the medium after a predetermined amount of the carbon source in the medium is consumed, and cleavage and degradation of the long-chain fatty acid or transfer of an amino group is catalyzed by the enzymes produced from the transformant, thereby producing various degradation products; the transformant is added to a reaction buffer solution containing long-chain fatty acid, and cleavage and degradation of the long-chain fatty acid or transfer of an amino group is catalyzed, thereby producing various degradation products; or each of the enzymes is separated from the transformant, and the separated enzymes are immobilized onto supports, respectively and then they are added to the reaction buffer solution containing long-chain fatty acid, and the cleavage or degradation of the long-chain fatty acid is catalyzed by the immobilized enzymes, thereby producing various degradation products. In this regard, the content of long-chain fatty acid in the medium or the buffer solution may be, but is not particularly limited to, preferably added to a final concentration of 0.1 to 100g/L.

[0033] The term "culture", as used herein, refers to culturing of a microorganism under artificially controlled environmental conditions. For the purpose of the present invention, the method for culturing the transformant may be conducted using a method widely known in the art. Specifically, examples of the culturing method include a batch process, a fed batch or repeated fed batch process in a continuous manner, but are not limited thereto.

[0034] The medium used for the culture should comply with the requirements of a specific strain in a proper manner while controlling temperature, pH, etc., under aerobic conditions in a typical medium containing a proper carbon source, nitrogen source, amino acids, vitamins, etc. Carbon sources to be used may include sugars such as glucose, sucrose, and lactose, lipids, fatty acids, glycerol. These materials may be used separately or in combination. Nitrogen sources to be used may include inorganic nitrogen sources such as ammonia, ammonium sulfate, ammonium chloride, and ammonium phosphate; and organic nitrogen sources such as amino acids including glutamic acid, peptone, meat extract, yeast extract, malt extract, corn steep liquor, casein hydrolysates, etc. These nitrogen sources may be used separately or in combination. In order to maintain aerobic condition, oxygen or oxygen-containing gas (e.g., air) is introduced into the culture broth. The temperature of the culture broth is normally 15°C to 37°C, preferably 20°C to 30°C, and the culture is conducted for 10 to 100 hours.

[0035] Furthermore, the step of recovering the degradation products such as medium-chain ω -hydroxy fatty acid, α,ω -dicarboxylic acid, ω -amino fatty acid, alcohol from the reaction solution may be performed by a method known in the art, such as dialysis, centrifugation, filtration, solvent extraction, chromatography, crystallization, etc. For example, the supernatant obtained after removing the transformant by centrifugation of the reaction solution, may be applied to a solvent extraction to recover the desired degradation products. Otherwise, any method may be used without limitation, as long as the degradation products are recovered by combinations of the known experimental methods suitable for the properties of each degradation product.

[0036] When the hydratase-encoding gene, alcohol dehydrogenase-encoding gene, BVMO-encoding gene, ester hydrolase-encoding gene, and aminotransferase-encoding gene provided in the present invention are introduced into a host cell in different combinations, various transformants having different functions can be prepared, and each of the transformants prepared is able to produce C5 to C14 medium-chain ω -hydroxy fatty acids, α,ω -dicarboxylic acids, ω -amino fatty acids or alcohols from C16 to C20 long-chain fatty acids (oleic acid, ricinoleic acid, linoleic acid, lesquerolic acid, etc.) present in the medium or reaction solution.

[0037] For example, a transformant prepared by introducing the alcohol dehydrogenase-encoding gene and the BVMO-encoding gene into *E. coli* is able to convert a long-chain fatty acid into a long-chain fatty acid having an ester group which is introduced into the chain thereof. When the transformant further includes ester hydrolase, alcohol dehydrogenase or aminotransferase individually or in combination, the long-chain fatty acid having an ester group introduced into the chain thereof can be converted into various types of medium-chain ω -hydroxy fatty acids, α,ω -dicarboxylic acids, ω -amino fatty acids or alcohols.

[0038] In one embodiment of the method of the present invention, the transformant capable of expressing hydratase, alcohol dehydrogenase, BVMO and ester hydrolase can produce medium-chain ω -hydroxy fatty acids such as ω -hydroxynonanoic acid (C9) from long-chain fatty acids such as oleic acid (C18), in which hydratase converts long-chain fatty acids into hydroxy fatty acids, alcohol dehydrogenase produces keto fatty acids by removing hydrogens from the

converted hydroxy fatty acids, BVMO oxidizes the produced keto fatty acids to produce long-chain fatty acids having an ester group introduced into the chain thereof, and ester hydrolase hydrolyzes an ester bond of the produced long-chain fatty acids having an ester group introduced into the chain thereof, thereby producing medium-chain ω -hydroxy fatty acids (FIG. 1a).

[0039] In another embodiment of the method of the present invention, the transformant capable of expressing alcohol dehydrogenase, BVMO and ester hydrolase can produce medium-chain ω -hydroxy fatty acids such as ω -hydroxyundec-9-enoic acid (C11) from hydroxy long-chain fatty acids such as ricinoleic acid (C18), in which alcohol dehydrogenase produces keto fatty acids by removing hydrogens from hydroxy long-chain fatty acids, BVMO oxidizes the produced keto fatty acids to produce long-chain fatty acids having an ester group introduced into the chain thereof, and ester hydrolase hydrolyzes an ester bond of the produced long-chain fatty acids having an ester group which is introduced into the chain thereof, thereby producing medium-chain ω -hydroxy fatty acids (FIG. 1b).

[0040] In still another embodiment of the method of the present invention, the transformant capable of expressing alcohol dehydrogenase, BVMO and ester hydrolase can produce medium-chain ω -hydroxy fatty acids such as ω -hydroxytridec-11-enoic acid (C13) from hydroxy long-chain fatty acids such as lesquerolic acid (C20), in which alcohol dehydrogenase produces keto fatty acids by removing hydrogens from hydroxy long-chain fatty acids, BVMO oxidizes the produced keto fatty acids to produce long-chain fatty acids having an ester group introduced into the chain thereof, and ester hydrolase hydrolyzes an ester bond of the produced long-chain fatty acids having an ester group which is introduced into the chain thereof, thereby producing medium-chain ω -hydroxy fatty acids (FIG. 1c).

[0041] In still another embodiment of the method of the present invention, the transformant capable of expressing hydratase, alcohol dehydrogenase, BVMO and ester hydrolase can produce α,ω -dicarboxylic acid such as α,ω -decanedioic acid (C10) and octanol (C8) from long-chain fatty acids such as oleic acid (C18), in which hydratase converts long-chain fatty acids into hydroxy fatty acids, alcohol dehydrogenase produces keto fatty acids by removing hydrogens from the converted hydroxy fatty acids, BVMO oxidizes the produced keto fatty acids to produce long-chain fatty acids having an ester group introduced into the chain thereof, and ester hydrolase hydrolyzes an ester bond of the produced long-chain fatty acids having an ester group introduced into the chain thereof, thereby producing α,ω -dicarboxylic acid and alcohol (FIG. 1d).

[0042] In still another embodiment of the method of the present invention, the transformant capable of expressing hydratase, *Micrococcus luteus*-derived alcohol dehydrogenase, BVMO, ester hydrolase, and *Pseudomonas putida*-derived alcohol dehydrogenase can produce α,ω -dicarboxylic acids such as α,ω -nonanedioic acid (C9) from long-chain fatty acids such as oleic acid (C18), in which hydratase converts long-chain fatty acids into hydroxy fatty acids, *Micrococcus luteus*-derived alcohol dehydrogenase produces keto fatty acids by removing hydrogens from the converted hydroxy fatty acids, BVMO oxidizes the produced keto fatty acids to produce long-chain fatty acids having an ester group introduced into the chain thereof, ester hydrolase hydrolyzes an ester bond of the produced long-chain fatty acids having an ester group introduced into the chain thereof, thereby producing ω -hydroxy fatty acids, and *Pseudomonas putida*-derived alcohol dehydrogenase produces α,ω -dicarboxylic acids by removing hydrogens from the ω -hydroxy fatty acids (FIG. 1e).

[0043] In still another embodiment of the method of the present invention, the transformant capable of expressing hydratase, *Micrococcus luteus*-derived alcohol dehydrogenase, BVMO, ester hydrolase, *Pseudomonas putida*-derived alcohol dehydrogenase and aminotransferase can produce ω -amino fatty acids such as ω -aminononanoic acid (C9) from long-chain fatty acids such as oleic acid (C18), in which hydratase converts long-chain fatty acids into hydroxy fatty acids, *Micrococcus luteus*-derived alcohol dehydrogenase produces keto fatty acids by removing hydrogens from the converted hydroxy fatty acids, BVMO oxidizes the produced keto fatty acids to produce long-chain fatty acids having an ester group introduced into the chain thereof, ester hydrolase hydrolyzes an ester bond of the produced long-chain fatty acids having an ester group introduced into the chain thereof, thereby producing ω -hydroxy fatty acids, *Pseudomonas putida*-derived alcohol dehydrogenase produces ω -keto fatty acids by removing hydrogens from the ω -hydroxy fatty acids, and aminotransferase transfers amino groups to the produced ω -keto fatty acid, thereby producing ω -amino fatty acids (FIG. 1f).

[0044] Meanwhile, ω -hydroxy fatty acids produced from long-chain fatty acids can be converted into α,ω -dicarboxylic acids by *Pseudomonas putida*-derived alcohol dehydrogenase (Examples 5 and 6), or into ω -amino fatty acids by successive reaction with aminotransferase (Example 7).

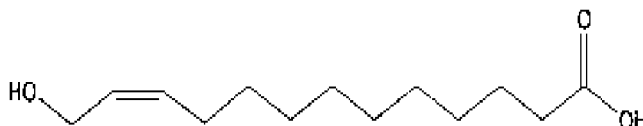
[0045] C5 to C14 ω -hydroxy fatty acids, α,ω -dicarboxylic acids, ω -amino fatty acids, and alcohols produced by the transformants are secreted into the medium or reaction buffer solution. Therefore, when the transformant is immobilized on an immobilization support or the transformant is cultured in a fed-batch or continuous mode, C5 to C14 ω -hydroxy fatty acids, α,ω -dicarboxylic acids, ω -amino fatty acids and alcohols can be produced in large amount.

[0046] According to one embodiment of the present invention, *Stenotrophomonas maltophilia*-derived oleic acid hydratase gene and *Micrococcus luteus*-derived alcohol dehydrogenase gene were introduced into *E. coli* BL21(DE3) to prepare a transformant expressing hydratase and alcohol dehydrogenase, and *Pseudomonas fluorescens*-derived BVMO gene was introduced into the transformant to prepare a transformant expressing hydratase, alcohol dehydrogenase

and BVMO (Example 4). Further, the transformant expressing hydratase, alcohol dehydrogenase, BVMO was cultured and reacted with oleic acid. As a result, 10-hydroxy stearic acid, 10-keto stearic acid, and fatty acid having an ester group which is introduced into the chain thereof were produced (FIG. 5), and *Pseudomonas fluorescens*-derived ester hydrolase was added thereto, thereby producing α,ω -decanedioic acid and octanol.

[0047] In still another aspect to achieve the above objects, the present invention provides a novel ω -hydroxy fatty acid represented by the following Chemical Formula 1, which is prepared by the above method.

[Chemical Formula 1]



[0048] The above compound can be prepared by the above described method. For example, the transformant expressing oleic acid hydratase, alcohol dehydrogenase and BVMO is cultured, and then reacted with lesquerolic acid to produce fatty acid having an ester group introduced into the chain thereof, and *Pseudomonas fluorescens*-derived ester hydrolase is added to and reacted with the produced fatty acid having an ester group introduced into the chain thereof, thereby producing the novel ω -hydroxy fatty acid of Chemical Formula 1. The novel ω -hydroxy fatty acid thus produced can be analyzed by GC/MS to examine its structure.

[0049] In still another aspect to achieve the above objects, the present invention provides a method for producing a fatty acid derivative having an ester group introduced into the chain thereof from keto fatty acid using BVMO.

Examples:

Example 1: Production of ω -hydroxynonanoic acid by multistep enzymatic synthesis

1) Gene cloning

[0050] To prepare a recombinant expression vector including oleic acid hydratase and alcohol dehydrogenase genes, *Stenotrophomonas maltophilia*-derived oleic acid hydratase gene and *Micrococcus luteus*-derived alcohol dehydrogenase gene were cloned.

[0051] First, oleic acid hydratase gene was amplified by PCR using a plasmid vector pET 28(+)/oleic acid hydratase (J. Biotechnol., 158:17-23, 2012) as a template and primers (SEQ ID NOS: 1 and 2) which were prepared by including PvuI and XhoI restriction enzyme sites.

Forward primer: 5'-gctagcatgtattacagtaatggaactatgaa-3' (SEQ ID NO: 1)

Reverse primer: 5'-ggctcgagctatattagtttacttcttca-3' (SEQ ID NO: 2)

[0052] The amplified PCR product was digested with restriction enzymes *PvuI* and *XhoI*, and inserted into a plasmid vector *pACYC* (manufactured by Novagen) to prepare a *pACYC*/oleic acid hydratase expression vector.

[0053] Further, alcohol dehydrogenase was amplified by PCR using DNA sequence (Genebank Accession No.ZP_07049769) of *Micrococcus luteus*-derived alcohol dehydrogenase as a template and primers (SEQ ID NOS: 3 and 4) which were prepared by including *EcoRI* and *HindIII* restriction enzyme sites.

Forward primer: 5'-atcgaattcgtccgagttcaccggttcga-3' (SEQ ID NO: 3)

Reverse primer: 5'-atatcaagcttcagccgagcgggtgtcct-3' (SEQ ID NO: 4)

[0054] The amplified PCR product was digested with restriction enzymes *EcoRI* and *HindIII*, and inserted into the prepared *pACYC*/oleic acid hydratase expression vector to prepare a *pACYC*/oleic acid hydratase/alcohol dehydrogenase expression vector.

2) Host cell culture

[0055] To maintain the plasmid, *E. coli* BL21(DE3) was cultured in a Riesenberg medium containing 10 g/l glucose and an appropriate antibiotic. At this time, the Riesenberg medium contained 4 g/l $N(NH_4)_2HPO_4$, 13.5 g/l KH_2PO_4 , 1.7 g/l citric acid, 1.4 g/l $MgSO_4$ and 10 ml/l trace metal solution (10 g/l $FeSO_4$, 2.25 g/l $ZnSO_4$, 1.0 g/l $CuSO_4$, 0.5 g/l $MnSO_4$,

0.23 g/l $\text{Na}_2\text{B}_4\text{O}_7$ 2.0 g/l CaCl_2 and 0.1 g/l $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, and *Micrococcus luteus* was cultured in LB medium.

3) Production of ω -hydroxynonanoic acid by transformant

[0056] First, the *pACYC*/oleic acid hydratase/alcohol dehydrogenase expression vector of Example 1-1), to which *Stenotrophomonas maltophilia*-derived oleic acid hydratase gene and *Micrococcus luteus*-derived alcohol dehydrogenase genes were inserted, was introduced into *E. coli* BL21(DE3) strain cultured in Example 1-2) to prepare a primary transformant.

[0057] Next, the primary transformant was introduced with the expression vector *pJOE-KT2440BVMO* (Biotechnol. Lett., 29:1393-1398, 2007) which was prepared to express *Pseudomonas putida*-derived BVMO gene, thereby preparing a secondary transformant capable of expressing oleic acid hydratase, alcohol dehydrogenase and BVMO.

[0058] Subsequently, while the secondary transformant was cultured in a Riesenbergs mineral medium at 30°C and 200 rpm, it was treated with IPTG and rhamnose to express oleic acid hydratase, alcohol dehydrogenase and BVMO, and reacted with 1 mM oleic acid to produce ω -hydroxynonanoic acid (FIG. 2). FIG. 2a is a graph showing the production change over time of fatty acid having an ester group introduced into the chain thereof, which was produced by using the secondary transformant, in which (●) indicates the concentration of oleic acid, (Δ) indicates the concentration of 10-hydroxystearic acid, (∇) indicates the concentration of 10-ketostearic acid, (■) indicates the concentration of fatty acid having an ester group introduced into the chain thereof, and (A) indicates the concentration of ω -hydroxynonanoic acid. FIG. 2b shows the results of GC/MS analysis of reaction products which were treated with ester hydrolase after termination of the reaction. Most of the fatty acid having an ester group introduced into the chain thereof which was prepared from oleic acid was converted into n-nonanoic acid and ω -hydroxynonanoic acid.

Example 2: Production of ω -hydroxyundec-9-enoic acid from ricinoleic acid

[0059] ω -hydroxyundec-9-enoic acid was produced from the substrate ricinoleic acid using the transformant which was prepared in Example 1.

[0060] That is, ω -hydroxyundec-9-enoic acid was produced in the same manner as in Example 1-3), except that 1 mM ricinoleic acid was added instead of oleic acid (FIG. 3). FIG. 3a is a graph showing the production change over time of fatty acid having an ester group introduced into the chain thereof, which was produced from ricinoleic acid by using the transformant, in which (Δ) indicates the concentration of ricinoleic acid, (∇) indicates the concentration of 12-ketooleic acid, (■) indicates the concentration of fatty acid having an ester group introduced into the chain thereof, and (A) indicates the concentration of ω -hydroxyundec-9-enoic acid. FIG. 3b shows the results of GC/MS analysis of reaction products which were treated with ester hydrolase after termination of the reaction. Most of the fatty acid having an ester group introduced into the chain thereof, which was prepared from ricinoleic acid, was converted into n-heptanoic acid and ω -hydroxyundec-9-enoic acid.

Example 3: Production of ω -hydroxytridec-11-enoic acid from lesquerolic acid

[0061] ω -hydroxytridec-11-enoic acid was produced from the substrate lesquerolic acid using the transformant prepared in Example 1.

[0062] That is, ω -hydroxytridec-11-enoic acid was produced in the same manner as in Example 1-3), except that 1 mM lesquerolic acid was added instead of oleic acid (FIG. 4).

[0063] FIG. 4 shows the results of GC/MS analysis of reaction products, after production of ω -hydroxytridec-11-enoic acid from lesquerolic acid using the transformant and ester hydrolase. Most of lesquerolic acid was converted into heptanoic acid and ω -hydroxytridec-11-enoic acid.

Example 4: Production of α,ω -decanedioic acid from oleic acid

[0064] *pACYC*/oleic acid hydratase/alcohol dehydrogenase expression vector prepared in Example 1 and *Pseudomonas fluorescens*-derived BVMO gene expression vector (Appl. Microbiol. Biotechnol. 73:1065-1072, 2007) were introduced into *E. coli* BL21(DE3) strain to prepare a transformant, which was used to produce α,ω -decanedioic acid (sebacic acid) from the substrate oleic acid (FIG. 5). FIG. 5a is a graph showing the production change over time of fatty acid having an ester group introduced into the chain thereof, which was produced by using the above transformant, in which (●) indicates the concentration of oleic acid, (Δ) indicates the concentration of 10-hydroxystearic acid, (∇) indicates the concentration of 10-ketostearic acid, and (■) indicates the concentration of fatty acid having an ester group introduced into the chain thereof. FIG. 5b shows the results of GC/MS analysis of reaction products which were treated with ester hydrolase after termination of the reaction. Most of the fatty acid having an ester group introduced into the chain thereof, which was prepared from oleic acid, was converted into sebacic acid and normal octanol.

Example 5: Production of α,ω -nonanedioic acid from oleic acid**1) Gene cloning**

[0065] To prepare a recombinant expression vector including ester hydrolase and alcohol dehydrogenase genes, *Pseudomonas fluorescens*-derived ester hydrolase gene and *Pseudomonas putida*-derived alcohol dehydrogenase gene were cloned.

[0066] First, ester hydrolase gene was amplified by PCR using a plasmid vector pGASTON/ester hydrolase (Agric Biol. Chem., 54:2039-2045, 1990) as a template and primers (SEQ ID NOS: 5 and 6) which were prepared by including NdeI and XhoI restriction enzyme sites.

Forward primer: 5'-gcgccatgatgagcacattgttgcaaaa-3' (SEQ ID NO: 5)

Reverse primer: 5'-gcgccctcgagtcagtggtgatggtgatgactccgccgacctt-3' (SEQ ID NO: 6)

[0067] The amplified PCR product was digested with restriction enzymes NdeI and XhoI, and inserted into a plasmid vector pCOLAduet-1 (manufactured by Novagen) to prepare a pCOLAduet-1/ester hydrolase expression vector.

[0068] Further, alcohol dehydrogenase was amplified by PCR using DNA sequence (J. Biotechnol. 262:17712-17718, 1987) of *Pseudomonas putida*-derived alcohol dehydrogenase as a template and primers (SEQ ID NOS: 7 and 8) which were prepared by including BamHI and NotI restriction enzyme sites.

Forward primer: 5'-gcgcgatccgatgtacgactatataatcggt-3' (SEQ ID NO: 7)

Reverse primer: 5'-gcgcgccgcccgttagtggtgatggtgatgcatgcagacagctat-3' (SEQ ID NO: 8)

[0069] The amplified PCR product was digested with restriction enzymes BamI and NotI, and inserted into the prepared pCOLAduet-1/ester hydrolase expression vector to prepare a pCOLAduet-1/ester hydrolase/alcohol dehydrogenase expression vector.

2) Production of α,ω -nonanedioic acid by transformant

[0070] First, the pCOLAduet-1/ester hydrolase/alcohol dehydrogenase expression vector of Example 5-1), to which *Pseudomonas fluorescens*-derived ester hydrolase and *Pseudomonas putida*-derived alcohol dehydrogenase genes were inserted, was introduced into *E. coli* BL21(DE3) strain cultured in Example 1-2) to prepare a transformant.

[0071] α,ω -nonanedioic acid (azelaic acid) was produced from the substrate oleic acid using the transformant prepared in Example 1 and the transformant prepared in Example 5-1). Fatty acid having an ester group introduced into the chain thereof was produced from oleic acid using the transformant prepared in Example 1, and azelaic acid was produced from the fatty acid having an ester group introduced into the chain thereof using the transformant prepared in Example 5-1) (FIG. 6). FIG. 6 is a graph showing the production change over time of azelaic acid produced, in which (■) indicates the concentration of fatty acid having an ester group introduced into the chain thereof, (A) indicates the concentration of ω -hydroxynonanoic acid, (◇) indicates the concentration of 9-oxononanoic acid, and (◆) indicates the concentration of azelaic acid.

Example 6: Production of α,ω -undec-2-enedioic acid from ricinoleic acid

[0072] *E. coli* BL21(DE3) strain was introduced with the pACYC/oleic acid hydratase/alcohol dehydrogenase expression vector prepared in Example 1, *Pseudomonas putida*-derived BVMO gene expression vector, and the pCOLAduet-1/ester hydrolase/alcohol dehydrogenase expression vector prepared in Example 5-1) to prepare a transformant.

[0073] α,ω -undec-2-enedioic acid was produced from ricinoleic acid ($C_{18}H_{34}O_3$) using the transformant (FIG. 7). FIG. 7 is a graph showing the production change over time of α,ω -undec-2-enedioic acid which was produced from ricinoleic acid using the above transformant, in which (Δ) indicates the concentration of ricinoleic acid, (▽) indicates the concentration of 12-ketooleic acid, (■) indicates the concentration of fatty acid having an ester group introduced into the chain thereof, (A) indicates the concentration of ω -hydroxyundec-9-enoic acid, (◇) indicates the concentration of 9-oxoundec-9-enoic acid, and (◆) indicates the concentration of α,ω -undec-2-enedioic acid.

[0074] Meanwhile, heptanoic acid and α,ω -tridec-2-enedioic acid could be produced from lesquerolic acid by using the above transformant.

Example 7: Production of α -aminononanoic acid from oleic acid**1) Preparation of transformant**

[0075] First, the pCOLAduet-1/ester hydrolase/alcohol dehydrogenase expression vector of Example 5-1), to which *Pseudomonas fluorescens*-derived ester hydrolase and *Pseudomonas putida*-derived alcohol dehydrogenase genes were inserted, was introduced into *E. coli* to prepare a primary transformant.

[0076] Next, *Silicibacter*-derived aminotransferase expression vector (ChemCatChem, 5:154-157, 2013) was introduced into the primary transformant to prepare a secondary transformant capable of expressing ester hydrolase, alcohol dehydrogenase and aminotransferase.

2) Production of α -aminononanoic acid by transformant

[0077] α -aminononanoic acid was produced from the substrate oleic acid using the transformant prepared in Example 1 and the secondary transformant prepared in Example 6-1). Fatty acid having an ester group introduced into the chain thereof was produced from oleic acid using the transformant prepared in Example 1, and α -aminononanoic acid was produced from the fatty acid having an ester group introduced into the chain thereof using the transformant prepared in Example 6-1) (FIG. 8). FIG. 8 is a graph showing the production change over time of α -aminononanoic acid produced, in which (■) indicates the concentration of fatty acid having an ester group introduced into the chain thereof, (A) indicates the concentration of ω -hydroxy nonanoic acid, (◇) indicates the concentration of 9-oxononanoic acid, and (◆) indicates the concentration of α -aminononanoic acid.

[0078] Meanwhile, heptanoic acid and α -aminoundec-9-enoic acid could be produced from ricinoleic acid by using the transformant.

<110> Ewha University-Industry Collaboration Foundation

<120> Bioprocess for preparing medium chain ω -hydroxyfatty acids, a, ω -dicarboxylic acids, and ω -aminofatty acids from long chain fatty acids

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Claims

1. A method for producing degradation products from long-chain fatty acids, comprising

- (a) reacting the transformant with a long-chain fatty acid to obtain reactants; and
- (b) recovering degradation products from the reactants.

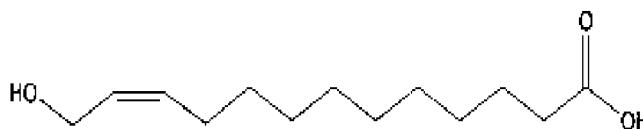
wherein the transformant is introduced with Baeyer-Villiger monooxygenase (BVMO)-encoding gene and has ability to produce degradation products from long-chain fatty acids,

wherein the long-chain fatty acid is C16 to C20 straight-chain fatty acid and

wherein the BVMO-encoding gene encodes an amino acid sequence of BVMO derived from *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas veronii*, *Rhodococcus jostii* or *Pseudomonas sp.* strain HI-70, or a sequence having 80% or more homology thereto which maintains BVMO activity.

2. The method according to claim 1, wherein a hydratase- or lipoxygenase-encoding gene, an alcohol dehydrogenase-encoding gene, an ester hydrolase-encoding gene, an aminotransferase-encoding gene or a combination thereof is further introduced into the transformant.
- 5 3. The method according to claim 2, wherein the hydratase-encoding gene, the alcohol dehydrogenase-encoding gene, the BVMO-encoding gene and the ester hydrolase-encoding gene are introduced into the transformant to produce medium-chain (C5-C14) ω -hydroxy fatty acids, or α,ω -dicarboxylic acids and alcohols from the long-chain fatty acids.
- 10 4. The method according to claim 3, wherein ω -hydroxynonanoic acid (C9) is produced as the medium-chain ω -hydroxy fatty acid, or α,ω -decanedioic acid (C10) is produced as the α,ω -dicarboxylic acid and octanol (C8) is produced as the alcohol, when oleic acid (C18) is used as the long-chain fatty acid.
- 15 5. The method according to claim 2, wherein the alcohol dehydrogenase-encoding gene, the BVMO-encoding gene and the ester hydrolase-encoding gene are introduced into transformant to produce medium-chain ω -hydroxy fatty acid from hydroxy long-chain fatty acids.
- 20 6. The method according to claim 5, wherein ω -hydroxyundec-9-enoic acid (C11) is produced as the medium-chain ω -hydroxy fatty acid, when ricinoleic acid (C18) is used as the hydroxy long-chain fatty acid, and ω -hydroxytridec-11-enoic acid (C13) is produced as the medium-chain ω -hydroxy fatty acid, when lesquerolic acid (C20) is used as the hydroxy long-chain fatty acid.
- 25 7. The method according to claim 2, wherein the hydratase-encoding gene, two different alcohol dehydrogenases-encoding genes, the BVMO-encoding gene and the ester hydrolase-encoding gene are introduced into the transformant to produce α,ω -dicarboxylic acids from long-chain fatty acids.
- 30 8. The method according to claim 7, wherein α,ω -nonanedioic acid (C9) is produced as the α,ω -dicarboxylic acid, when oleic acid (C18) is used as the long-chain fatty acid.
- 35 9. The method according to claim 2, wherein the hydratase-encoding gene, two different alcohol dehydrogenases-encoding genes, the BVMO-encoding gene, the ester hydrolase-encoding gene, and the aminotransferase-encoding gene are introduced into the transformant to produce ω -amino fatty acids from long-chain fatty acids.
10. The method according to claim 9, wherein ω -aminononanoic acid (C9) is produced as the ω -amino fatty acid, when oleic acid (C18) is used as the long-chain fatty acid.
- 40 11. The method according to claim 1, wherein the long-chain fatty acid is oleic acid, ricinoleic acid, 12-hydroxystearic acid, linoleic acid, palmitoleic acid, lesquerolic acid or a combination thereof.
12. The method according to claim 1, wherein the degradation products are medium-chain ω -hydroxy fatty acids, α,ω -dicarboxylic acids, ω -amino fatty acids, alcohols or combinations thereof.
- 45 13. The method according to claim 1, wherein the degradation products are ω -hydroxynonanoic acid, ω -hydroxyundec-9-enoic acid, ω -hydroxytridec-11-enoic acid, α,ω -nonanedioic acid (azelaic acid), α,ω -decanedioic acid (sebacic acid), ω -aminononanoic acid, heptanoic acid, nonanoic acid, ω -hydroxyundecanoic acid, ω -hydroxytridecenoic acid, α,ω -undec-2-enedioic acid cis-2-undecene-1,11-dioic acid), α -aminoundec-9-enoic acid, α,ω -tridec-2-enedioic acid, α -aminononanoic acid, normal octanol or a combination thereof.
- 50 14. ω -hydroxytridec-11-enoic acid represented by the following Chemical Formula 1.

[Chemical Formula 1]



15. A method for producing a fatty acid derivative having an ester group which is introduced into the chain thereof from

a keto fatty acid using Baeyer-Villiger monooxygenase (BVMO).

Patentansprüche

1. Verfahren zur Herstellung von Abbauprodukten von langkettigen Fettsäuren, umfassend

- (a) Reagieren des Transformanten mit einer langkettigen Fettsäure, um Reaktanten zu erhalten; und
- (b) Wiedergewinnen von Abbauprodukten von den Reaktanten,

wobei der Transformant mit Baeyer-Villiger-Monooxygenase (BVMO)-codierendem Gen eingeführt wird und die Fähigkeit aufweist, Abbauprodukte von langkettigen Fettsäuren herzustellen, wobei die langkettige Fettsäure C16 bis C20 geradkettige Fettsäure ist, und wobei das BVMO-codierende Gen eine Aminosäuresequenz von BVMO codiert, abgeleitet von *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas veronii*, *Rhodococcus jostii* oder *Pseudomonas sp.* Stamm HI-70, oder eine Sequenz, aufweisend 80 % oder mehr Homologie damit, die BVMO-Aktivität beibehält.

2. Verfahren nach Anspruch 1, wobei ein Hydratase- oder Lipoxygenase-codierendes Gen, ein Alkoholdehydrogenase-codierendes Gen, ein Esterhydrolase-codierendes Gen, ein Aminotransferasecodierendes Gen oder einer Kombination davon weiter in den Transformanten eingeführt wird.

3. Verfahren nach Anspruch 2, wobei das Hydratasecodierende Gen, das Alkoholdehydrogenase-codierende Gen, das BVMO-codierende Gen und das Esterhydrolase-codierende Gen in den Transformanten eingeführt werden, um mittelkettige (C5-C14) ω -Hydroxyfettsäuren oder α,ω -Dicarbonsäuren und Alkohole aus den langkettigen Fettsäuren herzustellen.

4. Verfahren nach Anspruch 3, wobei ω -Hydroxynonansäure (C9) als die mittelkettige ω -Hydroxyfettsäure hergestellt wird, oder α,ω -Decandionsäure (C10) als die α,ω -Dicarbonsäure hergestellt wird und Octanol (C8) als der Alkohol hergestellt wird, wenn Ölsäure (C18) als die langkettige Fettsäure verwendet wird.

5. Verfahren nach Anspruch 2, wobei das Alkoholdehydrogenase-codierende Gen, das BVMO-codierende Gen und das Esterhydrolase-codierende Gen in den Transformanten eingeführt werden, um mittelkettige ω -Hydroxyfettsäure aus langkettigen Hydroxyfettsäuren herzustellen.

6. Verfahren nach Anspruch 5, wobei ω -Hydroxyundec-9-ensäure (C11) als die mittelkettige ω -Hydroxyfettsäure hergestellt wird, wenn Ölsäure (C18) als die langkettige Hydroxy-Fettsäure verwendet wird und ω -Hydroxytridec-11-ensäure (C13) als die mittelkettige ω -Hydroxyfettsäure hergestellt wird, wenn Lesquerolsäure (C20) als die langkettige Hydroxy-Fettsäure verwendet wird.

7. Verfahren nach Anspruch 2, wobei das Hydratasecodierende Gen, zwei verschiedene Alkoholdehydrogenasen-codierende Gene, das BVMO-codierende Gen und das Esterhydrolase-codierende Gen in den Transformanten eingeführt werden, um α,ω -Dicarbonsäuren aus langkettigen Fettsäuren herzustellen.

8. Verfahren nach Anspruch 7, wobei α,ω -Nonandisäure (C9) als die α,ω -Dicarbonsäure hergestellt wird, wenn Ölsäure (C18) als die langkettige Fettsäure verwendet wird.

9. Verfahren nach Anspruch 2, wobei das Hydratasecodierende Gen, zwei verschiedene Alkoholdehydrogenasen-codierende Gene, das BVMO-codierende Gen, das Esterhydrolase-codierende Gen und das Aminotransferase-codierende Gen in den Transformanten eingeführt werden, um ω -Aminofettsäuren aus langkettigen Fettsäuren herzustellen.

10. Verfahren nach Anspruch 9, wobei ω -Aminononansäure (C9) als die ω -Aminofettsäure hergestellt wird, wenn Ölsäure (C18) als die langkettige Fettsäure verwendet wird.

11. Verfahren nach Anspruch 1, wobei die langkettige Fettsäure Ölsäure, Ricinolsäure, 12-Hydroxystearinsäure, Linolsäure, Palmitoleinsäure, Lesquerolsäure oder einer Kombination davon ist.

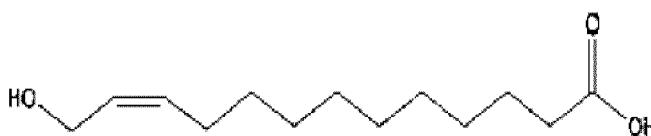
12. Verfahren nach Anspruch 1, wobei die Abbauprodukte mittelkettige ω -Hydroxyfettsäuren, α,ω -Dicarbonsäuren, ω -

Aminofettsäuren, Alkohole oder Kombinationen daraus sind.

13. Verfahren nach Anspruch 1, wobei die Abbauprodukte ω -Hydroxynonansäure, ω -Hydroxundec-9-ensäure, ω -Hydroxytridec-11-ensäure, α,ω -Nonandisäure (Azelaensäure), α,ω -Decandionsäure (Sebacinsäure), ω -Aminononansäure, Heptansäure, Nonansäure, ω -Hydroxyundecansäure, ω -Hydroxytridecensäure, α,ω -Undec-2-endisäure, cis-2-Undecen-1,11-disäure, α -Aminoundec-9-ensäure, α,ω -Tridec-2-endisäure, α -Aminononansäure, normales Octanol oder eine Kombination daraus sind.

14. ω -Hydroxytridec-11-ensäure, dargestellt durch die folgende chemische Formel 1.

[chemische Formel 1]



15. Verfahren zum Herstellen eines Fettsäurederivats, aufweisend eine Estergruppe, die in die Kette davon von einer Ketofettsäure unter Verwendung von Baeyer-Villiger-Monooxygenase (BVMO) eingeführt wird.

Revendications

1. Méthode de production de produits de dégradation à partir d'acides gras à longue chaîne, comprenant

- (a) la mise en réaction du transformant avec un acide gras à chaîne longue pour obtenir des réactifs ; et
(b) la récupération de produits de dégradation à partir des réactifs,

dans laquelle le transformant est introduit avec un gène codant pour une monooxygénase Baeyer-Villiger (BVMO) et a la capacité de produire des produits de réaction à partir d'acides gras à chaîne longue, dans laquelle l'acide gras à chaîne longue est l'acide gras à chaîne droite en C16 à C20 et dans laquelle le gène codant pour BVMO code pour une séquence d'acides aminés de BVMO dérivée de *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas veronii*, *Rhodococcus jostii* ou *Pseudomonas sp.* souche HI-70, ou une séquence ayant une homologie de 80 % ou plus avec celle-ci qui conserve l'activité de BVMO.

2. Méthode selon la revendication 1, dans laquelle un gène codant pour une hydratase ou lipoxygénase, un gène codant pour un alcool déshydrogénase, un gène codant pour une ester hydrolase, un gène codant pour une aminotransférase ou une combinaison de ceux-ci est en outre introduit dans le transformant.

3. Méthode selon la revendication 2, dans laquelle le gène codant pour une hydratase, le gène codant pour un alcool déshydrogénase, le gène codant pour BVMO et le gène codant pour une ester hydrolase sont introduits dans le transformant pour produire des acides gras ω -hydroxy à chaîne moyenne (C5 à C14), ou des acides α,ω -dicarboxyliques et des alcools à partir des acides gras à chaîne longue.

4. Méthode selon la revendication 3, dans laquelle un acide ω -hydroxynonanoïque (C9) est produit en tant qu'acide gras ω -hydroxy à chaîne moyenne, ou un acide α,ω -décanedioïque (C10) est produit en tant que l'acide α,ω -dicarboxylique et l'octanol (C8) est produit en tant qu'alcool, lorsque l'acide oléique (C18) est utilisé en tant qu'acide gras à chaîne longue.

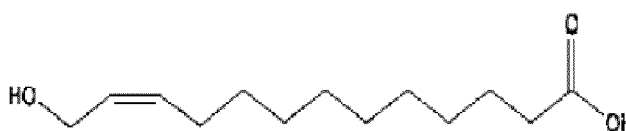
5. Méthode selon la revendication 2, dans laquelle le gène codant pour un alcool déshydrogénase, le gène codant pour BVMO, et le gène codant pour une ester hydrolase sont introduits dans le transformant pour produire un acide gras ω -hydroxy à chaîne moyenne à partir d'acides gras hydroxy à chaîne longue.

6. Méthode selon la revendication 5, dans laquelle l'acide ω -hydroxyundéc-9-énoïque (C11) est produit en tant qu'acide gras ω -hydroxy à chaîne moyenne, lorsque l'acide ricinoléique (C18) est utilisé en tant qu'acide gras hydroxy à

chaîne longue, et l'acide ω -hydroxytridéc-11-énoïque (C13) est produit en tant qu'acide gras ω -hydroxy à chaîne moyenne, lorsque l'acide lesquérolïque (C20) est utilisé en tant qu'acide gras hydroxy à chaîne longue.

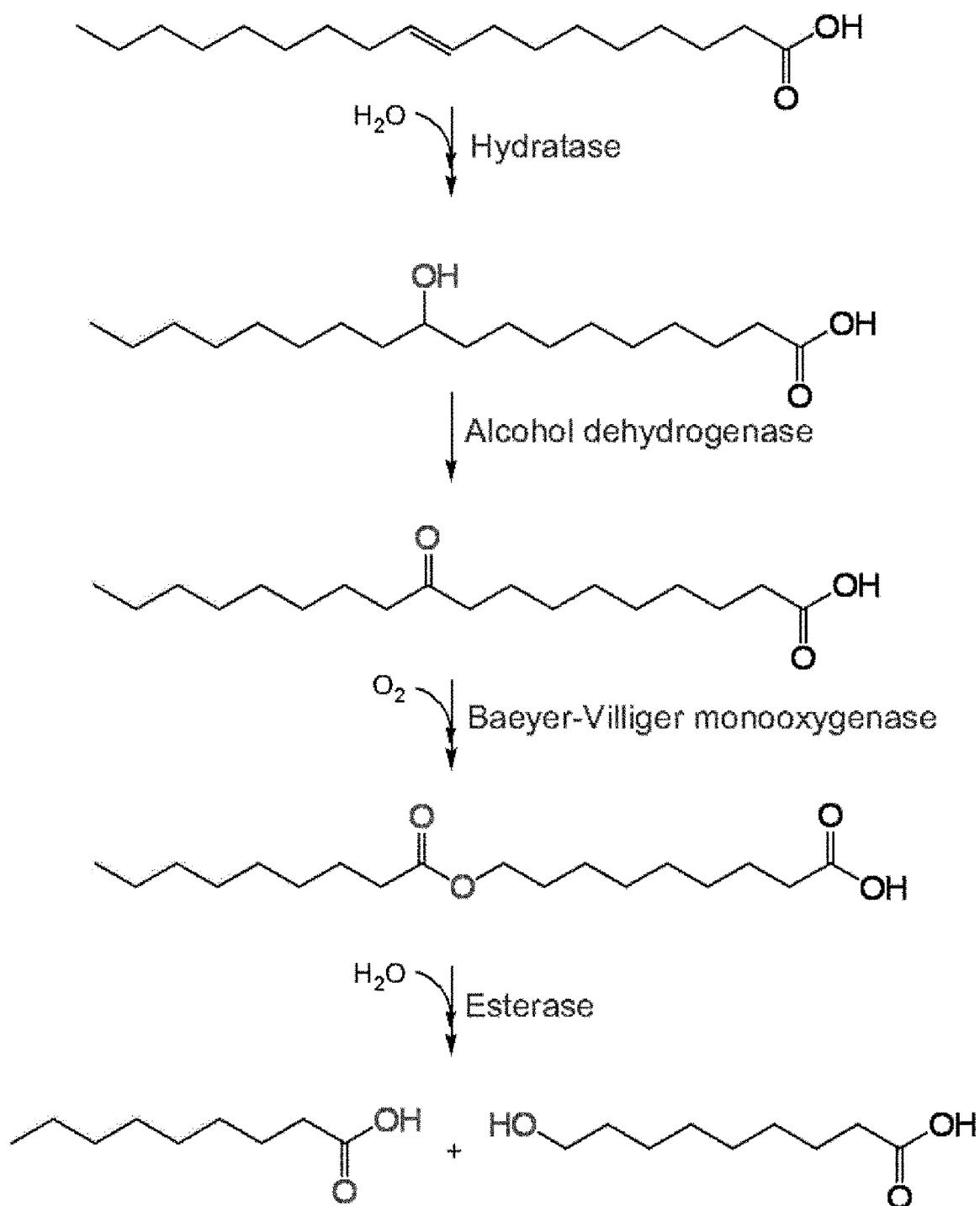
7. Méthode selon la revendication 2, dans laquelle le gène codant pour une hydratase, deux gènes différents codant pour des alcools déshydrogénases, le gène codant pour BVMO, et le gène codant pour une ester hydrolase sont introduits dans le transformant pour produire des acides α,ω -dicarboxyliques à partir d'acides gras à chaîne longue.
8. Méthode selon la revendication 7, dans laquelle l'acide α,ω -nonanedioïque (C9) est produit en tant qu'acide α,ω -dicarboxylique, lorsque l'acide oléique (C18) est utilisé en tant qu'acide gras à chaîne longue.
9. Méthode selon la revendication 2, dans laquelle le gène codant pour une hydratase, deux gènes différents codant pour des alcools déshydrogénases, le gène codant pour BVMO, le gène codant pour une ester hydrolase et le gène codant pour une aminotransférase sont introduits dans le transformant pour produire des acides gras ω -amino à partir d'acides gras à chaîne longue.
10. Méthode selon la revendication 9, dans laquelle l'acide ω -aminononanoïque (C9) est produit en tant qu'acide gras ω -amino, lorsque l'acide oléique (C18) est utilisé en tant qu'acide gras à chaîne longue.
11. Méthode selon la revendication 1, dans laquelle l'acide gras à chaîne longue est l'acide oléique, l'acide ricinoléique, l'acide 12-hydroxystéarique, l'acide linoléique, l'acide palmitoléique, l'acide lesquérolïque ou une combinaison de ceux-ci.
12. Méthode selon la revendication 1, dans laquelle les produits de dégradation sont des acides gras ω -hydroxy à chaîne moyenne, des acides α,ω -dicarboxyliques, des acides gras ω -amino, des alcools ou des combinaisons de ceux-ci.
13. Méthode selon la revendication 1, dans laquelle les produits de dégradation sont l'acide ω -hydroxynonanoïque, l'acide ω -hydroxyundéc-9-énoïque, l'acide ω -hydroxytridéc-11-énoïque, l'acide α,ω -nonanedioïque (acide azélaïque) l'acide α,ω -décanedioïque (acide sébacique), l'acide ω -aminononanoïque, l'acide heptanoïque, l'acide nonanoïque, l'acide ω -hydroxyundécanoïque, l'acide ω -hydroxytridécénoïque, l'acide α,ω -undéc-2-ènedioïque, l'acide cis-2-undécène-1,11-dioïque, l'acide α -aminoundéc-9-énoïque, l'acide α,ω -tridéc-2-ènedioïque, l'acide α -aminononanoïque, l'octanol normal ou une combinaison de ceux-ci.
14. Acide ω -hydroxytridéc-11-énoïque représenté par la formule chimique 1 suivante.

[Formule chimique 1]

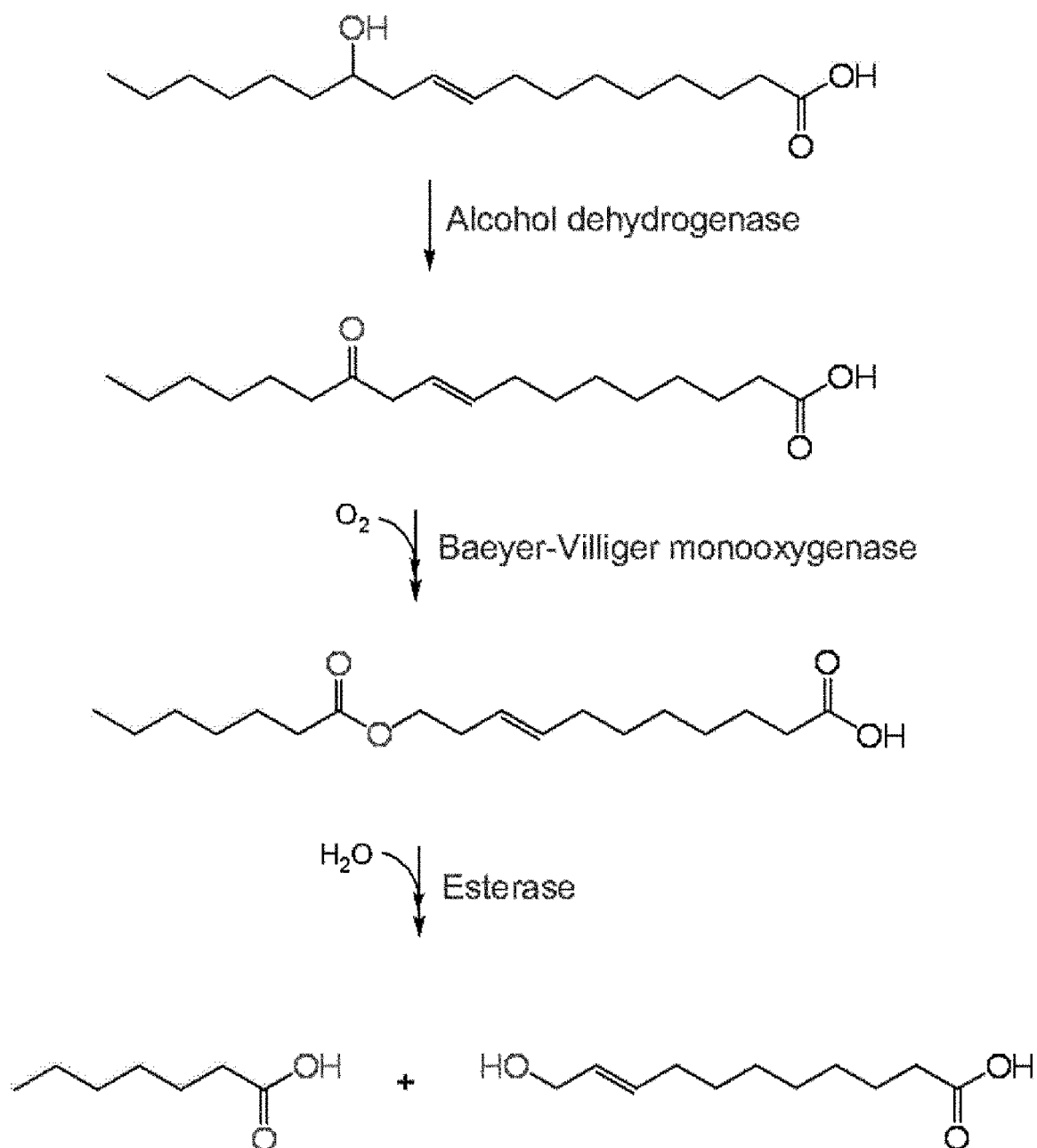


15. Méthode de production d'un dérivé d'acide gras ayant un groupe ester qui est introduit dans sa chaîne à partir d'un céto-acide gras à l'aide d'une monooxygénase Baeyer-Villiger (BVMO).

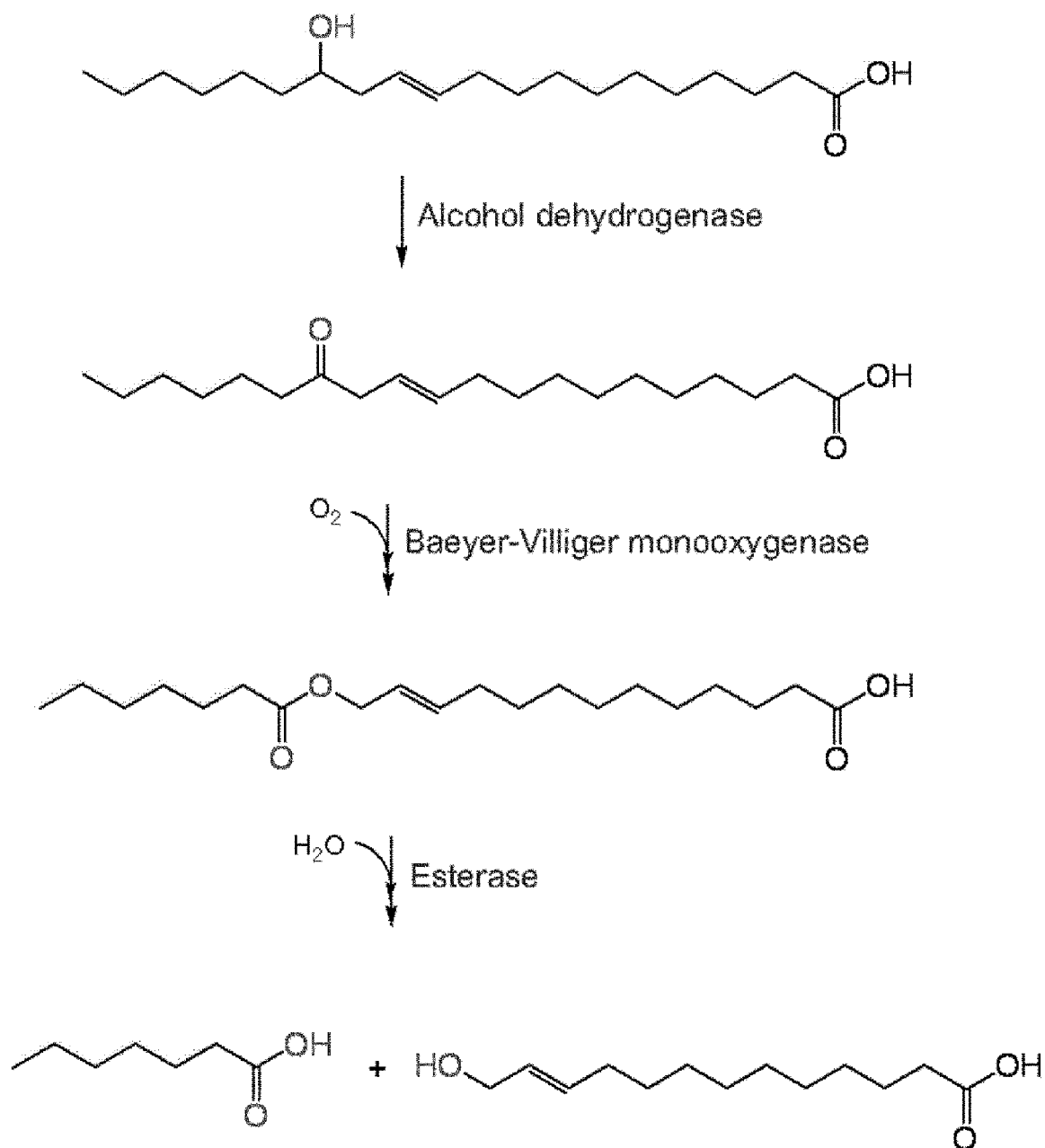
[FIG. 1a]



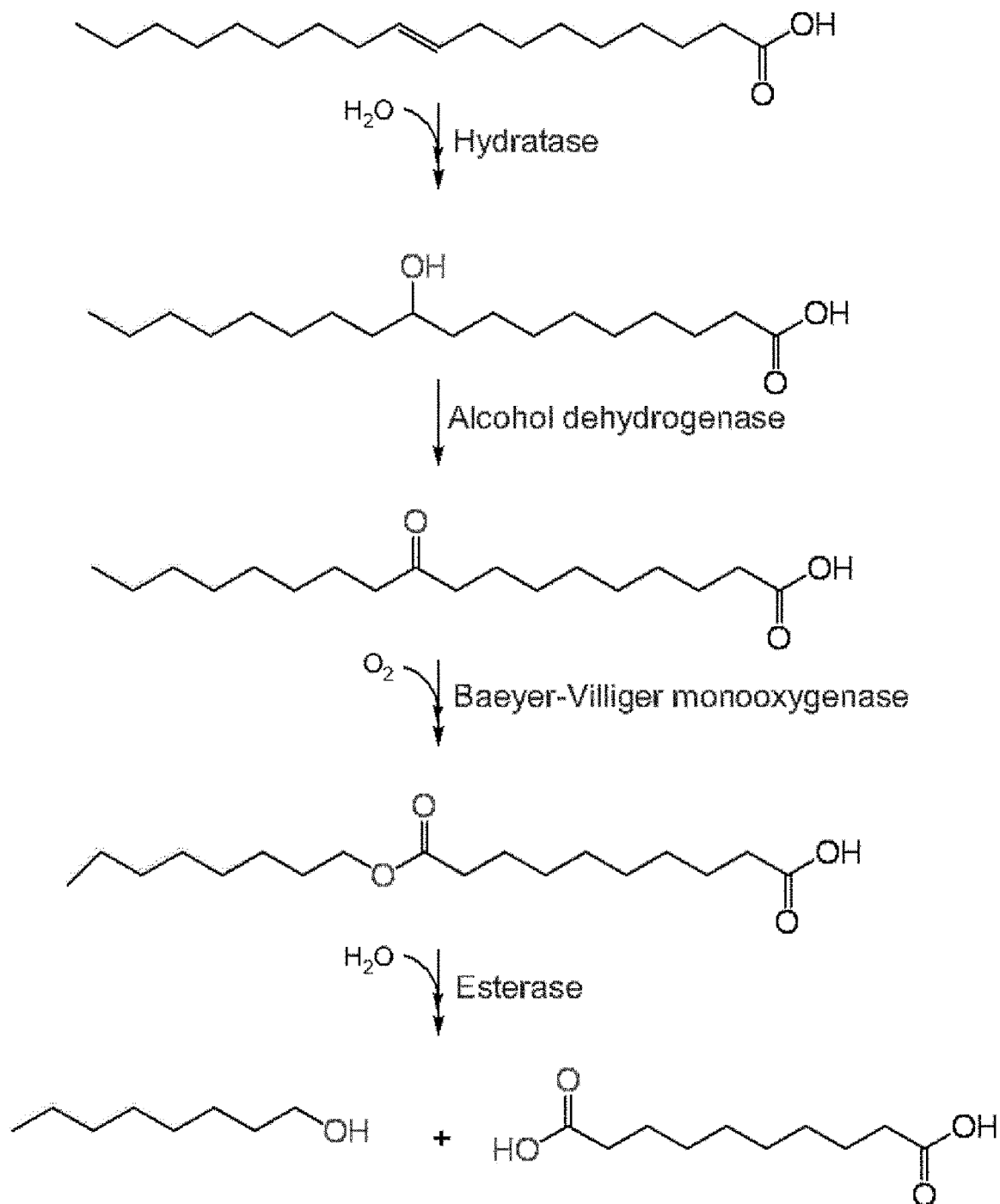
[FIG. 1b]



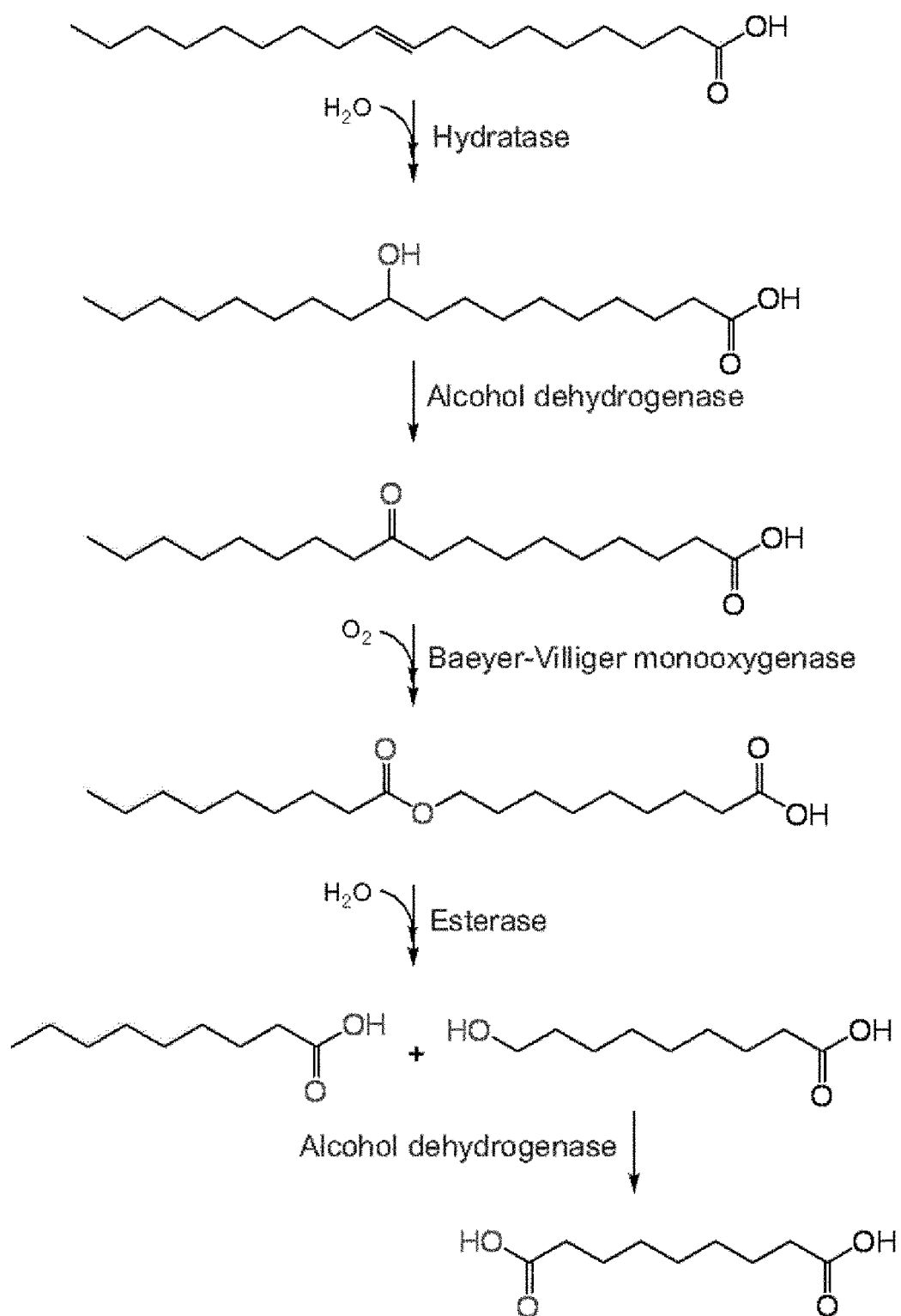
[FIG. 1c]



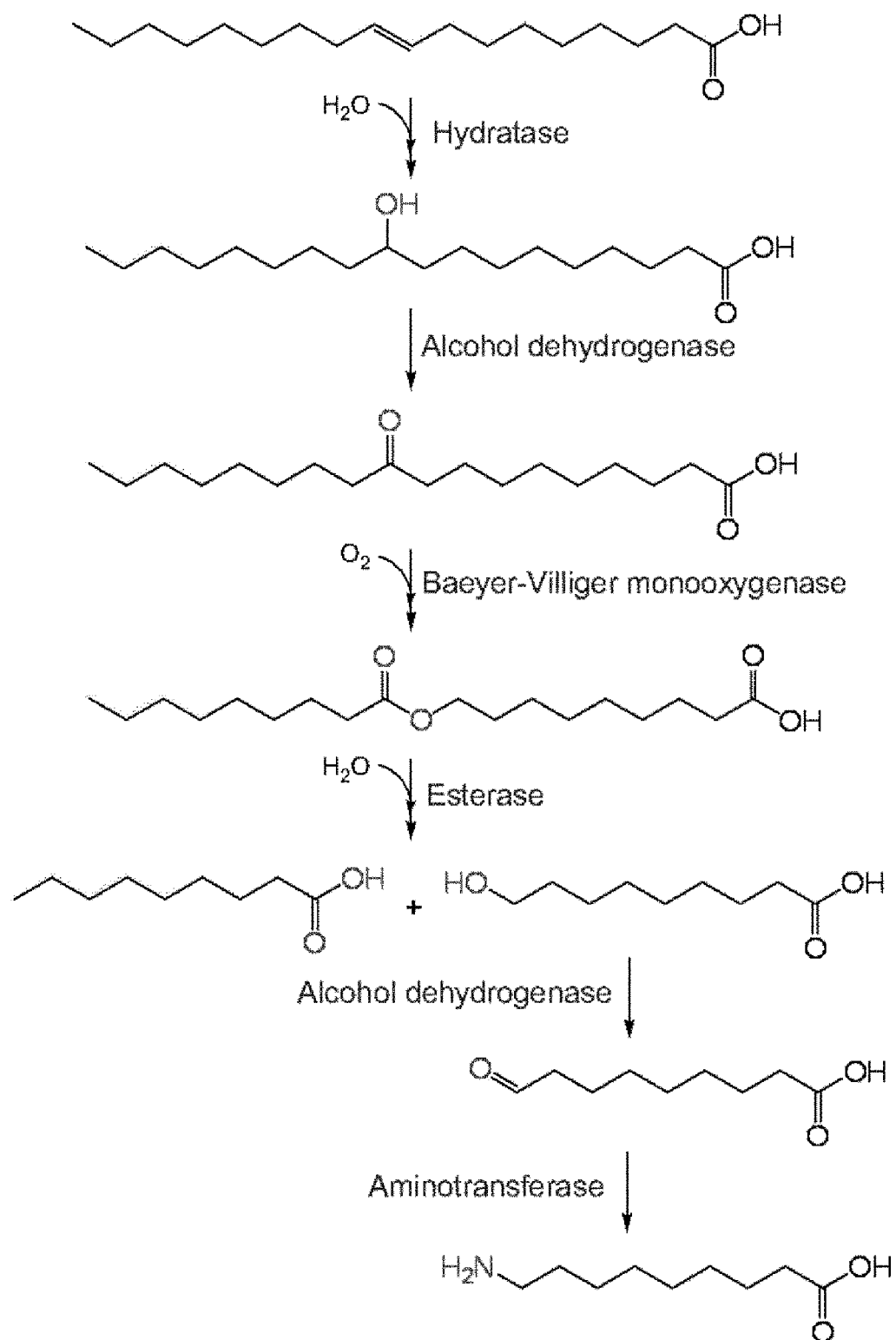
[FIG. 1d]



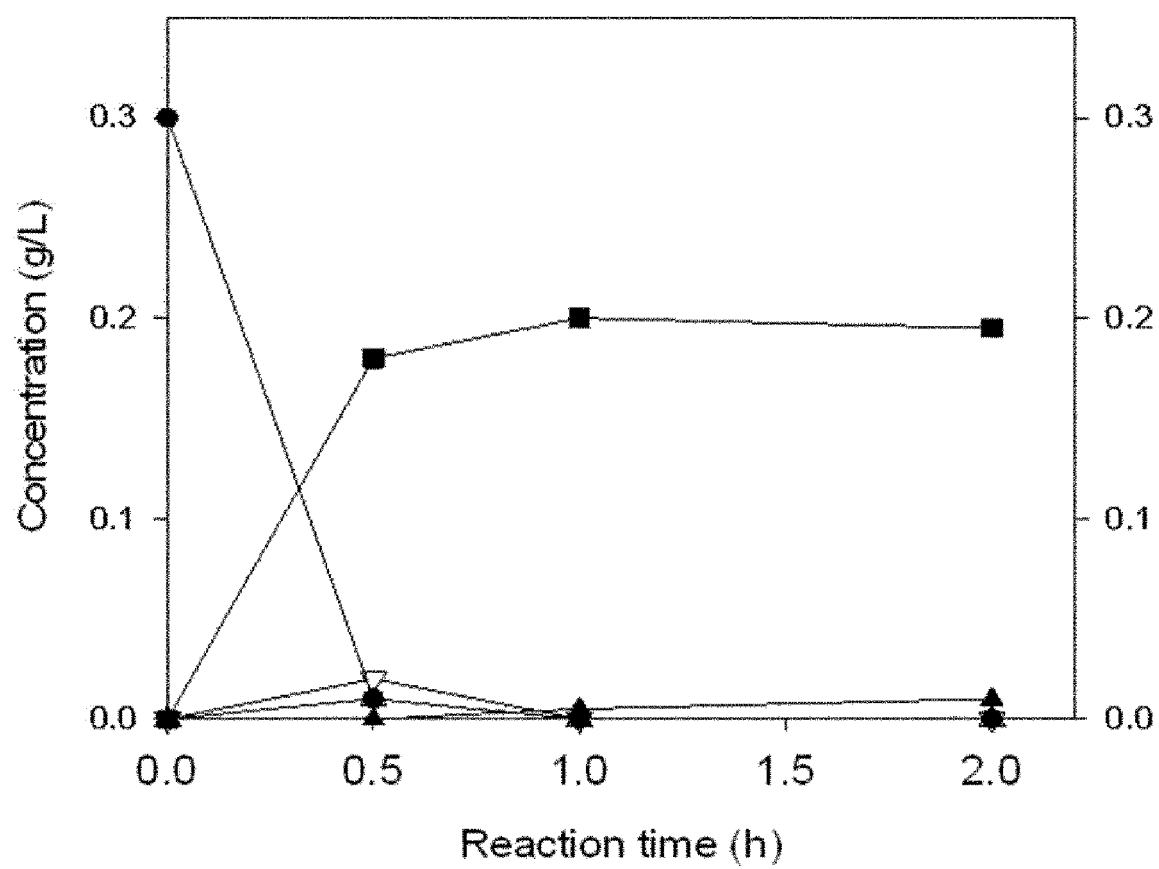
[FIG. 1e]



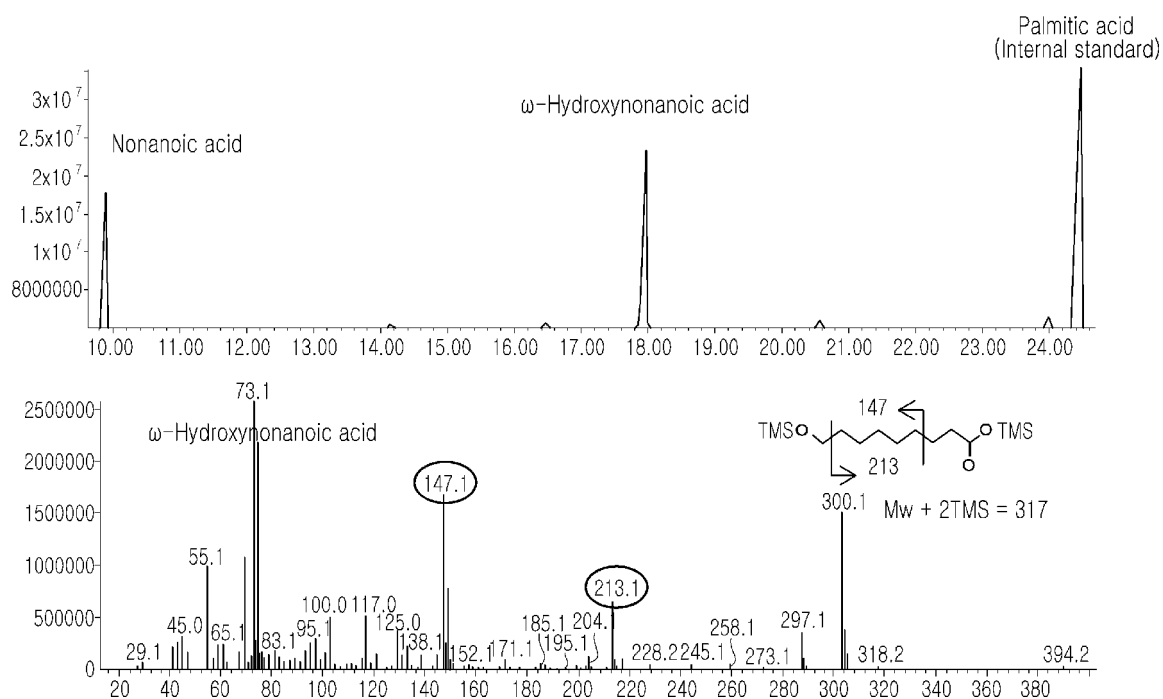
[FIG. 1f]



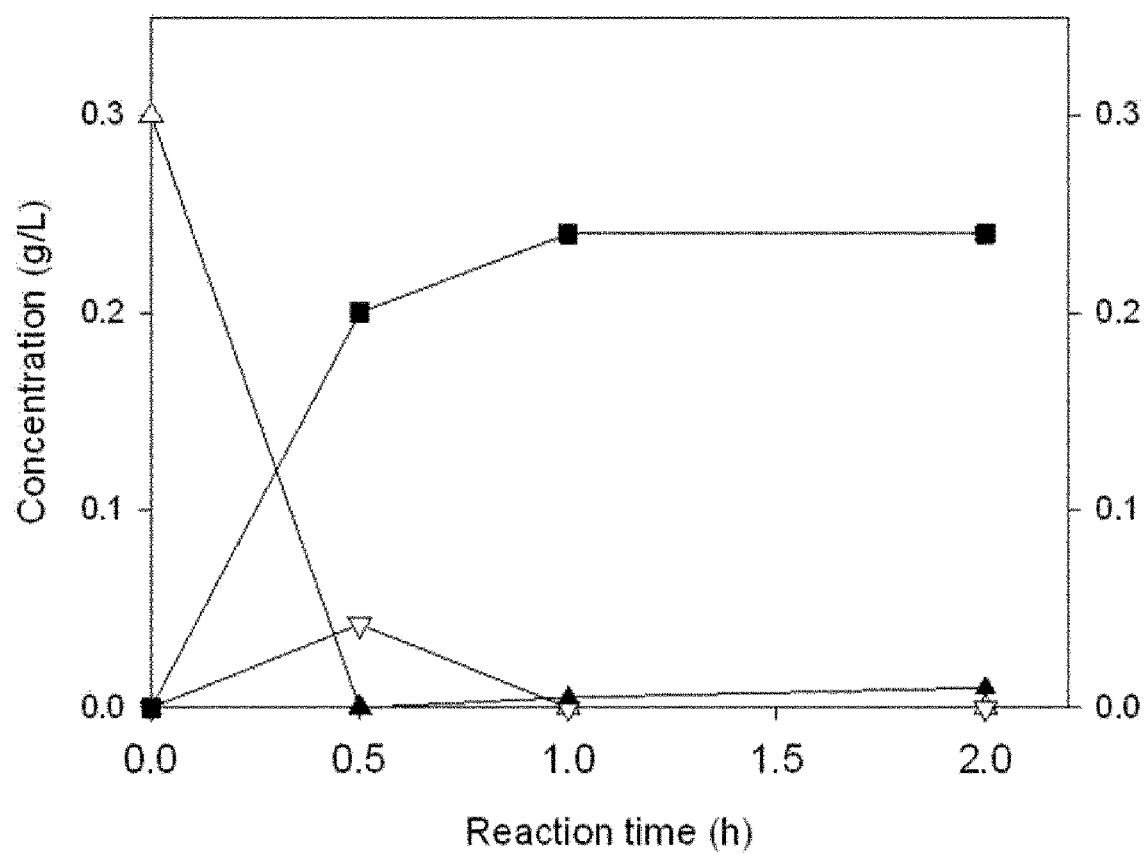
[FIG. 2a]



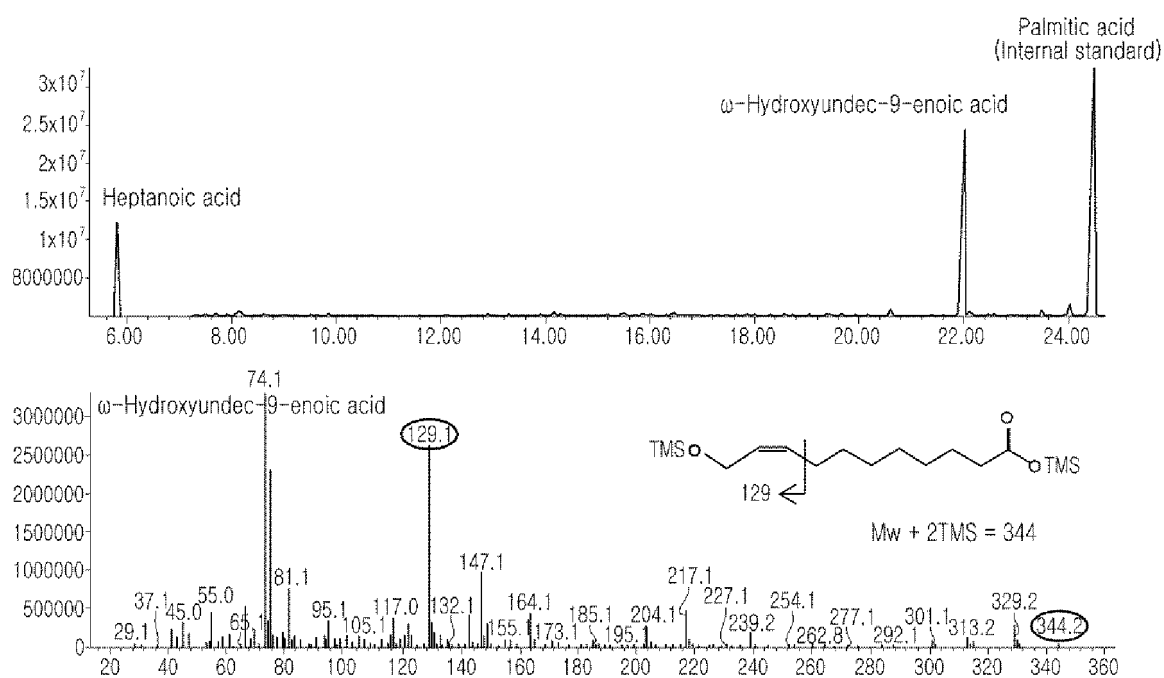
[FIG. 2b]



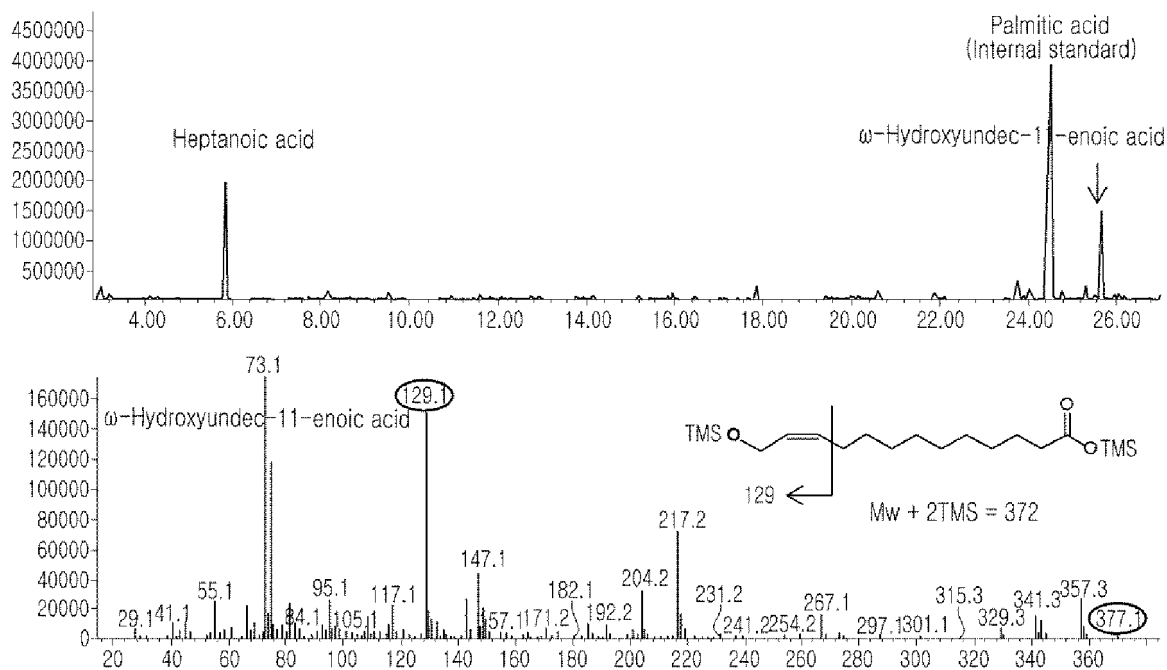
[FIG. 3a]



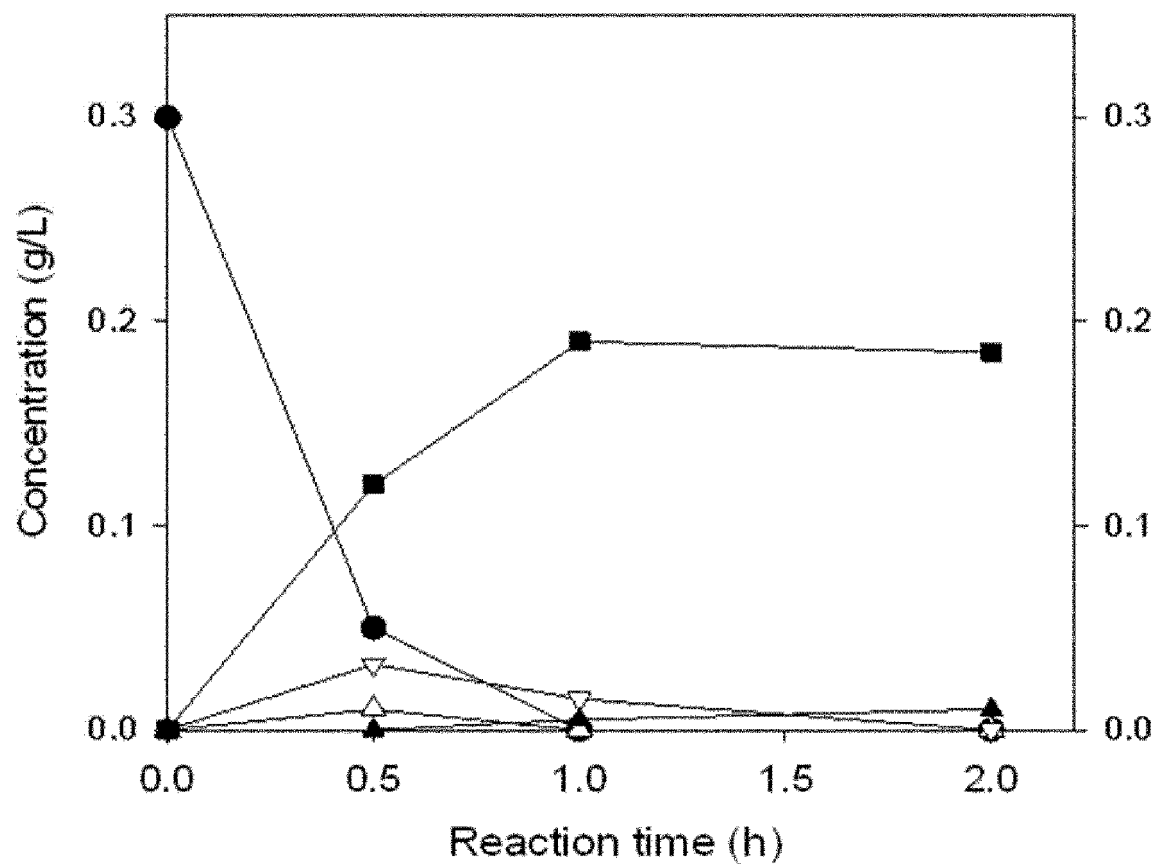
[FIG. 3b]



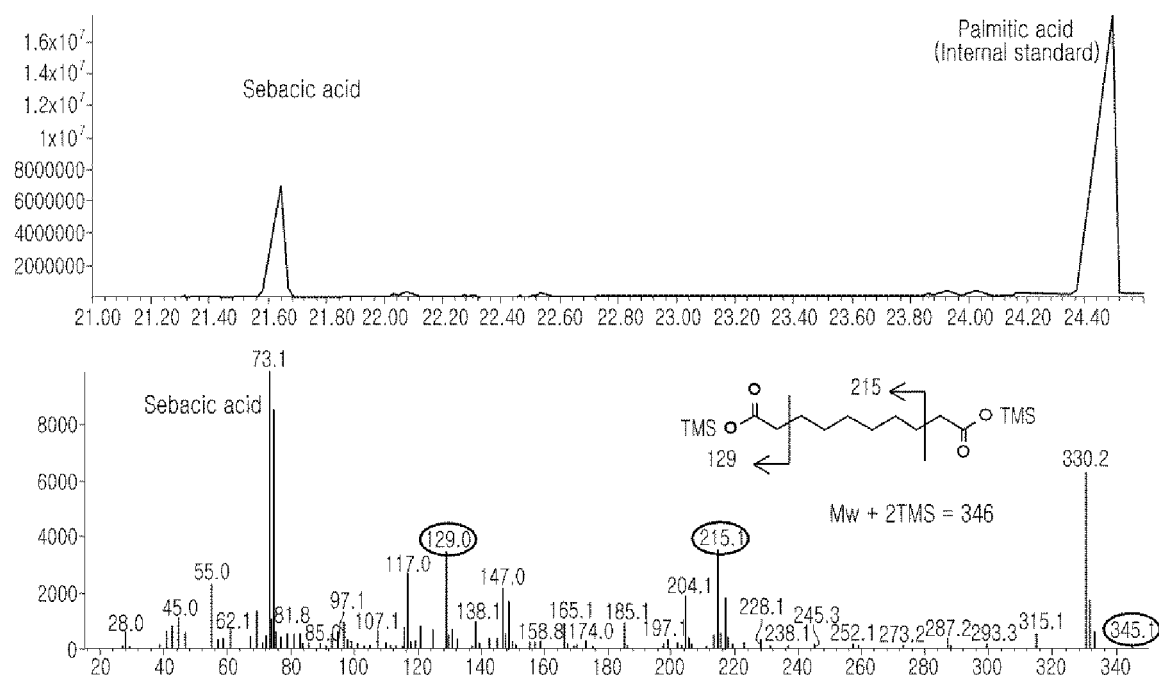
[FIG. 4]



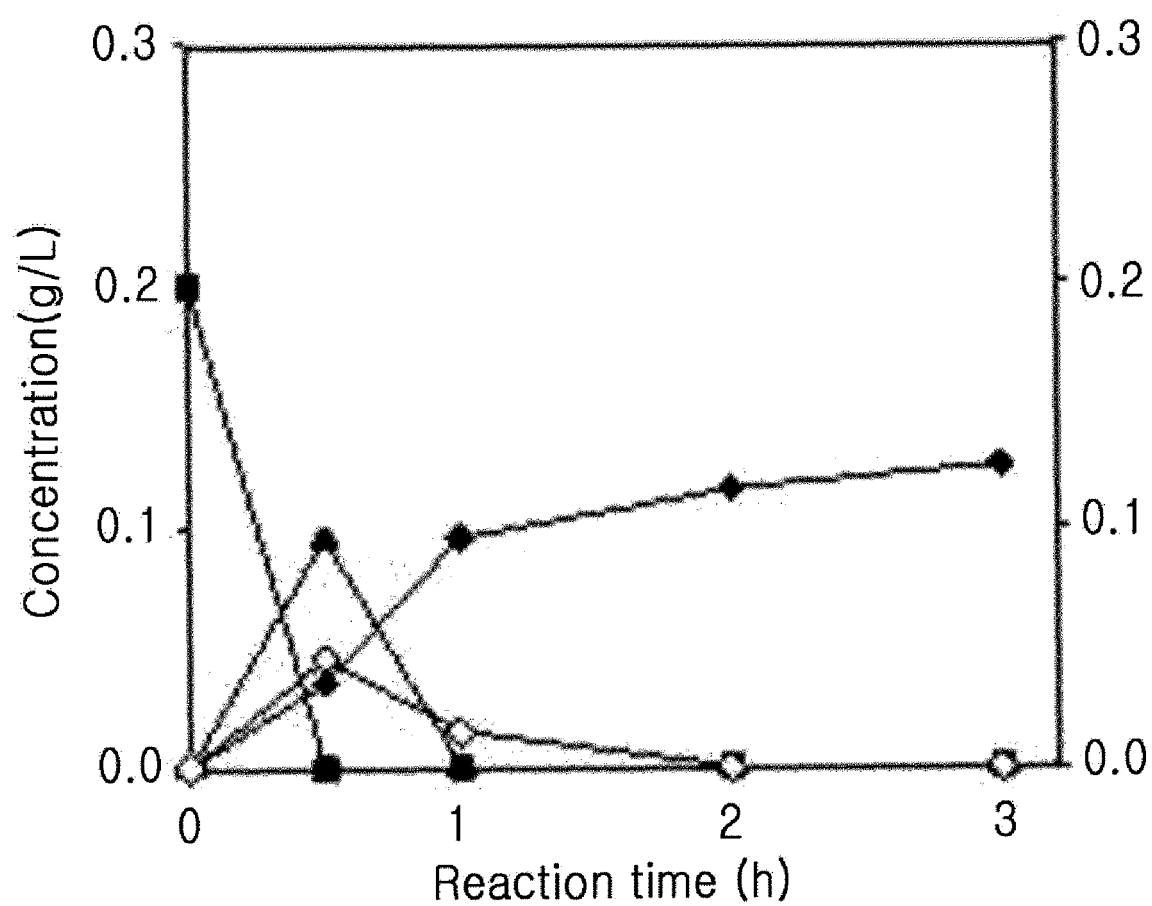
[FIG. 5a]



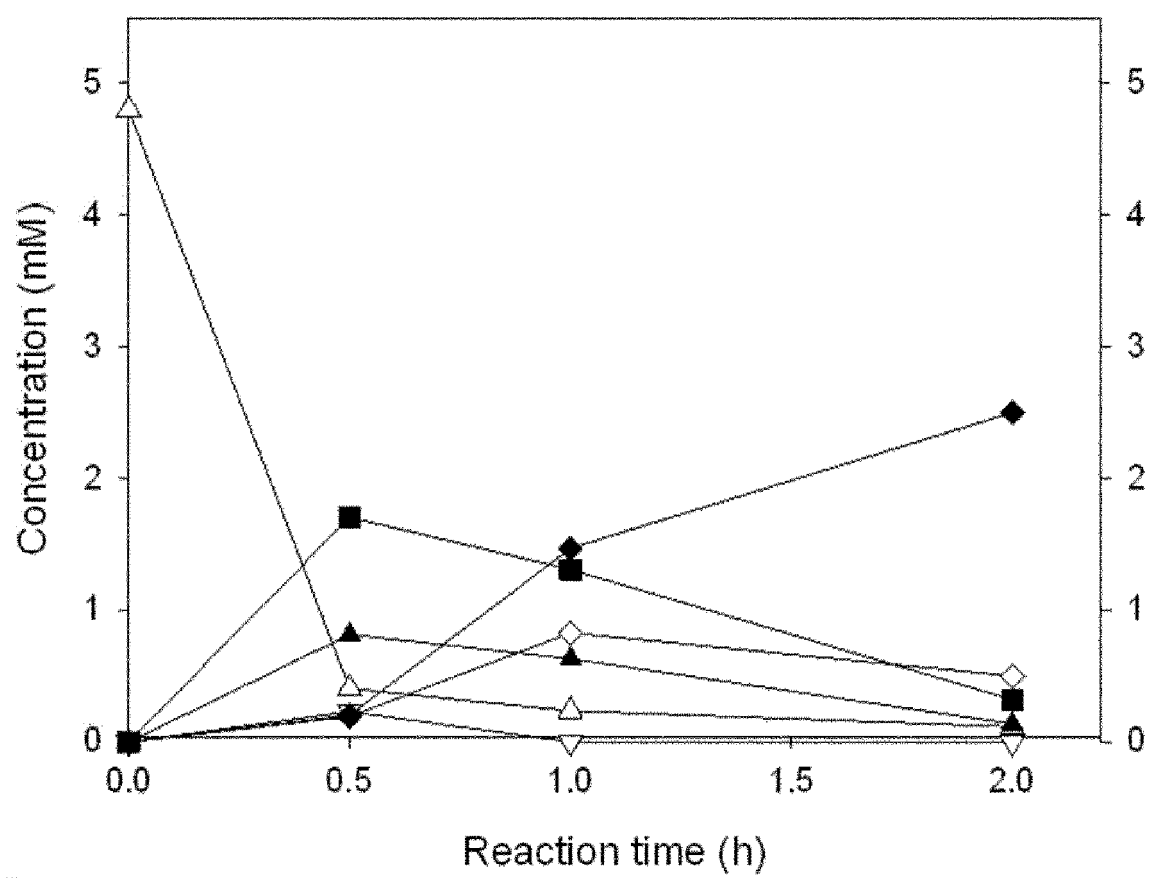
[FIG. 5b]



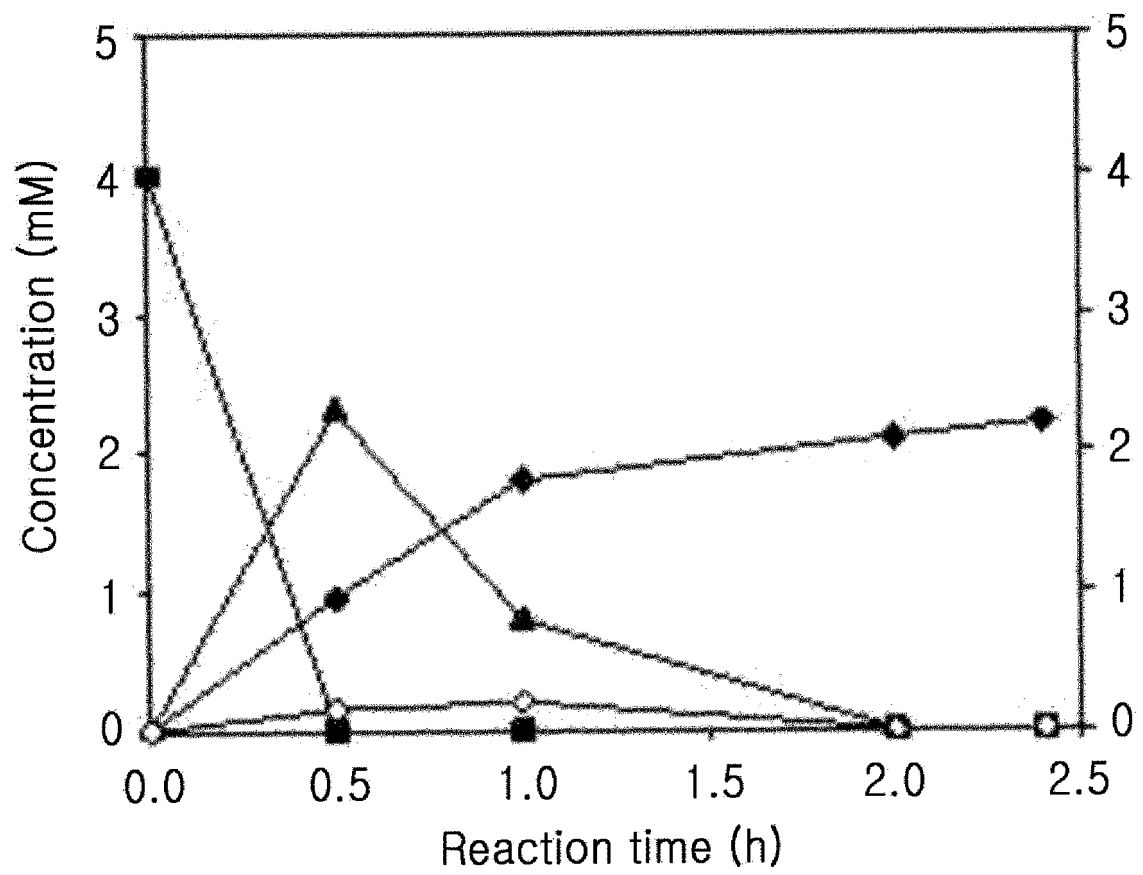
[FIG. 6]



[FIG. 7]



[FIG. 8]



REFERENCES CITED IN THE DESCRIPTION

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