

Improved methods for production, recovery and secretion of hydrophobic compounds in a fermentation

Technical field

The present invention relates to improved methods for producing a hydrophobic compound, in particular a hydrophobic compound which is a pheromone such as an insect pheromone, in a fermentation process involving cultivation of a microorganism such as a yeast, said microorganism producing said hydrophobic compound, wherein the methods facilitate recovery of the hydrophobic compound from the fermentation broth, increase the titer of the hydrophobic compound and/or increase secretion of the hydrophobic compound from the microorganism.

Background

Living cells, in particular microbial cells, are widely used nowadays for the biological production of a number of compounds. Examples of such compounds are fatty alcohols, fatty acyl acetates and fatty aldehydes, such as insect pheromones, which can be produced in e.g. yeast cells. Such compounds have applications in agriculture, and can for example be used as green pest repellents. Other useful compounds which can be produced by cells, e.g. genetically engineered cells, are terpenes and terpenoids. Terpenes are naturally produced by plants, and have a number of industrial applications in the field of food, pharmaceuticals, cosmetics and biotechnology. They are for example used as part of natural agricultural pesticides. Terpenoids (also termed isoprenoids) are modified terpenes containing additional groups, usually O-containing groups. They are often used for their aromatic qualities and as part of traditional herbal remedies. While terpenes and terpenoids occur widely, their extraction from natural sources is often problematic. Consequently, they are typically produced by chemical synthesis, usually from petrochemicals.

A common property of the above compounds is that they are hydrophobic or lipophilic. Their recovery from a fermentation broth typically involves several organic solvents, which introduces a number of challenges, including process safety, the need for multiple extraction steps, the need for removal of solvent residues from the final products, and significant labour and costs (both monetary and environmental).

Another challenge posed by fermentation processes involving recombinant microorganisms is that the compound of interest may largely retained intracellularly. Limited secretion of the product(s) from the cell into the fermentation broth may thus limit recovery of the compound of interest, or at best require additional steps involving cellular lysis in order to release the compound into the broth. Secretion of the product from the cell into the fermentation broth presents multiple advantages, such as reduced product inhibition and degradation, reduced effect on the host cell, higher titers, and easier and cheaper recovery process (Borodina I., 2019). Particular process advantage is achieved, when a secreted lipophilic product can be recovered in a separate phase.

Thus there is a need for improved methods for recovering hydrophobic fermentation products, as well as improved methods for increasing secretion of a hydrophobic compound from a cell in fermentation processes.

Summary

The present methods solve the above challenges.

Herein is provided a method for producing a hydrophobic compound such as a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate, a fatty aldehyde and/or a terpene such as a terpenoid in a fermentation, said method comprising the step of providing a microorganism capable of producing said hydrophobic compound and culturing said microorganism in a culture medium under conditions allowing production of said hydrophobic compound, wherein the culture medium comprises an extractant in an amount equal to or greater than its cloud concentration measured in an aqueous solution, wherein the extractant a non-ionic surfactant such as an antifoaming agent, preferably a polyethoxylated surfactant selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof, the method optionally further comprising the step of recovering the hydrophobic compound. Hence is provided herein a method for producing a hydrophobic compound selected from a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate, a fatty aldehyde and a terpene in a fermentation, said method comprising the step of providing a yeast cell capable of producing said hydrophobic compound and culturing said yeast

cell in a culture medium under conditions allowing production of said hydrophobic compound, wherein the culturing step is performed at a cultivation temperature, wherein the culture medium comprises an extractant in an amount equal to or greater than its cloud concentration measured in an aqueous solution such as the culture medium at the cultivation temperature, wherein the extractant is a non-ionic ethoxylated surfactant, the method further comprising the step of recovering the hydrophobic compound.

Also provided herein is a method for increasing the titer of a hydrophobic compound such as a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate, a fatty aldehyde and/or a terpene such as a terpenoid in a fermentation, said method comprising culturing a microorganism capable of producing said hydrophobic compound in a culture medium under conditions allowing production of said hydrophobic compound, wherein the culture medium comprises an extractant in an amount equal to or greater than its cloud concentration in an aqueous solution, wherein the extractant is a non-ionic surfactant such as an antifoaming agent, preferably a polyethoxylated surfactant selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof, whereby the titer of the hydrophobic compound is increased compared to a fermentation performed under similar conditions in the absence of extractant or in the presence of extractant in an amount lower than its cloud concentration in an aqueous solution. Also provided herein is a method for increasing the titer of a hydrophobic compound selected from a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate, a fatty aldehyde and a terpene in a fermentation, said method comprising culturing a yeast cell capable of producing said hydrophobic compound in a culture medium under conditions allowing production of said hydrophobic compound, wherein the culturing step is performed at a cultivation temperature, wherein the culture medium comprises an extractant in an amount equal to or greater than its cloud concentration measured in an aqueous solution at the cultivation temperature, wherein the extractant is a non-ionic ethoxylated surfactant, whereby the titer of the hydrophobic compound is increased compared to a fermentation performed under the same conditions but either in the absence of extractant or in the presence of extractant in an amount lower than its cloud concentration in an aqueous solution at the cultivation temperature.

Also provided herein is a method for increasing the secretion of a hydrophobic compound such as a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate, a fatty aldehyde and/or a terpene such as a terpenoid from a microorganism capable of producing said hydrophobic compound in a fermentation, said method comprising culturing said

5 microorganism in a culture medium under conditions allowing production of said hydrophobic compound, wherein the culture medium comprises an extractant in an amount equal to or greater than its cloud concentration measured in an aqueous solution, wherein the extractant is a non-ionic surfactant such as an antifoaming agent, preferably a polyethoxylated surfactant selected from: a polyethylene polypropylene glycol, a

10 mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof, whereby the secretion of the hydrophobic compound from the microorganism is increased compared to a fermentation performed under similar conditions

15 in the absence of extractant or in the presence of extractant in an amount lower than its cloud concentration measured in an aqueous solution. Also provided herein is a method for increasing the secretion of a hydrophobic compound selected from a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate, a fatty aldehyde and a terpene from a yeast cell capable of producing said hydrophobic compound in a fermentation, said

20 method comprising culturing said yeast cell in a culture medium under conditions allowing production of said hydrophobic compound, wherein the culturing step is performed at a cultivation temperature, wherein the culture medium comprises an extractant in an amount equal to or greater than its cloud concentration measured in an aqueous solution at the cultivation temperature, wherein the extractant is a non-ionic ethoxylated

25 surfactant, whereby the secretion of the hydrophobic compound from the yeast cell is increased compared to a fermentation performed under the same conditions but either in the absence of extractant or in the presence of extractant in an amount lower than its cloud concentration in an aqueous solution at the cultivation temperature.

30 Also provided herein is a hydrophobic compound obtainable by the methods disclosed herein, preferably wherein the hydrophobic compound is selected from a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate, a fatty aldehyde and a terpene.

Also provided herein is a method of monitoring the presence of pest or disrupting the

35 mating of pest, said method comprising the steps of:

- i) producing a hydrophobic compound by the methods described herein, wherein the hydrophobic compound is as defined herein,
- ii) formulating said desaturated fatty alcohol, desaturated fatty acyl acetate and/or desaturated fatty aldehyde as a pheromone composition, and
- 5 iii) employing said pheromone composition as an integrated pest management composition.

Description of the drawings

Figure 1 *In situ* extraction and recovery of fatty alcohols produced by fermentation. Antifoam A was added at a concentration of: 0% vol/vol (A); 0.4% vol/vol (B);

10 2% vol/vol (C) or 5% vol/vol (D) in a fermentation of a *Yarrowia lipolytica* strain capable of producing fatty alcohols. When antifoam A is absent (A) or at 0.4% vol/vol (B), the fermentation broth after centrifugation consists of two phases (a solid cellular fraction and a water phase). When antifoam A is added at 2% vol/vol (C) or 5% vol/vol (D), an additional immiscible phase is observed. The fermentation broth after centrifugation

15 consists of three phases: a solid cellular phase, a water phase and a product phase comprising the antifoam and the fatty alcohols. The fatty alcohols are thus isolated in this phase.

Figure 2 Antifoaming agents and oils as extractants. Various antifoams were

20 added at a concentration of 3% vol/vol in a fermentation of a *Yarrowia lipolytica* strain capable of producing fatty alcohols. (A) no antifoam; (B) corn oil; (C) oleic acid; (D) antifoam A; (E) Kolliphor® P407; (F) A-204; (G) simethicone; (H) dodecane. After centrifugation, three phases, including a product phase, were observed in fermentation broths to which antifoam A (D), Kolliphor® P407 (E), A-204 (F) or simethicone (G) were

25 added.

Detailed description of the invention

The present disclosure relates to the finding that fermentation of a microorganism, particularly a yeast, capable of producing a hydrophobic compound can be improved in

30 several ways by including a non-ionic surfactant, in particular a non-ionic ethoxylated surfactant, for example an antifoaming agent, in an amount equal to or greater than its cloud concentration in an aqueous system. Under such conditions, the non-ionic ethox-

ylated surfactant acts as an extractant, whereby recovery of the hydrophobic compound is facilitated. In addition, the presence of the non-ionic ethoxylated surfactant surprisingly increases the titer of the hydrophobic compound in the fermentation, and may also increase the secretion of the hydrophobic compound from the yeast cell, thereby further increasing production and facilitating recovery.

Definitions

Surfactant: the term refers to compounds that lower the surface tension (or interfacial tension) between two liquids, between a gas and a liquid, or between a liquid and a solid. Surfactants may act as detergents, wetting agents, emulsifiers, antifoaming agents, and dispersants. Surfactants are usually organic compounds that are amphiphilic, meaning they contain both hydrophobic groups (their tails) and hydrophilic groups (their heads). Therefore, a surfactant typically contains both a water-insoluble (or oil-soluble) component and a water-soluble component. Most commonly, surfactants are classified according to polar head group. A non-ionic surfactant has no charged groups in its head.

Extractant: the term "extractant" as used herein refers to a non-ionic surfactant, more particularly a non-ionic ethoxylated surfactant such as an agent that can be also used as antifoaming agent which facilitates recovery of hydrophobic compounds produced in a fermentation, in particular an ethoxylated surfactant such as a fatty alcohol alkoxylate or a polyethoxylated surfactant selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an agent or an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof. Non-ionic ethoxylated surfactants are often also referred to as low-foaming antifoaming agents.

Polyethoxylated surfactant: the term herein refers to ethoxylated surfactants which may be polyethoxylated surfactants, i.e. non-ionic surfactants.

Ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agent or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agent: the term refers to a group of polyethoxylated, non-ionic surfactants which comprise or mainly consist of ethoxylated and propoxylated alcohols in C₁₆-C₁₈, for example CAS number 68002-96-0, also termed

C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate or C₁₆-C₁₈ alcohols ethoxylated propoxylated polymer. Some compounds in this group are commonly used as antifoaming agents, while others are not and are thus generally referred to as "ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents" herein.

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Polyethylene polypropylene glycol: the term refers to a group of polyethoxylated non-ionic surfactants which comprise or mainly consist of PEG-PPG-PEG block copolymer antifoaming agents, for example Kolliphor® P407 (CAS number 9003-11-6), also termed poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol).

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Mixture of polyether dispersions: the term refers to a group of polyethoxylated non-ionic surfactants which comprise or mainly consist of a mixture of polyether dispersions, for example organic antifoam 204 from Sigma Aldrich (product number A6426 and A8311, MDL number MFCD00130523).

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Simethicone: the term refers to a group of polyethoxylated non-ionic surfactants which comprise or mainly consist of simethicone, also termed simeticone (CAS number 8050-81-5), dimethyl polysiloxane, or activated Polymethylsiloxane. Simethicone is a silicone-based emulsion containing also 1.2-1.6% polyethylene glycol monostearate.

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Cloud point: The cloud point of a surfactant, in particular non-ionic, or a glycol solution, in a solution, for example an aqueous solution, is the temperature at which a mixture of said surfactant and said solution, for example said aqueous solution, starts to phase-separate, and two phases appear, thus becoming cloudy. This behavior is characteristic of non-ionic surfactants containing polyoxyethylene chains, which exhibit reverse solubility versus temperature behavior in water and therefore "cloud out" at some point as the temperature is raised. Glycols demonstrating this behavior are known as "cloud-point glycols". The cloud point is affected by salinity, being generally lower in more saline fluids.

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Cloud concentration: the term will herein be used to refer to the concentration of a surfactant, in particular non-ionic, or a glycol solution, in a solution above which, at a given temperature, a mixture of said surfactant and said solution starts to phase-separate, and two phases appear, thus becoming cloudy. For example, the cloud concentration of a surfactant in an aqueous solution at a given temperature is the minimal concentration of said surfactant which, when mixed with the aqueous solution, gives rise to two

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phases. The cloud concentration can be obtained from the manufacturer of the surfactant, or it may be determined experimentally, by making a dosage curve and determining the concentration at which the mixture phase separates. For example, the method used in Example 7 can be applied. The cloud concentration can be determined at room temperature in an aqueous solution, for example in the culture medium which is used in the present methods. It can also be determined at the cultivation temperature at which the cultivation step is performed, for example at 30°C. In the case of surfactants that can be used as antifoaming agents, the cloud concentration is typically greater than the concentration recommended by the manufacturer for foam management.

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Non-ionic ethoxylated surfactant

The present methods rely on the use of a non-ionic ethoxylated surfactant, for example an antifoaming agent, which essentially acts as an extractant in the fermentation broth, where the non-ionic ethoxylated surfactant is present in an amount equal to or greater than its cloud concentration measured in an aqueous solution. Thereby the produced hydrophobic compound is produced with a higher titer, is secreted more readily from the producing microorganism, in particular the producing yeast cell, into the broth, and/or is more easily recovered compared to a fermentation performed with an amount of the same non-ionic ethoxylated surfactant lower than its cloud concentration measured in aqueous solution, such as in the absence of the non-ionic ethoxylated surfactant.

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While non-ionic surfactants, including non-ionic ethoxylated surfactants, in particular antifoaming agents, are routinely used in fermentation processes to prevent the formation of foam, the present inventors have found that non-ionic ethoxylated surfactants when included in the fermentation broth in an amount equal to or greater than their cloud concentration measured in an aqueous solution result in increased titer, increased secretion and facilitate recovery of a hydrophobic compound. The cloud concentration in an aqueous solution is determined at a given temperature, preferably at room temperature or at the temperature at which the fermentation is to be performed, for example 30°C; this temperature is herein referred to as "cultivation temperature". The term "extractant" as used herein refers to a non-ionic surfactant, more particularly a non-ionic ethoxylated surfactant, in particular an antifoaming agent, which facilitates recovery of hydrophobic compounds produced in a fermentation. For example, the non-ionic surfactant is a fatty alcohol alkoxylate or a polyethoxylated surfactant, for example

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selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof.

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Non-ionic surfactants particularly useful for the present methods are ethoxylated and polyethoxylated surfactants, some of which are also routinely used as antifoaming agents, although their application as antifoaming agent is normally associated with their use at lower concentrations than described herein, i.e. at concentrations lower than their cloud concentration as measured in an aqueous solution. These include polyethylene fatty alcohol alkoxylates, and polyethoxylated surfactants, such as polypropylene glycol, mixtures of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof.

Thus in one embodiment, the non-ionic surfactant acting as extractant is an antifoaming agent. In some embodiments, the non-ionic ethoxylated surfactant is a fatty alcohol alkoxylate. In some embodiments, the non-ionic ethoxylated surfactant is a polyethoxylated surfactant. In some embodiments, the antifoaming agent is polyethylene polypropylene glycol. In another embodiment, the antifoaming agent is a mixture of polyether dispersions. In another embodiment, the antifoaming agent is an antifoaming agent comprising polyethylene glycol monostearate or simethicone. In another embodiment, the antifoaming agent is an ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agent or an ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agent. In some embodiments, the extractant is a mixture of said agents or antifoaming agents and/or non-ionic ethoxylated surfactants.

In preferred embodiments of the present methods, the non-ionic surfactant is a non-ionic ethoxylated surfactant such as an antifoaming agent comprising or consisting of an ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agent or an ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agent. For example, C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate (CAS number 68002-96-0), Agnique BP420 (CAS number 68002-96-0), a polyethylene polypropylene glycol, antifoam 204, a surfactant comprising polyethylene glycol monostearate and fatty alcohol alkoxylates, in particular the fatty alcohol alkoxylates described below, have been found particularly advantageous.

Thus in one embodiment the antifoaming agent is C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate (CAS number 68002-96-0).

5 In another embodiment of the present methods, the non-ionic surfactant is a non-ionic ethoxylated surfactant such as an antifoaming agent comprising or consisting of a polyethylene polypropylene glycol. For example, the antifoaming agent is Kolliphor® P407 (CAS number 9003-11-6).

10 In another embodiment of the present methods, the non-ionic surfactant is a non-ionic ethoxylated surfactant such as an antifoaming agent comprising or consisting of a mixture of polyether dispersions. For example, the antifoaming agent is Antifoam 204 from Sigma Aldrich (product number A6426 or A8311).

15 In another embodiment of the present methods, the non-ionic surfactant is a non-ionic ethoxylated surfactant such as an antifoaming agent comprising or consisting of an antifoaming agent comprising polyethylene glycol monostearate or simethicone (CAS number 8050-81-5), preferably simethicone.

20 In another embodiment of the present methods, the non-ionic surfactant is a non-ionic ethoxylated surfactant such as Agnique BP420 (CAS number 68002-96-0).

In another embodiment of the present methods, the non-ionic surfactant is a non-ionic ethoxylated surfactant such as antifoam 204.

25 In some embodiments of the present methods, the non-ionic ethoxylated surfactant is a fatty alcohol alkoxylate, preferably selected from Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574.

30 In one embodiment, the extractant is Plurafac® LF300 (CAS number 196823-11-7). In another embodiment, the extractant is Plurafac® LF1300 (68002-96-0). In another embodiment, the extractant is Plurafac® SLF180 (CAS number 196823-11-7). In another embodiment, the extractant is Dehypon® 2574 (CAS number 68154-97-2). In another
35 embodiment, the extractant is Imbentin SG/251 (CAS number 68002-96-0).

The inventors have found that the above non-ionic surfactants, in particular the above non-ionic ethoxylated surfactants, some of which are routinely for foam management in fermentations, when added in an amount equal to or greater than their cloud concentration in an aqueous solution, i.e. an amount higher than required for foam management, result in increased titer, increased secretion and facilitate recovery of the hydrophobic compound produced in the fermentation. The cloud concentration of a surfactant is the concentration of surfactant at which, when it is mixed in an aqueous solution, the mixture starts to phase-separate, and two phases appear, thus the mixture becomes cloudy.

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In order to determine the cloud concentration of a surfactant, and hence determine the minimal amount of surfactant to use in the present methods, the person of skill in the art will know how to perform a dosage curve, where the surfactant is added to a solution, preferably an aqueous solution, at a given temperature, to determine the concentration of surfactant at which the appearance of two phases in the mixture is observed. The cloud concentration may be determined at room temperature, i.e. between 18 and 25°C, for example at 19°C, 20°C, 21°C, 22°C, 23°C or 24°C, or at a temperature suitable for the envisaged fermentation process, e.g. 30°C or 37°C. The temperature of the fermentation broth may be adjusted after fermentation in order to enhance the phase separation as described herein. Example 7 describes one way to determine the cloud concentration of a surfactant.

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In some embodiments, the non-ionic surfactant, or the non-ionic ethoxylated surfactant, is added in an amount greater than its cloud concentration measured in an aqueous solution. In some embodiments, the ethoxylated surfactant, such as the fatty alcohol alkoxyate or the polyethoxylated surfactant, is added in an amount greater than its cloud concentration measured in an aqueous solution. The cloud concentration may be determined at room temperature, or at the cultivation temperature.

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In some embodiments, the culture medium comprises the extractant, i.e. the non-ionic surfactant, in particular the non-ionic ethoxylated surfactant, in an amount greater than its cloud concentration by at least 50%, such as at least 100%, such as at least 150%, such as at least 200%, such as at least 250%, such as at least 300%, such as at least 350%, such as at least 400%, such as at least 500%, such as at least 750%, such as at least 1000%, or more, where the cloud concentration preferably is measured in an aqueous solution, for example at room temperature or at the cultivation temperature.

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The extractant is preferably a fatty alcohol alkoxylate or an ethoxylated surfactant, such as a polyethoxylated surfactant selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof.

In some embodiments, the culture medium comprises the extractant, i.e. a non-ionic ethoxylated surfactant such as a fatty alcohol alkoxylate or a polyethoxylated surfactant selected from: Agnique BP420 (CAS number 68002-96-0), antifoam 204, a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof, in an amount greater than its cloud concentration by at least 50%, such as at least 100%, such as at least 150%, such as at least 200%, such as at least 250%, such as at least 300%, such as at least 350%, such as at least 400%, such as at least 500%, such as at least 750%, such as at least 1000%, or more, where the cloud concentration preferably is measured in an aqueous solution, for example at room temperature or at the cultivation temperature.

In some embodiments, the culture medium comprises the extractant, i.e. a non-ionic ethoxylated surfactant such as a fatty alcohol alkoxylate selected from: Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof, in an amount greater than its cloud concentration by at least 50%, such as at least 100%, such as at least 150%, such as at least 200%, such as at least 250%, such as at least 300%, such as at least 350%, such as at least 400%, such as at least 500%, such as at least 750%, such as at least 1000%, or more, where the cloud concentration preferably is measured in an aqueous solution, for example at room temperature or at the cultivation temperature.

In other embodiments, the culture medium comprises the extractant, i.e. the non-ionic surfactant, in particular the non-ionic ethoxylated surfactant such as a fatty alcohol alkoxylate or the polyethoxylated surfactant, in an amount at least 2-fold its cloud concentration, such as at least 3-fold its cloud concentration, such as at least 4-fold its

cloud concentration, such as at least 5-fold its cloud concentration, such as at least 6-fold its cloud concentration, such as at least 7-fold its cloud concentration, such as at least 8-fold its cloud concentration, such as at least 9-fold its cloud concentration, such as at least 10-fold its cloud concentration, such as at least 12.5-fold its cloud concentration, such as at least 15-fold its cloud concentration, such as at least 17.5-fold its cloud concentration, such as at least 20-fold its cloud concentration, such as at least 25-fold its cloud concentration, such as at least 30-fold its cloud concentration, where the cloud concentration preferably is measured in an aqueous solution, for example at room temperature or at the cultivation temperature.

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In other embodiments, the culture medium comprises the extractant, i.e. a non-ionic ethoxylated surfactant such as a fatty alcohol alkoxylate selected from: Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof, in an amount at least 2-fold its cloud concentration, such as at least 3-fold its cloud concentration, such as at least 4-fold its cloud concentration, such as at least 5-fold its cloud concentration, such as at least 6-fold its cloud concentration, such as at least 7-fold its cloud concentration, such as at least 8-fold its cloud concentration, such as at least 9-fold its cloud concentration, such as at least 10-fold its cloud concentration, such as at least 12.5-fold its cloud concentration, such as at least 15-fold its cloud concentration, such as at least 17.5-fold its cloud concentration, such as at least 20-fold its cloud concentration, such as at least 25-fold its cloud concentration, such as at least 30-fold its cloud concentration, where the cloud concentration preferably is measured in an aqueous solution, for example at room temperature or at the cultivation temperature.

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In some embodiments, the culture medium comprises the extractant, i.e. the non-ionic ethoxylated surfactant such as a fatty alcohol alkoxylate or a polyethoxylated surfactant selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof, in an amount at least 2-fold its cloud concentration, such as at least 3-fold its cloud concentration, such as at least 4-fold its cloud concentration, such as at least 5-fold its cloud concentration, such as at least 6-fold its cloud concentration, such as at least 7-fold its

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cloud concentration, such as at least 8-fold its cloud concentration, such as at least 9-fold its cloud concentration, such as at least 10-fold its cloud concentration, such as at least 12.5-fold its cloud concentration, such as at least 15-fold its cloud concentration, such as at least 17.5-fold its cloud concentration, such as at least 20-fold its cloud concentration, such as at least 25-fold its cloud concentration, such as at least 30-fold its cloud concentration, where the cloud concentration preferably is measured in an aqueous solution, for example at room temperature or at the cultivation temperature.

In some embodiments, the culture medium comprises the extractant, i.e. the non-ionic ethoxylated surfactant such as a fatty alcohol alkoxylate selected from: Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof, in an amount at least 2-fold its cloud concentration, such as at least 3-fold its cloud concentration, such as at least 4-fold its cloud concentration, such as at least 5-fold its cloud concentration, such as at least 6-fold its cloud concentration, such as at least 7-fold its cloud concentration, such as at least 8-fold its cloud concentration, such as at least 9-fold its cloud concentration, such as at least 10-fold its cloud concentration, such as at least 12.5-fold its cloud concentration, such as at least 15-fold its cloud concentration, such as at least 17.5-fold its cloud concentration, such as at least 20-fold its cloud concentration, such as at least 25-fold its cloud concentration, such as at least 30-fold its cloud concentration, where the cloud concentration preferably is measured in an aqueous solution, for example at room temperature or at the cultivation temperature.

In some embodiments, the culture medium comprises at least 1% vol/vol extractant, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol extractant, wherein the extractant is a non-ionic surfactant. In some embodiments, the culture medium comprises at least 1% vol/vol extractant, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at

least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol extractant, wherein the extractant is a non-ionic ethoxylated surfactant such as a fatty alcohol alkoxylate such as a fatty alcohol alkoxylate selected from: Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof, or a polyethoxylated surfactant, such as selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an anti-foaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof.

In some embodiments, the non-ionic surfactant is a non-ionic ethoxylated surfactant, in particular an antifoaming agent comprising or consisting of an ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agent or an ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agent, for example, C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate (CAS number 68002-96-0). The cloud concentration of C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate (CAS number 68002-96-0) is about 1% vol/vol at room temperature. Accordingly, when this antifoaming agent is used, the culture medium preferably comprises at least 1% vol/vol of C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate, or more.

In some embodiments, the non-ionic surfactant is a non-ionic ethoxylated surfactant, in particular an antifoaming agent comprising or consisting of a polyethylene polypropylene glycol, for example Kolliphor® P407 (CAS number 9003-11-6), also termed poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol). The cloud concentration of Kolliphor® P407 is 10% at a temperature above 100°C. Accordingly, when a polyethylene polypropylene glycol such as Kolliphor® P407 is used, the culture medium preferably comprises at least 10% vol/vol of polyethylene polypropylene glycol such as Kolliphor® P407, such as at least 11% vol/vol, such as at least 12%

vol/vol, such as at least 13% vol/vol, such as at least 14% vol/vol, such as at least 15% vol/vol, such as at least 16% vol/vol, such as at least 17% vol/vol, such as at least 18% vol/vol, such as at least 19% vol/vol, such as at least 20% vol/vol, such as at least 25% vol/vol, such as at least 30% vol/vol, such as at least 35% vol/vol of polyethylene polypropylene glycol such as Kolliphor® P407, or more.

In some embodiments, the non-ionic surfactant is a non-ionic ethoxylated surfactant, in particular an antifoaming agent comprising or consisting of a mixture of polyether dispersions, such as antifoam 204 (product number A6426 or A8311 from Sigma Aldrich). The cloud concentration of antifoam 204 is 1% in an aqueous solution at a temperature of 18.0 to 21.0°C. Accordingly, when a mixture of polyether dispersions such as antifoam 204 is used, the culture medium preferably comprises at least 1% vol/vol of a mixture of polyether dispersions such as antifoam 204, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol of a mixture of polyether dispersions such as antifoam 204, or more.

In some embodiments, the non-ionic surfactant is a non-ionic ethoxylated surfactant, in particular an antifoaming agent comprising or consisting of an antifoaming agent comprising polyethylene glycol monostearate or simethicone. Simethicone comprises polyethylene glycol monostearate, which, without being bound by theory, appears to be the compound important for the ability of simethicone to act as an extractant. Polyethylene glycol monostearate has a cloud point of 1% in an aqueous solution at 5°C. Accordingly, when simethicone or a surfactant comprising polyethylene glycol monostearate is used as antifoaming agent, the culture medium preferably comprises at least 1% vol/vol of polyethylene glycol monostearate or simethicone, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol polyethylene glycol monostearate or simethicone, or more.

In some embodiments, the non-ionic surfactant is a non-ionic ethoxylated surfactant, in particular Agnique BP420 (CAS number 68002-96-0). Agnique BP420 (CAS number 68002-96-0) has a cloud point of 1% in an aqueous solution at room temperature. Accordingly, when Agnique BP420 (CAS number 68002-96-0) is used as antifoaming agent, the culture medium preferably comprises at least 1% vol/vol of Agnique BP420 (CAS number 68002-96-0), such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol Agnique BP420 (CAS number 68002-96-0).

In some embodiments, the non-ionic surfactant is a non-ionic ethoxylated surfactant, in particular a fatty alcohol alkoxylate such as Plurafac® LF300 (CAS number 196823-11-7). The cloud concentration of Plurafac® LF300 (CAS number 196823-11-7) is about 1% vol/vol at room temperature. Accordingly, when Plurafac® LF300 (CAS number 196823-11-7) is used, the culture medium preferably comprises at least 1% vol/vol of Plurafac® LF300 (CAS number 196823-11-7), such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol Plurafac® LF300 (CAS number 196823-11-7), or more.

In some embodiments, the non-ionic surfactant is a non-ionic ethoxylated surfactant, in particular a fatty alcohol alkoxylate such as Plurafac® LF1300 (68002-96-0). The cloud concentration of Plurafac® LF1300 (68002-96-0) is about 1% vol/vol at room temperature. Accordingly, when Plurafac® LF1300 (68002-96-0) is used, the culture medium preferably comprises at least 1% vol/vol of Plurafac® LF1300 (68002-96-0), such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol Plurafac® LF1300 (68002-96-0), or more.

In some embodiments, the non-ionic surfactant is a non-ionic ethoxylated surfactant, in particular a fatty alcohol alkoxylate such as Plurafac® SLF180 (CAS number 196823-11-7). The cloud concentration of Plurafac® SLF180 (CAS number 196823-11-7) is about 1% vol/vol at room temperature. Accordingly, when Plurafac® SLF180 (CAS number 196823-11-7) is used, the culture medium preferably comprises at least 1% vol/vol of Plurafac® SLF180 (CAS number 196823-11-7), such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol Plurafac® SLF180 (CAS number 196823-11-7), or more.

In some embodiments, the non-ionic surfactant is a non-ionic ethoxylated surfactant, in particular a fatty alcohol alkoxylate such as Dehypon® 2574 (CAS number 68154-97-2). The cloud concentration of Dehypon® 2574 (CAS number 68154-97-2) is about 1% vol/vol at room temperature. Accordingly, when Dehypon® 2574 (CAS number 68154-97-2) is used, the culture medium preferably comprises at least 1% vol/vol of Dehypon® 2574 (CAS number 68154-97-2), such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol Dehypon® 2574 (CAS number 68154-97-2), or more.

In some embodiments, the non-ionic surfactant is a non-ionic ethoxylated surfactant, in particular a fatty alcohol alkoxylate such as Imbentin SG/251 (CAS number 68002-96-0). The cloud concentration of Imbentin SG/251 (CAS number 68002-96-0) is about 1% vol/vol at room temperature. Accordingly, when Imbentin SG/251 (CAS number 68002-96-0) is used, the culture medium preferably comprises at least 1% vol/vol of Imbentin SG/251 (CAS number 68002-96-0), such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such

as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol Imbentin SG/251 (CAS number 68002-96-0), or more.

Hydrophobic compound

- 5 The herein disclosed methods are useful for facilitating recovery of a hydrophobic compound from a fermentation broth, for increasing the titer of the hydrophobic compound in the fermentation and for increasing secretion of the hydrophobic compound from the producing microorganism.
- 10 The hydrophobic compound may be any hydrophobic compound produced by the microorganism in the fermentation. The microorganism is in preferred embodiments a yeast cell. In particular, the hydrophobic compound may be selected from: a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate, a fatty aldehyde and a terpene such as a terpenoid. The hydrophobic compound may be several hydrophobic compounds, for
- 15 example a mixture of one or more of at least one fatty alcohol, at least one fatty acyl acetate, at least one fatty aldehyde and at least one terpene such as at least one terpenoid. In some embodiments the hydrophobic compound is a mixture of one or more fatty alcohols, one or more fatty acyl acetates and/or one or more fatty aldehydes. In some embodiments the hydrophobic compound is a mixture of one or more terpenes,
- 20 such as a mixture of one or more terpenoids or a mixture of one or more terpenes and one or more terpenoids.

The present methods are particularly useful for producing and recovering hydrophobic compounds which are pheromones, in particular insect pheromones.

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Fatty alcohols

- The fatty alcohol may be a saturated fatty alcohol, a desaturated fatty alcohol or a mixture thereof. In one embodiment, the fatty alcohol has a chain length of 8. In another embodiment, the fatty alcohol has a chain length of 9. In another embodiment, the fatty
- 30 alcohol has a chain length of 10. In another embodiment, the fatty alcohol has a chain length of 11. In another embodiment, the fatty alcohol has a chain length of 12. In another embodiment, the fatty alcohol has a chain length of 13. In another embodiment, the fatty alcohol has a chain length of 14. In another embodiment, the fatty alcohol has a chain length of 15. In another embodiment, the fatty alcohol has a chain length of 16.

In another embodiment, the fatty alcohol has a chain length of 17. In another embodiment, the fatty alcohol has a chain length of 18. In another embodiment, the fatty alcohol has a chain length of 19. In another embodiment, the fatty alcohol has a chain length of 20. In another embodiment, the fatty alcohol has a chain length of 21. In another embodiment, the fatty alcohol has a chain length of 22.

In some embodiments, the fatty alcohol is a desaturated fatty alcohol. Such compounds are naturally produced e.g. by insect cells, where they act as pheromones. The desaturated fatty alcohols may be:

- 10 - (Z)- Δ^3 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ^3 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ^5 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 15 - (E)- Δ^5 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ^6 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 20 - (E)- Δ^6 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ^7 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ^7 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 25 - (Z)- Δ^8 desaturated fatty alcohols having a carbon chain length of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ^8 desaturated fatty alcohols having a carbon chain length of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 30 - (Z)- Δ^9 desaturated fatty alcohols having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ^9 desaturated fatty alcohols having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ^{10} desaturated fatty alcohols having a carbon chain length of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 35 - (E)- Δ^{10} desaturated fatty alcohols having a carbon chain length of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;

- (E)- Δ 10 desaturated fatty alcohols having a carbon chain length of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ 11 desaturated fatty alcohols having a carbon chain length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 5 - (E)- Δ 11 desaturated fatty alcohols having a carbon chain length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ 12 desaturated fatty alcohols having a carbon chain length of 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ 12 desaturated fatty alcohols having a carbon chain length of 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 10 - (Z)- Δ 13 desaturated fatty alcohols having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22; and
- (E)- Δ 13 desaturated fatty alcohols having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22.

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In some embodiments, the fatty alcohols are desaturated fatty alcohols having a carbon chain length of 14, such as:

- (Z)- Δ 5 desaturated fatty alcohols having a carbon chain length of 14;
- (E)- Δ 5 desaturated fatty alcohols having a carbon chain length of 14;
- 20 - (Z)- Δ 6 desaturated fatty alcohols having a carbon chain length of 14;
- (E)- Δ 6 desaturated fatty alcohols having a carbon chain length of 14;
- (Z)- Δ 7 desaturated fatty alcohols having a carbon chain length of 14;
- (E)- Δ 7 desaturated fatty alcohols having a carbon chain length of 14;
- (Z)- Δ 8 desaturated fatty alcohols having a carbon chain length of 14;
- 25 - (E)- Δ 8 desaturated fatty alcohols having a carbon chain length of 14;
- (Z)- Δ 9 desaturated fatty alcohols having a carbon chain length of 14;
- (E)- Δ 9 desaturated fatty alcohols having a carbon chain length of 14;
- (Z)- Δ 10 desaturated fatty alcohols having a carbon chain length of 14;
- (E)- Δ 10 desaturated fatty alcohols having a carbon chain length of 14;
- 30 - (Z)- Δ 11 desaturated fatty alcohols having a carbon chain length of 14;
- (E)- Δ 11 desaturated fatty alcohols having a carbon chain length of 14;
- (Z)- Δ 12 desaturated fatty alcohols having a carbon chain length of 14;
- (E)- Δ 12 desaturated fatty alcohols having a carbon chain length of 14;
- (Z)- Δ 13 desaturated fatty alcohols having a carbon chain length of 14; and
- 35 - (E)- Δ 13 desaturated fatty alcohols having a carbon chain length of 14.

In some embodiments, the fatty alcohols are desaturated fatty alcohols having a carbon chain length of 16, such as:

- (Z)- Δ 5 desaturated fatty alcohols having a carbon chain length of 16;
- (E)- Δ 5 desaturated fatty alcohols having a carbon chain length of 16;
- 5 - (Z)- Δ 6 desaturated fatty alcohols having a carbon chain length of 16;
- (E)- Δ 6 desaturated fatty alcohols having a carbon chain length of 16;
- (Z)- Δ 7 desaturated fatty alcohols having a carbon chain length of 16;
- (E)- Δ 7 desaturated fatty alcohols having a carbon chain length of 16;
- (Z)- Δ 8 desaturated fatty alcohols having a carbon chain length of 16;
- 10 - (E)- Δ 8 desaturated fatty alcohols having a carbon chain length of 16;
- (Z)- Δ 9 desaturated fatty alcohols having a carbon chain length of 16;
- (E)- Δ 9 desaturated fatty alcohols having a carbon chain length of 16;
- (Z)- Δ 10 desaturated fatty alcohols having a carbon chain length of 16;
- (E)- Δ 10 desaturated fatty alcohols having a carbon chain length of 16;
- 15 - (Z)- Δ 11 desaturated fatty alcohols having a carbon chain length of 16;
- (E)- Δ 11 desaturated fatty alcohols having a carbon chain length of 16;
- (Z)- Δ 12 desaturated fatty alcohols having a carbon chain length of 16;
- (E)- Δ 12 desaturated fatty alcohols having a carbon chain length of 16;
- (Z)- Δ 13 desaturated fatty alcohols having a carbon chain length of 16; and
- 20 - (E)- Δ 13 desaturated fatty alcohols having a carbon chain length of 16.

The desaturated fatty alcohols may be desaturated in more than one position. The desaturated fatty alcohols may be desaturated in at least two positions, such as at least three positions, such as four positions.

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- For example, the fatty alcohol is an (E)7, (Z)9 desaturated fatty alcohol having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22. In some embodiments, the fatty alcohol is an (E)3, (Z)8, (Z)11 desaturated fatty alcohol having a carbon chain length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22, for example 14. In
- 30 some embodiments, the fatty alcohol is a (Z)9, (E)11, (E)13 desaturated fatty alcohol having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22. In other embodiments, the fatty alcohol is an (E)7, (Z)9 desaturated fatty alcohol having a carbon chain length of 14. In other embodiments, the desaturated fatty alcohol is an (E)3, (Z)8, (Z)11 desaturated fatty alcohol having a carbon chain length of 14. In other embodiments, the
- 35 desaturated fatty alcohol is a (Z)9, (E)11, (E)13 desaturated fatty alcohol having a carbon chain length of 14. For example, the fatty alcohol is an (E)7, (Z)9 desaturated fatty

alcohol having a carbon chain length of 12. In other embodiments, the desaturated fatty alcohol is an (E)3, (Z)8, (Z)11 desaturated fatty alcohol having a carbon chain length of 12. In other embodiments, the desaturated fatty alcohol is a (Z)9, (E)11, (E)13 desaturated fatty alcohol having a carbon chain length of 12. In other embodiments, the desaturated fatty alcohol is a (E)8, (E)10 desaturated fatty alcohol having a carbon chain length of 12.

In a particular embodiment, the fatty alcohol is (Z)-11-hexadecen-1-ol or (Z)-9-tetradecen-1-ol.

Fatty alcohols esters

The fatty alcohol ester may be a saturated fatty alcohol ester, a desaturated fatty alcohol ester or a mixture thereof. In one embodiment, the fatty alcohol ester has a chain length of 8. In another embodiment, the fatty alcohol ester has a chain length of 9. In another embodiment, the fatty alcohol ester has a chain length of 10. In another embodiment, the fatty alcohol ester has a chain length of 11. In another embodiment, the fatty alcohol ester has a chain length of 12. In another embodiment, the fatty alcohol ester has a chain length of 13. In another embodiment, the fatty alcohol ester has a chain length of 14. In another embodiment, the fatty alcohol ester has a chain length of 15. In another embodiment, the fatty alcohol ester has a chain length of 16. In another embodiment, the fatty alcohol ester has a chain length of 17. In another embodiment, the fatty alcohol ester has a chain length of 18. In another embodiment, the fatty alcohol ester has a chain length of 19. In another embodiment, the fatty alcohol ester has a chain length of 20. In another embodiment, the fatty alcohol ester has a chain length of 21. In another embodiment, the fatty alcohol ester has a chain length of 22.

In some embodiments, the fatty alcohol ester is a desaturated fatty alcohol ester. Such compounds are naturally produced e.g. by insect cells, where they act as pheromones.

The desaturated fatty alcohol esters may be:

- (Z)- Δ^3 desaturated fatty alcohol esters having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ^3 desaturated fatty alcohol esters having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ^5 desaturated fatty alcohol esters having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;

- (E)- Δ 5 desaturated fatty alcohol esters having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ 6 desaturated fatty alcohol esters having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 5 - (E)- Δ 6 desaturated fatty alcohol esters having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ 7 desaturated fatty alcohol esters having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 10 - (E)- Δ 7 desaturated fatty alcohol esters having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ 8 desaturated fatty alcohol esters having a carbon chain length of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ 8 desaturated fatty alcohol esters having a carbon chain length of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 15 - (Z)- Δ 9 desaturated fatty alcohol esters having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ 9 desaturated fatty alcohol esters having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ 10 desaturated fatty alcohol esters having a carbon chain length of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 20 - (E)- Δ 10 desaturated fatty alcohol esters having a carbon chain length of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ 11 desaturated fatty alcohol esters having a carbon chain length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 25 - (E)- Δ 11 desaturated fatty alcohol esters having a carbon chain length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ 12 desaturated fatty alcohol esters having a carbon chain length of 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ 12 desaturated fatty alcohol esters having a carbon chain length of 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 30 - (Z)- Δ 13 desaturated fatty alcohol esters having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22; and
- (E)- Δ 13 desaturated fatty alcohol esters having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22.

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In some embodiments, the fatty alcohol esters are desaturated fatty alcohol esters having a carbon chain length of 14, such as:

- (Z)- Δ 5 desaturated fatty alcohol esters having a carbon chain length of 14;
- (E)- Δ 5 desaturated fatty alcohol esters having a carbon chain length of 14;
- 5 - (Z)- Δ 6 desaturated fatty alcohol esters having a carbon chain length of 14;
- (E)- Δ 6 desaturated fatty alcohol esters having a carbon chain length of 14;
- (Z)- Δ 7 desaturated fatty alcohol esters having a carbon chain length of 14;
- (E)- Δ 7 desaturated fatty alcohol esters having a carbon chain length of 14;
- (Z)- Δ 8 desaturated fatty alcohol esters having a carbon chain length of 14;
- 10 - (E)- Δ 8 desaturated fatty alcohol esters having a carbon chain length of 14;
- (Z)- Δ 9 desaturated fatty alcohol esters having a carbon chain length of 14;
- (E)- Δ 9 desaturated fatty alcohol esters having a carbon chain length of 14;
- (Z)- Δ 10 desaturated fatty alcohol esters having a carbon chain length of 14;
- (E)- Δ 10 desaturated fatty alcohol esters having a carbon chain length of 14;
- 15 - (Z)- Δ 11 desaturated fatty alcohol esters having a carbon chain length of 14;
- (E)- Δ 11 desaturated fatty alcohol esters having a carbon chain length of 14;
- (Z)- Δ 12 desaturated fatty alcohol esters having a carbon chain length of 14;
- (E)- Δ 12 desaturated fatty alcohol esters having a carbon chain length of 14;
- (Z)- Δ 13 desaturated fatty alcohol esters having a carbon chain length of 14; and
- 20 - (E)- Δ 13 desaturated fatty alcohol esters having a carbon chain length of 14.

In some embodiments, the fatty alcohol esters are desaturated fatty alcohol esters having a carbon chain length of 16, such as:

- (Z)- Δ 5 desaturated fatty alcohol esters having a carbon chain length of 16;
- 25 - (E)- Δ 5 desaturated fatty alcohol esters having a carbon chain length of 16;
- (Z)- Δ 6 desaturated fatty alcohol esters having a carbon chain length of 16;
- (E)- Δ 6 desaturated fatty alcohol esters having a carbon chain length of 16;
- (Z)- Δ 7 desaturated fatty alcohol esters having a carbon chain length of 16;
- (E)- Δ 7 desaturated fatty alcohol esters having a carbon chain length of 16;
- 30 - (Z)- Δ 8 desaturated fatty alcohol esters having a carbon chain length of 16;
- (E)- Δ 8 desaturated fatty alcohol esters having a carbon chain length of 16;
- (Z)- Δ 9 desaturated fatty alcohol esters having a carbon chain length of 16;
- (E)- Δ 9 desaturated fatty alcohol esters having a carbon chain length of 16;
- (Z)- Δ 10 desaturated fatty alcohol esters having a carbon chain length of 16;
- 35 - (E)- Δ 10 desaturated fatty alcohol esters having a carbon chain length of 16;
- (Z)- Δ 11 desaturated fatty alcohol esters having a carbon chain length of 16;

- (E)- Δ 11 desaturated fatty alcohol esters having a carbon chain length of 16;
- (Z)- Δ 12 desaturated fatty alcohol esters having a carbon chain length of 16;
- (E)- Δ 12 desaturated fatty alcohol esters having a carbon chain length of 16;
- (Z)- Δ 13 desaturated fatty alcohol esters having a carbon chain length of 16; and
- 5 - (E)- Δ 13 desaturated fatty alcohol esters having a carbon chain length of 16.

The desaturated fatty alcohol esters may be desaturated in more than one position.

The desaturated fatty alcohol esters may be desaturated in at least two positions, such as at least three positions, such as four positions.

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For example, the fatty alcohol ester is an (E)7, (Z)9 desaturated fatty alcohol ester having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22. In

some embodiments, the fatty alcohol ester is an (E)3, (Z)8, (Z)11 desaturated fatty alcohol ester having a carbon chain length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22,

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for example 14. In some embodiments, the fatty alcohol ester is a (Z)9, (E)11, (E)13 desaturated fatty alcohol ester having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22. In other embodiments, the fatty alcohol ester is an (E)7, (Z)9 desaturated

fatty alcohol ester having a carbon chain length of 14. In other embodiments, the desaturated fatty alcohol ester is an (E)3, (Z)8, (Z)11 desaturated fatty alcohol ester hav-

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ing a carbon chain length of 14. In other embodiments, the desaturated fatty alcohol ester is a (Z)9, (E)11, (E)13 desaturated fatty alcohol ester having a carbon chain length of 14. For example, the fatty alcohol ester is an (E)7, (Z)9 desaturated fatty alcohol ester having a carbon chain length of 12. In other embodiments, the desaturated

fatty alcohol ester is an (E)3, (Z)8, (Z)11 desaturated fatty alcohol ester having a carbon chain length of 12. In other embodiments, the desaturated fatty alcohol ester is a

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(Z)9, (E)11, (E)13 desaturated fatty alcohol ester having a carbon chain length of 12. In other embodiments, the desaturated fatty alcohol ester is a (E)8, (E)10 desaturated fatty alcohol ester having a carbon chain length of 12.

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In a particular embodiment, the fatty alcohol ester is (Z)-11-hexadecen-1-ol ester or (Z)-9-tetradecen-1-ol ester.

The fatty alcohol ester may be a fatty alcohol acetate ester.

Fatty acyl acetates

The fatty acyl acetates may be saturated fatty acyl acetates or desaturated fatty acyl acetates or a mixture thereof. Fatty acyl acetates, in particular desaturated fatty acyl acetates, are also naturally comprised within pheromones, in particular pheromones produced by species belonging to the *Lepidoptera* order.

In one embodiment, the fatty acyl acetate has a chain length of 8. In another embodiment, the fatty acyl acetate has a chain length of 9. In another embodiment, the fatty acyl acetate has a chain length of 10. In another embodiment, the fatty acyl acetate has a chain length of 11. In another embodiment, the fatty acyl acetate has a chain length of 12. In another embodiment, the fatty acyl acetate has a chain length of 13. In another embodiment, the fatty acyl acetate has a chain length of 14. In another embodiment, the fatty acyl acetate has a chain length of 15. In another embodiment, the fatty acyl acetate has a chain length of 16. In another embodiment, the fatty acyl acetate has a chain length of 17. In another embodiment, the fatty acyl acetate has a chain length of 18. In another embodiment, the fatty acyl acetate has a chain length of 19. In another embodiment, the fatty acyl acetate has a chain length of 20. In another embodiment, the fatty acyl acetate has a chain length of 21. In another embodiment, the fatty acyl acetate has a chain length of 22.

In some embodiments, the fatty acyl acetate is a desaturated fatty acyl acetate. The desaturated fatty acyl acetate may be a desaturated fatty acyl acetate having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22, such as:

- (Z)- Δ^3 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ^3 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ^5 desaturated fatty acyl acetates having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ^5 desaturated fatty acyl acetates having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ^6 desaturated fatty acyl acetates having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ^6 desaturated fatty acyl acetates having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;

- (Z)- Δ^7 desaturated fatty acyl acetates having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ^7 desaturated fatty acyl acetates having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 5 - (Z)- Δ^8 desaturated fatty acyl acetates having a carbon chain length of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ^8 desaturated fatty acyl acetates having a carbon chain length of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 10 - (Z)- Δ^9 desaturated fatty acyl acetates having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ^9 desaturated fatty acyl acetates having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ^{10} desaturated fatty acyl acetates having a carbon chain length of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 15 - (E)- Δ^{10} desaturated fatty acyl acetates having a carbon chain length of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ^{11} desaturated fatty acyl acetates having a carbon chain length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ^{11} desaturated fatty acyl acetates having a carbon chain length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 20 - (Z)- Δ^{12} desaturated fatty acyl acetates having a carbon chain length of 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ^{12} desaturated fatty acyl acetates having a carbon chain length of 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 25 - (Z)- Δ^{13} desaturated fatty acyl acetates having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22; and
- (E)- Δ^{13} desaturated fatty acyl acetates having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22.

- 30 In some embodiments, the fatty acyl acetates are desaturated fatty acyl acetates having a carbon chain length of 14, such as:
 - (Z)- Δ^5 desaturated fatty acyl acetates having a carbon chain length of 14;
 - (E)- Δ^5 desaturated fatty acyl acetates having a carbon chain length of 14;
 - (Z)- Δ^6 desaturated fatty acyl acetates having a carbon chain length of 14;
 - 35 - (E)- Δ^6 desaturated fatty acyl acetates having a carbon chain length of 14;
 - (Z)- Δ^7 desaturated fatty acyl acetates having a carbon chain length of 14;

- (E)- Δ 7 desaturated fatty acyl acetates having a carbon chain length of 14;
- (Z)- Δ 8 desaturated fatty acyl acetates having a carbon chain length of 14;
- (E)- Δ 8 desaturated fatty acyl acetates having a carbon chain length of 14;
- (Z)- Δ 9 desaturated fatty acyl acetates having a carbon chain length of 14;
- 5 - (E)- Δ 9 desaturated fatty acyl acetates having a carbon chain length of 14;
- (Z)- Δ 10 desaturated fatty acyl acetates having a carbon chain length of 14;
- (E)- Δ 10 desaturated fatty acyl acetates having a carbon chain length of 14;
- (Z)- Δ 11 desaturated fatty acyl acetates having a carbon chain length of 14;
- (E)- Δ 11 desaturated fatty acyl acetates having a carbon chain length of 14;
- 10 - (Z)- Δ 12 desaturated fatty acyl acetates having a carbon chain length of 14;
- (E)- Δ 12 desaturated fatty acyl acetates having a carbon chain length of 14;
- (Z)- Δ 13 desaturated fatty acyl acetates having a carbon chain length of 14; and
- (E)- Δ 13 desaturated fatty acyl acetates having a carbon chain length of 14.

15 In some embodiments, the fatty acyl acetates are desaturated fatty acyl acetates having a carbon chain length of 16, such as:

- (Z)- Δ 5 desaturated fatty acyl acetates having a carbon chain length of 16;
- (E)- Δ 5 desaturated fatty acyl acetates having a carbon chain length of 16;
- (Z)- Δ 6 desaturated fatty acyl acetates having a carbon chain length of 16;
- 20 - (E)- Δ 6 desaturated fatty acyl acetates having a carbon chain length of 16;
- (Z)- Δ 7 desaturated fatty acyl acetates having a carbon chain length of 16;
- (E)- Δ 7 desaturated fatty acyl acetates having a carbon chain length of 16;
- (Z)- Δ 8 desaturated fatty acyl acetates having a carbon chain length of 16;
- (E)- Δ 8 desaturated fatty acyl acetates having a carbon chain length of 16;
- 25 - (Z)- Δ 9 desaturated fatty acyl acetates having a carbon chain length of 16;
- (E)- Δ 9 desaturated fatty acyl acetates having a carbon chain length of 16;
- (Z)- Δ 10 desaturated fatty acyl acetates having a carbon chain length of 16;
- (E)- Δ 10 desaturated fatty acyl acetates having a carbon chain length of 16;
- (Z)- Δ 11 desaturated fatty acyl acetates having a carbon chain length of 16;
- 30 - (E)- Δ 11 desaturated fatty acyl acetates having a carbon chain length of 16;
- (Z)- Δ 12 desaturated fatty acyl acetates having a carbon chain length of 16;
- (E)- Δ 12 desaturated fatty acyl acetates having a carbon chain length of 16;
- (Z)- Δ 13 desaturated fatty acyl acetates having a carbon chain length of 16; and
- (E)- Δ 13 desaturated fatty acyl acetates having a carbon chain length of 16.

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The desaturated fatty acyl acetates may be desaturated in more than one position. The desaturated fatty acyl acetates may be desaturated in at least two positions, such as at least three positions, such as four positions.

- 5 For example, the fatty acyl acetate is an (E)7, (Z)9 desaturated fatty acyl acetate having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22. In some embodiments, the fatty acyl acetate is an (E)3, (Z)8, (Z)11 desaturated fatty acyl acetate having a carbon chain length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22. In some embodiments, the fatty acyl acetate is an (Z)9, (E)11, (E)13 desaturated fatty
10 acyl acetate having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22. In other embodiments, the fatty acyl acetate is an (E)7, (Z)9 desaturated fatty acyl acetate having a carbon chain length of 14. In some embodiments, the fatty acyl acetate is an (E)3, (Z)8, (Z)11 desaturated fatty acyl acetate having a carbon chain length of 14. In some embodiments, the fatty acyl acetate is a (Z)9, (E)11, (E)13 desaturated fatty acyl
15 acetate having a carbon chain length of 14. In other embodiments, the fatty acyl acetate is an (E)7, (Z)9 desaturated fatty acyl acetate having a carbon chain length of 12. In some embodiments, the fatty acyl acetate is an (E)3, (Z)8, (Z)11 desaturated fatty acyl acetate having a carbon chain length of 12.
- 20 In a particular embodiment, the fatty acyl acetate is (Z)-11-hexadecen-1-yl acetate or (Z)-9-tetradecen-1-yl acetate.

The fatty acyl acetates may be produced by the microorganism in the fermentation, e.g. where the microorganism is capable of converting a fatty alcohol to the corresponding
25 fatty acyl acetate, or they may be obtained by chemical conversion as is known in the art.

Fatty aldehydes

The fatty aldehydes may be saturated fatty aldehydes or desaturated fatty aldehydes or a mixture thereof. Fatty aldehydes, in particular desaturated fatty aldehydes, are also
30 naturally comprised within pheromones, in particular insect pheromones.

In one embodiment, the fatty aldehyde has a chain length of 8. In another embodiment, the fatty aldehyde has a chain length of 9. In another embodiment, the fatty aldehyde
35 has a chain length of 10. In another embodiment, the fatty aldehyde has a chain length

of 11. In another embodiment, the fatty aldehyde has a chain length of 12. In another embodiment, the fatty aldehyde has a chain length of 13. In another embodiment, the fatty aldehyde has a chain length of 14. In another embodiment, the fatty aldehyde has a chain length of 15. In another embodiment, the fatty aldehyde has a chain length of 16. In another embodiment, the fatty aldehyde has a chain length of 17. In another embodiment, the fatty aldehyde has a chain length of 18. In another embodiment, the fatty aldehyde has a chain length of 19. In another embodiment, the fatty aldehyde has a chain length of 20. In another embodiment, the fatty aldehyde has a chain length of 21. In another embodiment, the fatty aldehyde has a chain length of 22.

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In some embodiments, the fatty aldehyde is a desaturated fatty aldehyde. The desaturated fatty aldehyde may have a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22, such as:

- (Z)- Δ^3 desaturated fatty aldehydes having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ^3 desaturated fatty aldehydes having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ^5 desaturated fatty aldehydes having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ^5 desaturated fatty aldehydes having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ^6 desaturated fatty aldehydes having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ^6 desaturated fatty aldehydes having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ^7 desaturated fatty aldehydes having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ^7 desaturated fatty aldehydes having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ^8 desaturated fatty aldehydes having a carbon chain length of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ^8 desaturated fatty aldehydes having a carbon chain length of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ^9 desaturated fatty aldehydes having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;

- (E)- Δ 9 desaturated fatty aldehydes having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ 10 desaturated fatty aldehydes having a carbon chain length of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 5 - (E)- Δ 10 desaturated fatty aldehydes having a carbon chain length of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ 11 desaturated fatty aldehydes having a carbon chain length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 10 - (E)- Δ 11 desaturated fatty aldehydes having a carbon chain length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (Z)- Δ 12 desaturated fatty aldehydes having a carbon chain length of 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (E)- Δ 12 desaturated fatty aldehydes having a carbon chain length of 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- 15 - (Z)- Δ 13 desaturated fatty aldehydes having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22; and
- (E)- Δ 13 desaturated fatty aldehydes having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22.

- 20 In some embodiments, the fatty aldehydes are desaturated fatty aldehydes having a carbon chain length of 14, such as:
 - (Z)- Δ 5 desaturated fatty aldehydes having a carbon chain length of 14;
 - (E)- Δ 5 desaturated fatty aldehydes having a carbon chain length of 14;
 - (Z)- Δ 6 desaturated fatty aldehydes having a carbon chain length of 14;
 - 25 - (E)- Δ 6 desaturated fatty aldehydes having a carbon chain length of 14;
 - (Z)- Δ 7 desaturated fatty aldehydes having a carbon chain length of 14;
 - (E)- Δ 7 desaturated fatty aldehydes having a carbon chain length of 14;
 - (Z)- Δ 8 desaturated fatty aldehydes having a carbon chain length of 14;
 - (E)- Δ 8 desaturated fatty aldehydes having a carbon chain length of 14;
 - 30 - (Z)- Δ 9 desaturated fatty aldehydes having a carbon chain length of 14;
 - (E)- Δ 9 desaturated fatty aldehydes having a carbon chain length of 14;
 - (Z)- Δ 10 desaturated fatty aldehydes having a carbon chain length of 14;
 - (E)- Δ 10 desaturated fatty aldehydes having a carbon chain length of 14;
 - (Z)- Δ 11 desaturated fatty aldehydes having a carbon chain length of 14;
 - 35 - (E)- Δ 11 desaturated fatty aldehydes having a carbon chain length of 14;
 - (Z)- Δ 12 desaturated fatty aldehydes having a carbon chain length of 14;

- (E)- Δ 12 desaturated fatty aldehydes having a carbon chain length of 14;
- (Z)- Δ 13 desaturated fatty aldehydes having a carbon chain length of 14; and
- (E)- Δ 13 desaturated fatty aldehydes having a carbon chain length of 14.

5 In some embodiments, the fatty aldehydes are desaturated fatty aldehydes having a carbon chain length of 16, such as:

- (Z)- Δ 5 desaturated fatty aldehydes having a carbon chain length of 16;
- (E)- Δ 5 desaturated fatty aldehydes having a carbon chain length of 16;
- (Z)- Δ 6 desaturated fatty aldehydes having a carbon chain length of 16;
- 10 - (E)- Δ 6 desaturated fatty aldehydes having a carbon chain length of 16;
- (Z)- Δ 7 desaturated fatty aldehydes having a carbon chain length of 16;
- (E)- Δ 7 desaturated fatty aldehydes having a carbon chain length of 16;
- (Z)- Δ 8 desaturated fatty aldehydes having a carbon chain length of 16;
- (E)- Δ 8 desaturated fatty aldehydes having a carbon chain length of 16;
- 15 - (Z)- Δ 9 desaturated fatty aldehydes having a carbon chain length of 16;
- (E)- Δ 9 desaturated fatty aldehydes having a carbon chain length of 16;
- (Z)- Δ 10 desaturated fatty aldehydes having a carbon chain length of 16;
- (E)- Δ 10 desaturated fatty aldehydes having a carbon chain length of 16;
- (Z)- Δ 11 desaturated fatty aldehydes having a carbon chain length of 16;
- 20 - (E)- Δ 11 desaturated fatty aldehydes having a carbon chain length of 16;
- (Z)- Δ 12 desaturated fatty aldehydes having a carbon chain length of 16;
- (E)- Δ 12 desaturated fatty aldehydes having a carbon chain length of 16;
- (Z)- Δ 13 desaturated fatty aldehydes having a carbon chain length of 16; and
- (E)- Δ 13 desaturated fatty aldehydes having a carbon chain length of 16.

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The desaturated fatty aldehydes produced may be desaturated in more than one position. The desaturated fatty aldehydes may be desaturated in at least two positions, such as at least three positions, such as four positions.

30 For example, the fatty aldehyde is an (E)7, (Z)9 desaturated fatty aldehyde having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22, such as 14. In some embodiments, the fatty aldehyde is an (E)3, (Z)8, (Z)11 desaturated fatty aldehyde having a carbon chain length of 14. In some embodiments, the fatty aldehyde is a (Z)9, (E)11, (E)13 desaturated fatty aldehydes having a carbon chain length of 14, 15,

35 16, 17, 18, 19, 20, 21 or 22, such as 14. In some embodiments, the desaturated fatty aldehyde is an (E)7, (Z)9 desaturated fatty aldehyde having a carbon chain length of

12. In other embodiments, the fatty aldehyde is an (*E*)3, (*Z*)8, (*Z*)11 desaturated fatty aldehydes having a carbon chain length of 12. In some embodiments, the fatty aldehyde is a (*Z*)9, (*E*)11, (*E*)13 desaturated fatty aldehyde having a carbon chain length of 12.

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In a particular embodiment, the fatty aldehyde is (*Z*)-11-hexadecenal.

The fatty aldehydes may be produced by the microorganism in the fermentation, e.g. where the microorganism is capable of converting a fatty alcohol to the corresponding fatty aldehyde, or they may be obtained by chemical conversion as is known in the art.

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Terpenes and terpenoids

Terpenes are naturally produced by plants, and have a number of industrial applications in the field of food, pharmaceuticals, cosmetics and biotechnology. They are for example used as part of natural agricultural pesticides. Terpenoids (also termed isoprenoids) are modified terpenes containing additional groups, usually O-containing groups. They are often used for their aromatic qualities and as part of traditional herbal remedies.

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In some embodiments of the present methods, the hydrophobic compound is a terpene, such as a hemiterpene, a monoterpene, a sesquiterpene, a disesterterpene, a triterpene, a sesquaraterpene, a tetraterpene, or a polyterpene. In some embodiments, the terpene is a monoterpene such as geraniol, terpineol, limonene, myrcene, linalool, pinene or menthol. In some embodiments, the terpene is a sesquiterpene such as humulene, farnesene or farnesol. In some embodiments, the terpene is a triterpene such as squalene. In some embodiments, the terpene is a tetraterpene such as lycopene, and carotenes such as α -carotene, β -carotene and γ -carotene.

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In some embodiments the hydrophobic compound is a terpene such as a terpenoid, such as a hemiterpenoid, a monoterpenoid, a sesquiterpenoid, a disesterterpenoid, a triterpenoid, a sesquaraterpenoid, a tetraterpenoid or a polyterpenoid. In some embodiments, the terpenoid is a monoterpenoid such as monocyclic monoterpenoids, e.g. menthol, thymol or carvacrol, or bicyclic monoterpenoids, for example camphor, borneol or eucalyptol. In some embodiments, the terpenoid is a sesquiterpenoid such as geosmin, vetivazulene, guaiazulene or farnesol. In some embodiments, the terpenoid is

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a diterpenoid such as a taxene, retinol or phytol. In some embodiments, the terpenoid is a triterpenoid such as a steroid, for example a sterol or a cucurbitacin. In some embodiments the terpenoid is a tetraterpenoid such as a carotenoid.

5 **Microorganism**

The present methods are useful for recovering hydrophobic compounds produced in a fermentation by a microorganism, and/or for increasing the titer of the hydrophobic compound and/or for increasing the secretion of the hydrophobic compound from the microorganism. Preferably, the microorganism is a yeast.

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The microorganism may be a bacteria or a eukaryote. In some embodiments, the microorganism is a yeast cell.

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In some embodiments, the microorganism or the yeast cell has been modified at the genomic level, e.g. by gene editing in the genome. The cell may also be modified by insertion of at least one nucleic acid construct such as at least one vector. The vector may be designed as is known to the skilled person to either enable integration of nucleic acid sequences in the genome, or to enable expression of a polypeptide encoded by a nucleic acid sequence comprised in the vector without genome integration. In other embodiments, the microorganism is a natural producer of the desired hydrophobic compound, for example a yeast which naturally produces a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate, a fatty aldehyde and a terpene.

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In certain embodiments of the disclosure, yeast or fungi of genera including, but not limited to, *Blakeslea*, *Candida*, *Cryptococcus*, *Cunninghamella*, *Lipomyces*, *Mortierella*, *Mucor*, *Phycomyces*, *Pythium*, *Rhodosporidium*, *Rhodotorula*, *Trichosporon*, *Saccharomyces* and *Yarrowia* are employed. In certain particular embodiments, organisms of species that include, but are not limited to, *Blakeslea trispora*, *Candida pulcherrima*, *C. revkaufi*, *C. tropicalis*, *Cryptococcus curvatus*, *Cunninghamella echinulata*, *C. elegans*, *C. japonica*, *Lipomyces starkeyi*, *L. lipoferus*, *Mortierella alpina*, *M. isabellina*, *M. rammanniana*, *M. vinacea*, *Mucor circinelloides*, *Phycomyces blakesleanus*, *Pythium irregulare*, *Rhodosporidium toruloides*, *Rhodotorula glutinis*, *R. gracilis*, *R. graminis*, *R. mucilaginosa*, *R. pinicola*, *Trichosporon pullans*, *T. cutaneum*, *Saccharomyces cerevisiae* and *Yarrowia lipolytica* are used. In preferred embodiments, the microorganism is a yeast, in particular *Yarrowia lipolytica* or *Saccharomyces cerevisiae*.

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Several microorganisms, in particular yeast cells, have been described which can produce hydrophobic compounds, in particular fatty alcohols, fatty acyl acetates and fatty aldehydes, which can be formulated in pheromone compositions and used as pest repellants. The present methods can be employed in fermentation processes where such yeast cells are cultivated to produce such compounds and facilitate their recovery, increase their titer and/or increase their secretion from the cell. Such yeast cells and the resulting products are described in detail in e.g. WO 2016/207339, WO 2018/109163, WO 2018/109167, international application PCT/EP2020/053306 and EP application 19218703.7 filed on 20 December 2019 by same applicant and entitled "Yeast cells and methods for production of *E8,E10*-dodecadienyl coenzyme A, codlemone and derivatives thereof".

In general, yeast cells useful for production of such compounds rely on the expression of several enzymes, particularly heterologous enzymes, for example a desaturase such as a $\Delta 11$ desaturase (EC 1.14.19.5), a fatty acyl reductase (FAR) (EC 1.2.1.84), a fatty acyl-CoA synthetase (FAA) (EC 2.3.1.86), an acetyltransferase (EC 2.3.1.84) or an acyl-CoA oxidase (EC 1.3.3.6).

Herein below are described some specific embodiments.

Desaturated fatty alcohols

In some embodiments, the microorganism is yeast cell such as an oleaginous yeast cell and the hydrophobic compound is a desaturated fatty alcohol. The yeast cell, for example a *Yarrowia* cell such as a *Yarrowia lipolytica* cell, capable of producing the desaturated fatty alcohol:

- expresses at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and
- expresses at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol; and
- has a mutation resulting in reduced activity of Fao1 (SEQ ID NO: 11) and a mutation resulting in reduced activity of at least one of Hfd1 (SEQ ID NO: 12), Hfd4 (SEQ ID NO: 13), Pex10 (SEQ ID NO: 14) and GPAT (SEQ ID NO: 15) or has

a mutation resulting in reduced activity of at least one protein having at least 90% homology to Fao1 (SEQ ID NO: 11) and a mutation resulting in reduced activity of at least one of Hfd1 (SEQ ID NO: 12), Hfd4 (SEQ ID NO: 13), Pex10 (SEQ ID NO: 14) and GPAT (SEQ ID NO: 15), such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Fao1 (SEQ ID NO: 11) and at least one of Hfd1 (SEQ ID NO: 12), Hfd4 (SEQ ID NO: 13), Pex10 (SEQ ID NO: 14) and GPAT (SEQ ID NO: 15).

Throughout the present disclosure, it will be understood that mutations resulting in reduced activity of a protein or enzyme are preferably mutations in the genes encoding said protein or enzyme. The mutation is preferably in the promoter of the gene, or in the coding sequence of the gene, or both.

The desaturase is preferably selected from the group consisting of a $\Delta 3$ desaturase, a $\Delta 5$ desaturase, a $\Delta 6$ desaturase, a $\Delta 7$ desaturase, a $\Delta 8$ desaturase, a $\Delta 9$ desaturase, a $\Delta 10$ desaturase, a $\Delta 11$ desaturase, a $\Delta 12$ desaturase, a $\Delta 13$ desaturase and a $\Delta 14$ desaturase, preferably wherein the desaturase is derived from an insect, such as from the *Lepidoptera* order, preferably the desaturase is a $\Delta 11$ desaturase having at least 60% homology to the $\Delta 11$ desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 1 or a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 16. In some embodiments, the fatty acyl reductase is selected from:

- i) a FAR having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 5;
 - ii) a FAR having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 7;
 - iii) a FAR having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 6; and
 - iv) a FAR having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 17,
- preferably the FAR has at least 80% homology to the FAR from *Helicoverpa armigera* or to the FAR from *Heliothis subflexa*.

Such yeast cells are well suited for producing hydrophobic compounds as defined herein, in particular desaturated fatty alcohols, fatty acyl acetates and fatty aldehydes, and are described in detail in WO 2016/207339.

- 5 In some embodiments, the microorganism is a yeast cell such as a *Yarrowia* cell, for example a *Yarrowia lipolytica* cell, capable of producing said desaturated fatty alcohol, said yeast cell expressing:
- at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA having a carbon chain length of 14; and
 - 10 - at least one heterologous fatty acyl-CoA reductase (FAR), capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol.

Preferably, the desaturase in such embodiments has a higher specificity towards tetradecanoyl-CoA than towards hexadecanoyl-CoA and/or wherein the fatty acyl-CoA reductase has a higher specificity towards desaturated tetradecanoyl-CoA than towards desaturated hexadecanoyl-CoA. Such yeast cells are well suited for producing desaturated fatty alcohols of carbon chain length 14, and are described in detail in WO 2018/109167.

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In such embodiments, the at least one heterologous desaturase may be derived from an organism selected from *Pelargonium hortorum*, *Ricinus communis*, *Drosophila melanogaster*, *Spodoptera litura* and *Tribolium castaneum*, preferably the desaturase is derived from *Drosophila melanogaster*, preferably the at least one heterologous desaturase is selected from the group consisting of:

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- i) a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 16;
- ii) a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Spodoptera litura* as set forth in SEQ ID NO: 18;
- 30 iii) a desaturase having at least 60% homology to the desaturase from *Lobesia botrana* as set forth in SEQ ID NO: 43;
- iv) a desaturase having at least 60% homology to the desaturase from *Drosophila grimshawi* as set forth in SEQ ID NO: 44; and
- v) a desaturase having at least 60% homology to the desaturase from *Drosophila virilis* as set forth in SEQ ID NO: 45,

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- vi) a $\Delta 11$ desaturase having at least 60% homology to the $\Delta 11$ desaturase from *Choristoneura parallela* as set forth in SEQ ID NO: 42;
- vii) a $\Delta 11$ desaturase having at least 60% homology to the $\Delta 11$ desaturase from *Choristoneura rosaceana* as set forth in SEQ ID NO: 35.

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A desaturase having at least 60% homology to a given desaturase has at least 60% homology, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72% homology, such as at least 73% homology, such as at least 74% homology, such as at least 75% homology, such as at least 76% homology, such as at least 77% homology, such as at least 78% homology, such as at least 79% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology.

The fatty acyl reductase may be selected from:

- i) a FAR having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 5;
- ii) a FAR having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 7;
- iii) a FAR having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 6; and
- iv) a FAR having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 17,

preferably the FAR is a FAR having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 5.

A FAR having at least 80% homology to a given FAR has at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology.

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In some embodiments, the hydrophobic compound is a desaturated fatty alcohol and the microorganism is a yeast cell capable of producing said desaturated fatty alcohol, which yeast cell:

- has one or more mutations resulting in reduced activity of one or more native acyl-CoA oxidases; and
- expresses at least one first group of enzymes comprising at least one acyl-CoA oxidase capable of oxidising a fatty acyl-CoA, wherein the first group of enzymes is capable of shortening a fatty acyl-CoA of a first carbon chain length X to a shortened fatty acyl-CoA having a second carbon chain length X' , wherein $X' \leq X-2$; and
- expresses at least one heterologous desaturase capable of introducing at least one double bond in said fatty acyl-CoA and/or in said shortened fatty acyl-CoA; and
- expresses at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol.

Such yeast cells are described in detail in application WO 2020/169389.

The native acyl-CoA oxidase and/or the heterologous acyl-CoA oxidase may be a peroxisomal acyl-CoA oxidase. In some embodiments, the at least one acyl-CoA oxidase of the first group of enzymes is a native acyl-CoA oxidase or a heterologous acyl-CoA oxidase, which may be overexpressed compared to a reference yeast strain not expressing said at least one first group of enzymes. In some embodiments, the at least one acyl-CoA oxidase of the first group of enzymes is a heterologous acyl-CoA oxidase. In some embodiments, the at least one first group of enzymes comprises an

acyl-CoA oxidase derived from an organism of a genus selected from *Yarrowia*, *Agrotis*, *Arabidopsis*, *Aspergillus*, *Cucurbita*, *Homo*, *Paenarthrobacter* and *Rattus*. Preferably the at least one first group of enzymes comprises an acyl-CoA oxidase derived from *Yarrowia lipolytica*, *Agrotis segetum*, *Arabidopsis thaliana*, *Aspergillus nidulans*, *Cucurbita maxima*, *Homo sapiens*, *Paenarthrobacter ureafaciens* or *Rattus norvegicus*. In particular embodiments, preferably the at least one acyl-CoA oxidase of the first group of enzymes is an acyl-CoA oxidase selected from the group consisting of Yli_POX1 (SEQ ID NO: 19), Yli_POX2 (SEQ ID NO: 20), Yli_POX3 (SEQ ID NO: 21), Yli_POX4 (SEQ ID NO: 22), Yli_POX5 (SEQ ID NO: 23), Yli_POX6 (SEQ ID NO: 24), Ase_POX (SEQ ID NO: 25), Ath_POX1 (SEQ ID NO: 26), Ath_POX2 (SEQ ID NO: 27), Ani_POX (SEQ ID NO: 28), Cma_POX (SEQ ID NO: 29), Hsa_POX1-2 (SEQ ID NO: 30), Pur_POX (SEQ ID NO: 31), and Rno_POX2 (SEQ ID NO: 32), or a functional variant thereof having at least 60% homology thereto, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology thereto.

In some embodiments, the at least one heterologous desaturase is selected from the group consisting of a $\Delta 3$ desaturase, a $\Delta 5$ desaturase, a $\Delta 6$ desaturase, a $\Delta 7$ desaturase, a $\Delta 8$ desaturase, a $\Delta 9$ desaturase, a $\Delta 10$ desaturase, a $\Delta 11$ desaturase, a $\Delta 12$ desaturase, a $\Delta 13$ desaturase and a $\Delta 14$ desaturase, and/or wherein the desaturase is derived from a yeast such as *Saccharomyces* or *Yarrowia*, such as *Saccharomyces cerevisiae* or *Yarrowia lipolytica*, or from an insect, such as from the *Diptera*, the *Coleoptera*, or the *Lepidoptera* order, such as of the genus *Amyelois*, *Choristoneura*, *Drosophila*, *Ostrinia*, *Thaumetopoea*, *Dendrophilus*, *Grapholita*, *Cydia*, *Epiphyas*, or *Spodoptera*, such as *Drosophila melanogaster*, *Amyelois transitella*, *Choristoneura rosaceana*, *Ostrinia nubilalis*, *Thaumetopoea pityocampa*, *Dendrophilus punctatus*, *Grapholita molesta*, *Cydia pomonella*, *Epiphyas postvittana*, *Spodoptera littoralis* or *Choristoneura parallela*. For example, the desaturase is a Δ_{Z9} -desaturase such as Sce_OLE1 (SEQ ID NO: 33), Yli_OLE1 (SEQ ID NO: 34) or Dme_D9 (SEQ ID NO: 16), a Δ_{Z11} -desaturase such as Atr_D11 (SEQ ID NO: 1), Cro_Z11 (SEQ ID NO: 35), Onu_11 (SEQ ID NO: 36), Tpi_D13 (SEQ ID NO: 37), a Δ_{E9} -desaturase such as Dpu_E9-14 (SEQ ID NO: 38), a $\Delta_{Z/E10}$ -desaturase such as Gmo_CPRQ (SEQ ID NO:

39), or a desaturase such as Epo_E11 (SEQ ID NO: 40), Sls_ZE11 (SEQ ID NO: 41), Lbo_PPTQ (SEQ ID NO: 43), Dgd9 (SEQ ID NO: 44), Dvd9 (SEQ ID NO: 45) or Cpa_E11 (SEQ ID NO: 42), or a functional variant thereof having at least 60% homology thereto, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology thereto.

In some embodiments, the fatty acyl-CoA reductase is derived from an insect such as an insect of the *Lepidoptera* order, such as of the genus *Helicoverpa*, *Heliothis* or *Bicyclus*, preferably the fatty acyl-CoA reductase is a fatty acyl-CoA reductase native to *Helicoverpa armigera*, *Helicoverpa assulta*, *Heliothis subflexa*, *Bicyclus anynana*, or a functional variant thereof, preferably the fatty acyl-CoA reductase is selected from the group consisting of a fatty acyl-CoA reductase having at least 80% homology to Har_FAR (SEQ ID NO: 5), Has_FAR (SEQ ID NO: 7), Ban_FAR (SEQ ID NO: 17) or Hs_FAR (SEQ ID NO: 6).

The yeast cell producing the desaturated fatty alcohols may further express a fatty acyl synthetase (FAA) such as Sc_FAA1 (SEQ ID NO: 8) or YI_FAA (SEQ ID NO: 9) or a variant thereof having at least 75% homology, such as at least 80% homology, such as at least 85% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology, such as 100% homology to Sc_FAA1 (SEQ ID NO: 8) or YI_FAA (SEQ ID NO: 9).

The microorganism may be further modified to express an acetyltransferase such as a heterologous acetyltransferase (AcT) or to overexpress a native acetyltransferase, wherein said acetyltransferase is capable of converting at least part of the produced desaturated fatty alcohols into the corresponding fatty acyl acetates. In some embodiments the acetyltransferase is Sc_Atf1 (SEQ ID NO: 10) or a variant thereof having at least 75% homology, such as at least 80% homology, such as at least 85% homology,

such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology, such as 100% homology to Sc_Atf1 (SEQ ID NO: 10).

The desaturated fatty alcohols may also be converted to the corresponding fatty acyl acetates by chemical conversion, for example by performing an acetylation reaction using the desaturated fatty alcohols produced by the cell as substrate.

(Z)-11-hexadecen-1-ol

In some embodiments, the hydrophobic compound is (Z)-11-hexadecen-1-ol. In some embodiments, the microorganism is a yeast cell capable of producing (Z)-11-hexadecen-1-ol with a titer of at least 0.2 mg/L. The yeast cell expresses:

- 15 - a $\Delta 11$ -desaturase selected from the group consisting of the *Amyelois transitella* $\Delta 11$ -desaturase (Atr_ $\Delta 11$; SEQ ID NO: 1), the *Spodoptera littoralis* $\Delta 11$ -desaturase (SI_ $\Delta 11$; SEQ ID NO: 2), the *Agrotis segetum* $\Delta 11$ -desaturase (As_ $\Delta 11$; SEQ ID NO: 3) and the *Trichoplusia ni* $\Delta 11$ -desaturase (Tni_ $\Delta 11$; SEQ ID NO: 4) or a variant thereof having at least 65% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%,
20 such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 95%, such as 100% homology to Atr_ $\Delta 11$ (SEQ ID NO: 1), SI_ $\Delta 11$ (SEQ ID NO: 2), As_ $\Delta 11$ (SEQ ID NO: 3), or Tni_ $\Delta 11$ (SEQ ID NO: 4), and
- 25 - an alcohol-forming fatty acyl-CoA reductase (FAR) selected from the group consisting of Har_FAR (SEQ ID NO: 5), Hs_FAR (SEQ ID NO: 6), and Has_FAR (SEQ ID NO: 7), or a variant thereof having at least 80% homology, such as at least 85%, such as at least 90%, such as at least 95%, such as 100% homology to Har_FAR (SEQ ID NO: 5), Hs_FAR (SEQ ID NO: 6), or Has_FAR (SEQ
30 ID NO: 7);
 whereby
- the $\Delta 11$ -desaturase is capable of converting at least part of said hexadecanoyl-CoA to (Z)11-hexadecenoyl-CoA; and

- the FAR is capable of converting at least part of said (Z)11-hexadecenoyl-CoA to (Z)-11-hexadecenol. In some embodiments, the yeast cell is a *Saccharomyces cerevisiae* cell.

5 The yeast cell producing (Z)-11-hexadecen-1-ol may further express a fatty acyl synthetase (FAA) such as Sc_FAA1 (SEQ ID NO: 8) or Yl_FAA (SEQ ID NO: 9) or a variant thereof having at least 75% homology, such as at least 80% homology, such as at least 85% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94%
10 homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology, such as 100% homology to Sc_FAA1 (SEQ ID NO: 8) or Yl_FAA (SEQ ID NO: 9).

The microorganism may be further modified to express an acetyltransferase such as a
15 heterologous acetyltransferase (AcT) or to overexpress a native acetyltransferase, wherein said acetyltransferase is capable of converting at least part of the (Z)-11-hexadecen-1-ol into (Z)11-hexadecen-1-yl acetate. In some embodiments the acetyltransferase is Sc_Atf1 (SEQ ID NO: 10) or a variant thereof having at least 75% homology, such as at least 80% homology, such as at least 85% homology, such as at least 90%
20 homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology, such as 100% homology to Sc_Atf1 (SEQ ID NO: 10).

25 (Z)-11-hexadecen-1-ol may also be converted to (Z)11-hexadecen-1-yl acetate by chemical conversion, for example by performing an acetylation reaction using the (Z)11-hexadecen-1-ol produced by the cell as substrate.

30 Such yeast cells are well suited for producing hydrophobic compounds as defined herein, in particular desaturated fatty alcohols, fatty acyl acetates and fatty aldehydes, and are described in detail in WO 2016/207339.

Codlemone

In some embodiments, the hydrophobic compound is codlemone (*E8,E10*-dodecadien-1-ol), or one or more of its derivatives *E8,E10*-dodecadienyl acetate and/or *E8,E10*-dodecadienal.

5

Yeast cells capable of producing codlemone or one or more of its derivatives preferably express at least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty acyl-CoA, wherein at least part of said desaturated fatty acyl-CoA is *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA), and further express at least one heterologous fatty acyl-CoA reductase (EC 1.2.1.84) capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, wherein the fatty acyl-CoA reductase is capable of converting at least part of said *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA) to *E8,E10*-dodecadien-1-ol.

Such yeast cells are described in detail in European application 19218703.7, entitled "Yeast cells and methods for production of *E8,E10*-dodecadienyl coenzyme A, codlemone and derivatives thereof" filed on 20 December 2019 by the same applicant as the present application. This application describes desaturases and fatty acyl-CoA reductases which are particularly useful for production of codlemone and its derivatives, in particular in the section entitled "Desaturase" (p. 12 to 16 of EP 19218703.7) and in the section entitled "Fatty acyl-CoA reductase (EC 1.2.1.84)" (p. 16 to 20 of EP 19218703.7). Codlemone can be further converted to *E8,E10*-dodecadienyl acetate; this can be done *ex vivo*, as is known in the art, e.g. by chemical conversion, or it can be done *in vivo* by the action of an acetyltransferase (EC 2.3.1.84) capable of converting at least part of the *E8,E10*-dodecadien-1-ol produced by the cell into *E8,E10*-dodecadienyl acetate, as described in the section entitled "Production of *E8,E10*-dodecadienyl acetate" (p. 37-38 of EP 19218703.7). It may also be of interest to further convert at least part of the *E8,E10*-dodecadien-1-ol produced by the cell into *E8,E10*-dodecadienal. This can be done by chemical conversion or by further engineering the yeast cell, for example as described in the section entitled "Production of *E8,E10*-dodecadienal" (p. 39-40 of EP 19218703.7).

Method for producing a hydrophobic compound

Herein are disclosed methods for producing a hydrophobic compound, which may be any of the hydrophobic compounds described herein above. The methods comprise the step of providing a microorganism capable of producing said hydrophobic compound,

and culturing said microorganism in a culture medium under conditions allowing production of said hydrophobic compound, wherein the culture medium comprises an extractant in an amount equal to or greater than its cloud concentration measured in an aqueous solution, preferably at the cultivation temperature, or at room temperature. As
5 detailed above, such agents are routinely used in fermentations for foam management, however when used as antifoaming agents the agents are used at a concentration lower than the cloud concentration measured in an aqueous solution. Preferably the microorganism is a yeast. The extractant is a non-ionic surfactant, in particular a non-ionic ethoxylated surfactant, such as a fatty alcohol alkoxylate, preferably selected
10 from: Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof, or a polyethoxylated surfactant such as an antifoaming agent, for example a polyethoxylated surfactant selected from: a polyeth-
15 ylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof. The method may also further comprise a step of recovering the hydrophobic compound from the fermentation
20 broth.

The present methods are particularly useful for facilitating recovery of hydrophobic compounds produced by fermentation of a microorganism capable of producing these compounds, for example any of the microorganisms described in the above section
25 "Microorganism". The hydrophobic compound may be any compound described in the above section "Hydrophobic compound", in particular a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate, a fatty aldehyde and/or a terpene such as a terpenoid.

The present inventors have found that when a non-ionic surfactant, in particular a non-ionic ethoxylated surfactant which is preferably a fatty alcohol alkoxylate or a polyeth-
30 oxylated surfactant, such as an antifoaming agent, in particular any of the non-ionic surfactants and antifoaming agents described in the above section "Non-ionic ethoxylated surfactant", is included in the culture medium or fermentation broth in an amount equal to or greater than its cloud concentration measured in an aqueous solution, pref-
35 erably at the cultivation temperature, the non-ionic surfactant acts as an *in situ* extractant and facilitates recovery of the hydrophobic compound from the fermentation broth.

Accordingly, herein is provided a method for producing a hydrophobic compound such as a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate, a fatty aldehyde and/or a terpene such as a terpenoid in a fermentation, said method comprising the step of

5 providing a microorganism capable of producing said hydrophobic compound and culturing said microorganism in a culture medium under conditions allowing production of said hydrophobic compound, wherein the culture medium comprises an extractant in an amount equal to or greater than its cloud concentration measured in an aqueous so-

10 lution, wherein the extractant is a non-ionic surfactant such as an antifoaming agent, preferably a polyethoxylated surfactant selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof, the method optionally further comprising the step

15 of recovering the hydrophobic compound from the fermentation broth. Hence is provided herein a method for producing a hydrophobic compound selected from a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate, a fatty aldehyde and a terpene in a fermentation, said method comprising the step of providing a yeast cell capable of producing said hydrophobic compound and culturing said yeast cell in a culture medium under

20 conditions allowing production of said hydrophobic compound, wherein the culturing step is performed at a cultivation temperature, wherein the culture medium comprises an extractant in an amount equal to or greater than its cloud concentration measured in an aqueous solution such as the culture medium at the cultivation temperature, wherein the extractant is a non-ionic ethoxylated surfactant, the method further comprising the step of recovering the hydrophobic compound.

25

In some embodiments, the hydrophobic compound is a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate or a fatty aldehyde as described herein. In other embodiments, the hydrophobic compound is a terpene such as a terpenoid as described herein. In

30 some embodiments, the hydrophobic compound is a mixture of hydrophobic compounds, such as a mixture of fatty alcohols, fatty acyl acetates, fatty aldehydes and/or terpenes such as terpenoids as described herein. In particular embodiments, the hydrophobic compound is a desaturated fatty alcohol, a desaturated fatty acyl acetate or a desaturated fatty aldehyde as described herein.

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In some embodiments, the non-ionic surfactant is a non-ionic ethoxylated surfactant, for example a fatty alcohol alkoxylate, preferably selected from: Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof, or a non-ionic polyethoxylated surfactant, for example an antifoaming agent. The antifoaming agent is preferably a polyethoxylated surfactant, such as a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents, or a combination thereof.

In some embodiments, the non-ionic surfactant is added in an amount greater than its cloud concentration measured in an aqueous solution. In some embodiments, the non-ionic ethoxylated surfactant is added in an amount greater than its cloud concentration measured in an aqueous solution. In some embodiments, the polyethoxylated surfactant is added in an amount greater than its cloud concentration in an aqueous solution. In some embodiments, the fatty alcohol alkoxylate is added in an amount greater than its cloud concentration measured in an aqueous solution. The cloud concentration may be determined in the cultivation medium, for example at room temperature or at the cultivation temperature, as detailed herein elsewhere.

In some embodiments, the non-ionic surfactant is present in an amount greater than its cloud concentration by at least 50%, such as at least 100%, such as at least 150%, such as at least 200%, such as at least 250%, such as at least 300%, such as at least 350%, such as at least 400%, such as at least 500%, such as at least 750%, such as at least 1000%, or more. Preferably the cloud concentration is determined in the cultivation medium, for example at room temperature or at the cultivation temperature.

In some embodiments, the non-ionic surfactant is a non-ionic ethoxylated surfactant present in an amount greater than its cloud concentration by at least 50%, such as at least 100%, such as at least 150%, such as at least 200%, such as at least 250%, such as at least 300%, such as at least 350%, such as at least 400%, such as at least 500%, such as at least 750%, such as at least 1000%, or more. Preferably the cloud concentration is determined in the cultivation medium, for example at room temperature or at the cultivation temperature.

In some embodiments, the non-ionic surfactant is a fatty alcohol alkoxylate present in an amount greater than its cloud concentration by at least 50%, such as at least 100%, such as at least 150%, such as at least 200%, such as at least 250%, such as at least 300%, such as at least 350%, such as at least 400%, such as at least 500%, such as at least 750%, such as at least 1000%, or more. Preferably the cloud concentration is determined in the cultivation medium, for example at room temperature or at the cultivation temperature. In some embodiments, the fatty alcohol alkoxylate is selected from: Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof.

In some embodiments, the non-ionic surfactant is a polyethoxylated surfactant, which is present in an amount greater than its cloud concentration by at least 50%, such as at least 100%, such as at least 150%, such as at least 200%, such as at least 250%, such as at least 300%, such as at least 350%, such as at least 400%, such as at least 500%, such as at least 750%, such as at least 1000%, or more. Preferably the cloud concentration is determined in the cultivation medium, for example at room temperature or at the cultivation temperature. In some embodiments the polyethoxylated surfactant is selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof.

In some embodiments, the amount of non-ionic surfactant (extractant) is at least 2-fold its cloud concentration, such as at least 3-fold its cloud concentration, such as at least 4-fold its cloud concentration, such as at least 5-fold its cloud concentration, such as at least 6-fold its cloud concentration, such as at least 7-fold its cloud concentration, such as at least 8-fold its cloud concentration, such as at least 9-fold its cloud concentration, such as at least 10-fold its cloud concentration, such as at least 12.5-fold its cloud concentration, such as at least 15-fold its cloud concentration, such as at least 17.5-fold its cloud concentration, such as at least 20-fold its cloud concentration, such as at least 25-fold its cloud concentration, such as at least 30-fold its cloud concentration. In some embodiments the polyethoxylated surfactant is selected from: a polyethylene polypro-

pylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof. Preferably the cloud concentration is determined
5 in the cultivation medium, for example at room temperature or at the cultivation temperature.

In some embodiments, the amount of non-ionic ethoxylated surfactant (extractant) is at least 2-fold its cloud concentration, such as at least 3-fold its cloud concentration, such as at least 4-fold its cloud concentration, such as at least 5-fold its cloud concentration, such as at least 6-fold its cloud concentration, such as at least 7-fold its cloud concentration, such as at least 8-fold its cloud concentration, such as at least 9-fold its cloud concentration, such as at least 10-fold its cloud concentration, such as at least 12.5-fold its cloud concentration, such as at least 15-fold its cloud concentration, such as at least 17.5-fold its cloud concentration, such as at least 20-fold its cloud concentration, such as at least 25-fold its cloud concentration, such as at least 30-fold its cloud concentration. In some embodiments the ethoxylated surfactant is a fatty alcohol alkoxylate. Preferably the cloud concentration is determined in the cultivation medium, for example at room temperature or at the cultivation temperature.

20 In some embodiments, the non-ionic surfactant is a polyethoxylated surfactant, and the amount of polyethoxylated surfactant (extractant) is at least 2-fold its cloud concentration, such as at least 3-fold its cloud concentration, such as at least 4-fold its cloud concentration, such as at least 5-fold its cloud concentration, such as at least 6-fold its cloud concentration, such as at least 7-fold its cloud concentration, such as at least 8-fold its cloud concentration, such as at least 9-fold its cloud concentration, such as at least 10-fold its cloud concentration, such as at least 12.5-fold its cloud concentration, such as at least 15-fold its cloud concentration, such as at least 17.5-fold its cloud concentration, such as at least 20-fold its cloud concentration, such as at least 25-fold its cloud concentration, such as at least 30-fold its cloud concentration. In some embodiments the polyethoxylated surfactant is selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof. Preferably the cloud concentration is determined
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in the cultivation medium, for example at room temperature or at the cultivation temperature.

5 In some embodiments, the non-ionic surfactant is a fatty alcohol alkoxylate, and the amount of fatty alcohol alkoxylate (extractant) is at least 2-fold its cloud concentration, such as at least 3-fold its cloud concentration, such as at least 4-fold its cloud concentration, such as at least 5-fold its cloud concentration, such as at least 6-fold its cloud concentration, such as at least 7-fold its cloud concentration, such as at least 8-fold its cloud concentration, such as at least 9-fold its cloud concentration, such as at least 10-
10 fold its cloud concentration, such as at least 12.5-fold its cloud concentration, such as at least 15-fold its cloud concentration, such as at least 17.5-fold its cloud concentration, such as at least 20-fold its cloud concentration, such as at least 25-fold its cloud concentration, such as at least 30-fold its cloud concentration. In some embodiments the fatty alcohol alkoxylate is selected from: Plurafac® LF300 (CAS number 196823-
15 11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof. Preferably the cloud concentration is determined in the cultivation medium, for example at room temperature or at the cultivation temperature.

20 In some embodiments, the culture medium comprises at least 1% vol/vol extractant, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%,
25 such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol extractant, wherein the extractant is a non-ionic surfactant, preferably a non-ionic ethoxylated surfactant such as a fatty alcohol alkoxylate or a non-ionic polyethoxylated surfactant. In some embodiments the non-ionic surfactant is a polyethoxylated surfactant, such as selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monos-
30 tearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof. In some embodiments the fatty alcohol alkoxylate is selected from: Plu-
35 rafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and

Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof.

5 In some embodiments, the non-ionic ethoxylated surfactant is an ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agent or an ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agent, for example, C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate (CAS number 68002-96-0). The cloud concentration of C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate (CAS number 68002-96-0) is about 1% vol/vol at room temperature. Accordingly, when this antifoaming agent is used, the culture medium preferably
10 comprises at least 1% vol/vol of C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such
15 as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate, or more.

In some embodiments, the non-ionic ethoxylated surfactant is a polyethylene polypropylene glycol, for example Kolliphor® P407 (CAS number 9003-11-6), also termed
20 poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol). The cloud concentration of Kolliphor® P407 is 10% at a temperature above 100°C. Accordingly, when a polyethylene polypropylene glycol such as Kolliphor® P407 is used, the culture medium preferably comprises at least 10% vol/vol of polyethylene polypropylene glycol such as Kolliphor® P407, such as at least 11% vol/vol, such as at least 12%
25 vol/vol, such as at least 13% vol/vol, such as at least 14% vol/vol, such as at least 15% vol/vol, such as at least 16% vol/vol, such as at least 17% vol/vol, such as at least 18% vol/vol, such as at least 19% vol/vol, such as at least 20% vol/vol, such as at least 25% vol/vol, such as at least 30% vol/vol, such as at least 35% vol/vol of polyethylene polypropylene glycol such as Kolliphor® P407, or more.

30 In some embodiments, the non-ionic ethoxylated surfactant is a mixture of polyether dispersions, such as antifoam 204 (product number A6426 or A8311 from Sigma Aldrich). The cloud concentration of antifoam 204 is 1% in an aqueous solution at a temperature of 18.0 to 21.0°C. Accordingly, when a mixture of polyether dispersions such
35 as antifoam 204 is used, the culture medium preferably comprises at least 1% vol/vol of a mixture of polyether dispersions such as antifoam 204, such as at least 1.5%, such

as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%,
5 such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol of a mixture of polyether dispersions such as antifoam 204, or more.

In some embodiments, the non-ionic ethoxylated surfactant is Agnique BP420 (CAS number 68002-96-0). The cloud concentration of Agnique BP420 (CAS number 68002-96-0) is 1% in an aqueous solution at a temperature of 18.0 to 21.0°C. Accordingly,
10 when a mixture of polyether dispersions such as antifoam 204 is used, the culture medium preferably comprises at least 1% vol/vol of Agnique BP420 (CAS number 68002-96-0), such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%,
15 such as at least 30% vol/vol of a mixture of Agnique BP420 (CAS number 68002-96-0), or more.

In some embodiments, the non-ionic ethoxylated surfactant is an antifoaming agent comprising polyethylene glycol monostearate or simethicone. Simethicone comprises polyethylene glycol monostearate, which, without being bound by theory, appears to be the compound important for the ability of simethicone to act as an extractant. Polyethylene glycol monostearate has a cloud point of 1% in an aqueous solution at 5°C. Accordingly, when simethicone or a surfactant comprising polyethylene glycol monostearate is used, the culture medium preferably comprises at least 1% vol/vol of polyethylene glycol monostearate or simethicone, such as at least 1.5%, such as at least 2%,
20 such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol polyethylene glycol monostearate or simethicone, or more.

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In some embodiments, the non-ionic surfactant is a fatty alcohol alkoxylate such as Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2) or Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574. The cloud concentration of these surfactants is about 1% vol/vol at room temperature. Accordingly, when Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2) or Imbentin SG/251 (CAS number 68002-96-0) is used, the culture medium preferably comprises at least 1% vol/vol of Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2) or Imbentin SG/251 (CAS number 68002-96-0), such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2) or Imbentin SG/251 (CAS number 68002-96-0), or more.

The fermentation itself may be performed as is known in the art. In some embodiments, the fermentation is performed in a bioreactor. The fermentation is conducted under conditions that allow the microorganism present in the fermentation to produce the hydrophobic compound of interest.

The addition of an extractant, i.e. a non-ionic ethoxylated surfactant, preferably a fatty alcohol alkoxylate or a polyethoxylated surfactant such as any of the antifoaming agents described herein, results in the generation of an emulsion in the fermentation broth, where the hydrophobic compound produced by the microorganism, preferably a yeast cell, is present in the emulsion. The method thus may also comprise a step of breaking the emulsion to recover a product phase comprising the extractant and the hydrophobic compound. Once the emulsion is broken, the fermentation broth is separated in three phases: a water phase, comprising mainly water and aqueous compounds, a phase comprising cells and cellular debris, and a product phase mainly comprising the extractant and the hydrophobic compound. Thus a composition is obtained

consisting of three phases. In preferred embodiments, most of the hydrophobic compound of the fermentation broth is present in the product phase. For example, at least 50% of the hydrophobic compound is present in the product phase, such as at least 55%, such as at least 60%, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the hydrophobic compound is present in the product phase. In some embodiments, the product phase comprises at least 50% of the hydrophobic compound initially present in the fermentation broth, such as at least 55%, such as at least 60%, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the hydrophobic compound initially present in the fermentation broth.

The step of breaking the emulsion may be performed as is known in the art, for example by submitting the emulsion to a step of phase separation as is known in the art. In some embodiments, the step of phase separation is a centrifugation, for example 5 minutes at 10 000 g. In some embodiments, the centrifugation is performed for 1 minute or more, such as for 2 minutes or more, such as for 3 minutes or more, such as for 4 minutes or more, such as for 5 minutes or more, such as for 6 minutes or more, such as for 7 minutes or more, such as for 8 minutes or more, such as for 9 minutes or more, such as for 10 minutes or more. In some embodiments, the centrifugation is performed at 3 000 g or more, such as at 4 000 g or more, such as at 5 000 g or more, such as at 6 000 g or more, such as at 7 000 g or more, such as at 8 000 g or more, such as at 9 000 g or more, such as at 10 000 g or more, such as at 11 000 g or more, such as at 12 000 g or more, such as at 13 000 g or more, such as at 14 000 g or more, such as at 15 000 g or more, such as at 17 500 g or more, such as at 20 000 g or more.

Following the step of breaking the emulsion, the product phase comprising the extractant and the hydrophobic compound may be recovered from the composition. The method may in such embodiments further comprise the step of separating the hydrophobic compound from the extractant. This can be performed by methods known in the

art, such as by distillation, for example a distillation under reduced pressure, or a column purification. The extractant may be recycled, e.g. it may be recirculated back to the fermentation.

- 5 In some embodiments, the method involves culturing a microorganism capable of producing a fatty alcohol, such as a desaturated fatty alcohol or a mixture of (saturated and/or desaturated) fatty alcohols. In some embodiments, the method involves culturing a yeast cell capable of producing a fatty alcohol, such as a desaturated fatty alcohol or a mixture of (saturated and/or desaturated) fatty alcohols. The desaturated fatty alcohol may be recovered as described above. In such embodiments, the method may further comprise a step of recovering the produced fatty alcohol and chemically converting at least part thereof to the corresponding fatty acyl acetate and/or to the corresponding fatty aldehyde. The term "corresponding" here refers to a compound, fatty acyl acetate or fatty aldehyde, having the same carbon chain length and double bond position(s) as the fatty alcohol it is obtained from.
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Thus, when a microorganism such as a yeast cell produces fatty alcohols, the methods may further comprise the step of recovering said fatty alcohols, for example as described above, and chemically converting at least part of the fatty alcohols to the corresponding fatty acyl acetates. This can be done by performing an acetylation reaction as is known in the art, for example as described in Fritz et al., 1959, or Mattson et al., 1964. The methods may additionally or alternatively comprise the step of chemically converting at least part of the fatty alcohols to the corresponding fatty aldehydes. This can be done by performing an oxidation reaction as is known in the art, for example as described in Steves et al., 2013. The resulting fatty acyl acetates and/or fatty aldehydes may then be recovered.

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Acetylation for example may be carried out with acetic anhydride using pyridine as catalyst. The resulting fatty acetates are then extracted from the reaction mix with an organic solvent and the solvent is removed by evaporation.

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Oxidation may for example be carried out using known procedures for the oxidation of primary alcohols including, but not limited to, those published by Hoover et al., 2011, using Tetrakisacetonitrile copper(I) triflate/TEMPO catalyst system, Omura et al. (1978), Corey et al. (1972), Ratcliffe et al. (1970), Ley et al. (1994), or Anelli et al. (1987). The resulting fatty aldehydes are then extracted from the reaction mix with an

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organic solvent and the solvent can be removed by evaporation and the aldehydes are purified using distillation or column chromatography.

Method for increasing the titer of a hydrophobic compound in a fermentation

- 5 Herein are disclosed methods for increasing the titer of a hydrophobic compound in a fermentation. The methods comprise the step of culturing a microorganism capable of producing said hydrophobic compound in a culture medium under conditions allowing production of said hydrophobic compound, wherein the culture medium comprises an extractant in an amount equal to or greater than its cloud concentration measured in an
- 10 aqueous solution. Preferably, the microorganism is a yeast cell. Preferably, the cloud concentration is determined at room temperature or at the cultivation temperature. The extractant is a non-ionic surfactant, in particular a non-ionic ethoxylated surfactant, preferably selected from a fatty alcohol alkoxylate, preferably selected from Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac®
- 15 SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2) or Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and a polyethoxylated surfactant, such as an antifoaming agent, for example a polyethoxylated non-ionic surfactant selected from: a polyethylene polypropylene gly-
- 20 col, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof. The method may also further comprise a step of re-
- 25 covering the hydrophobic compound from the fermentation broth.
- 30 The present methods are particularly useful for increasing the titer of hydrophobic compounds produced by fermentation of a microorganism, for example a yeast cell, capable of producing these compounds, for example any of the microorganisms described in the above section "Microorganism". The hydrophobic compound may be any compound described in the above section "Hydrophobic compound", in particular a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate, a fatty aldehyde and a terpene such as
- 35 a terpenoid. The presence of a non-ionic surfactant, in particular a non-ionic ethoxylated surfactant, preferably selected from a fatty alcohol alkoxylate such as Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2) or Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon®

- 2574, or an antifoaming agent, in particular a polyethoxylated surfactant, to the culture medium, results in an increase in the titer of the hydrophobic compound compared to the titer obtained in a fermentation performed in similar conditions but with an amount of non-ionic surfactant which is lower than its cloud concentration. Thus the present
- 5 methods are useful for increasing the titer of the hydrophobic compound compared to a fermentation performed under the same conditions but either in the absence of extractant or in the presence of extractant in an amount lower than its cloud concentration in an aqueous solution at the cultivation temperature or at room temperature.
- 10 In some embodiments, the hydrophobic compound is a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate or a fatty aldehyde as described herein. In other embodiments, the hydrophobic compound is a terpene such as a terpenoid as described herein. In some embodiments, the hydrophobic compound is a mixture of hydrophobic compounds, such as a mixture of fatty alcohols, fatty alcohol esters, fatty acyl acetates,
- 15 fatty aldehydes and terpenes such as terpenoids as described herein. In particular embodiments, the hydrophobic compound is a desaturated fatty alcohol, a desaturated fatty alcohol ester, a desaturated fatty acyl acetate or a desaturated fatty aldehyde as described herein.
- 20 The non-ionic surfactant is preferably a non-ionic ethoxylated surfactant or a fatty alcohol alkoxylate, preferably selected from Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2) or Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, or an antifoaming agent such as a
- 25 polyethoxylated surfactant, for example selected from: polyethoxylated non-ionic surfactants, such as a polyethylene polypropylene glycol, mixtures of polyether dispersions, antifoaming agents comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents, or a combination thereof.
- 30 In some embodiments, the non-ionic surfactant or the non-ionic ethoxylated surfactant is added in an amount greater than its cloud concentration measured in an aqueous solution, preferably at room temperature or at the cultivation temperature. In some embodiments, the non-ionic ethoxylated surfactant, preferably a fatty alcohol alkoxylate or

a polyethoxylated surfactant, is added in an amount greater than its cloud concentration measured in an aqueous solution, preferably at room temperature or at the cultivation temperature.

5 In some embodiments, the non-ionic surfactant is present in an amount greater than its cloud concentration by at least 50%, such as at least 100%, such as at least 150%, such as at least 200%, such as at least 250%, such as at least 300%, such as at least 350%, such as at least 400%, such as at least 500%, such as at least 750%, such as at least 1000%, or more. The cloud concentration may be determined in the cultivation
10 medium, for example at room temperature or at the cultivation temperature.

In some embodiments, the non-ionic surfactant is an antifoaming agent such as a polyethoxylated surfactant. The polyethoxylated surfactant is then preferably present in an amount greater than its cloud concentration by at least 50%, such as at least 100%,
15 such as at least 150%, such as at least 200%, such as at least 250%, such as at least 300%, such as at least 350%, such as at least 400%, such as at least 500%, such as at least 750%, such as at least 1000%, or more. In some embodiments the polyethoxylated surfactant is selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monos-
20 tearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof. The cloud concentration may be determined in the cultivation medium, for example at room temperature or at the cultivation temperature.

25 In some embodiments, the non-ionic surfactant is a fatty alcohol alkoxylate such as Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2) or Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574. The fatty alcohol alkoxylate is then preferably present in an amount
30 greater than its cloud concentration by at least 50%, such as at least 100%, such as at least 150%, such as at least 200%, such as at least 250%, such as at least 300%, such as at least 350%, such as at least 400%, such as at least 500%, such as at least 750%, such as at least 1000%, or more. In some embodiments the fatty alcohol alkoxylate is selected from: Plurafac® LF300 (CAS number 196823-11-7), Plurafac®
35 LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2) or Imbentin SG/251 (CAS number 68002-96-0), preferably

Plurafac® LF300 or Dehypon® 2574, and combinations thereof. The cloud concentration may be determined in the cultivation medium, for example at room temperature or at the cultivation temperature.

5 In some embodiments, the amount of non-ionic surfactant (extractant) is at least 2-fold its cloud concentration, such as at least 3-fold its cloud concentration, such as at least 4-fold its cloud concentration, such as at least 5-fold its cloud concentration, such as at least 6-fold its cloud concentration, such as at least 7-fold its cloud concentration, such as at least 8-fold its cloud concentration, such as at least 9-fold its cloud concentration,
10 such as at least 10-fold its cloud concentration, such as at least 12.5-fold its cloud concentration, such as at least 15-fold its cloud concentration, such as at least 17.5-fold its cloud concentration, such as at least 20-fold its cloud concentration, such as at least 25-fold its cloud concentration, such as at least 30-fold its cloud concentration. The cloud concentration may be determined in the cultivation medium, for example at room
15 temperature or at the cultivation temperature.

In some embodiments the non-ionic surfactant is a polyethoxylated surfactant. In some embodiments the amount of polyethoxylated surfactant (extractant) is at least 2-fold its cloud concentration, such as at least 3-fold its cloud concentration, such as at least 4-
20 fold its cloud concentration, such as at least 5-fold its cloud concentration, such as at least 6-fold its cloud concentration, such as at least 7-fold its cloud concentration, such as at least 8-fold its cloud concentration, such as at least 9-fold its cloud concentration, such as at least 10-fold its cloud concentration, such as at least 12.5-fold its cloud concentration, such as at least 15-fold its cloud concentration, such as at least 17.5-fold its
25 cloud concentration, such as at least 20-fold its cloud concentration, such as at least 25-fold its cloud concentration, such as at least 30-fold its cloud concentration. In some embodiments the polyethoxylated surfactant is selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈
30 alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof. The cloud concentration may be determined in the cultivation medium, for example at room temperature or at the cultivation temperature.

35 In some embodiments the non-ionic surfactant is a fatty alcohol alkoxylate. In some embodiments the amount of fatty alcohol alkoxylate (extractant) is at least 2-fold its

cloud concentration, such as at least 3-fold its cloud concentration, such as at least 4-fold its cloud concentration, such as at least 5-fold its cloud concentration, such as at least 6-fold its cloud concentration, such as at least 7-fold its cloud concentration, such as at least 8-fold its cloud concentration, such as at least 9-fold its cloud concentration, such as at least 10-fold its cloud concentration, such as at least 12.5-fold its cloud concentration, such as at least 15-fold its cloud concentration, such as at least 17.5-fold its cloud concentration, such as at least 20-fold its cloud concentration, such as at least 25-fold its cloud concentration, such as at least 30-fold its cloud concentration. In some embodiments the fatty alcohol alkoxylate is selected from: Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2) or Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof. The cloud concentration may be determined in the cultivation medium, for example at room temperature or at the cultivation temperature.

In some embodiments, the culture medium comprises at least 1% vol/vol extractant, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol extractant, wherein the extractant is a non-ionic surfactant. In some embodiments the non-ionic surfactant is a non-ionic ethoxylated surfactant such as a polyethoxylated surfactant or a fatty alcohol alkoxylate. In some embodiments the polyethoxylated surfactant is selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof. In some embodiments the fatty alcohol alkoxylate is selected from: Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2) or Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof.

In some embodiments, the non-ionic surfactant is an antifoaming agent. In some embodiments, the antifoaming agent is an ethoxylated and propoxylated C₁₆-C₁₈ alcohol-

based agent or an ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agent, for example, C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate (CAS number 68002-96-0). The cloud concentration of C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate (CAS number 68002-96-0) is about 1% vol/vol at room temperature. Accordingly, when this

5 antifoaming agent is used, the culture medium preferably comprises at least 1% vol/vol of C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%,

10 such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate, or more.

In some embodiments, the antifoaming agent is a polyethylene polypropylene glycol, for example Kolliphor® P407 (CAS number 9003-11-6), also termed poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol). The cloud concentration of Kolliphor® P407 is 10% at a temperature above 100°C. Accordingly, when a polyethylene polypropylene glycol such as Kolliphor® P407 is used, the culture medium preferably comprises at least 10% vol/vol of polyethylene polypropylene glycol such as Kol-

15 liphor® P407, such as at least 11% vol/vol, such as at least 12% vol/vol, such as at least 13% vol/vol, such as at least 14% vol/vol, such as at least 15% vol/vol, such as at least 16% vol/vol, such as at least 17% vol/vol, such as at least 18% vol/vol, such as at least 19% vol/vol, such as at least 20% vol/vol, such as at least 25% vol/vol, such as at least 30% vol/vol, such as at least 35% vol/vol of polyethylene polypropylene glycol

20 such as Kolliphor® P407, or more.

In some embodiments, the antifoaming agent is a mixture of polyether dispersions, such as antifoam 204 (product number A6426 or A8311 from Sigma Aldrich). The cloud concentration of antifoam 204 is 1% in an aqueous solution at a temperature of 18.0 to

30 21.0°C. Accordingly, when a mixture of polyether dispersions such as antifoam 204 is used, the culture medium preferably comprises at least 1% vol/vol of a mixture of polyether dispersions such as antifoam 204, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%,

35 such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at

least 25%, such as at least 27.5%, such as at least 30% vol/vol of a mixture of polyether dispersions such as antifoam 204, or more.

5 In some embodiments, the antifoaming agent is Agnique BP420 (CAS number 68002-96-0). The cloud concentration of Agnique BP420 (CAS number 68002-96-0) is 1% in an aqueous solution at a temperature of 18.0 to 21.0°C. Accordingly, when Agnique BP420 (CAS number 68002-96-0) is used, the culture medium preferably comprises at least 1% vol/vol of a mixture of polyether dispersions such as antifoam 204, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol of Agnique BP420 (CAS number 68002-96-0), or more.

15 In some embodiments, the antifoaming agent is an antifoaming agent comprising polyethylene glycol monostearate or simethicone. Simethicone comprises polyethylene glycol monostearate, which, without being bound by theory, appears to be the compound important for the ability of simethicone to act as an extractant. Polyethylene glycol monostearate has a cloud point of 1% in an aqueous solution at 5°C. Accordingly, when simethicone or a surfactant comprising polyethylene glycol monostearate is used, the culture medium preferably comprises at least 1% vol/vol of polyethylene glycol monostearate or simethicone, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol polyethylene glycol monostearate or simethicone, or more.

30 In some embodiments, the extractant is a fatty alcohol alkoxylate, preferably selected from: Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof. These fatty alcohol alkoxylates have a cloud point of 1% in an aqueous solution at room temperature. Accordingly, when a

fatty alcohol alkoxylate, preferably selected from: Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof, is used, the culture medium preferably comprises at least 1% vol/vol of said fatty alcohol alkoxylate, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol fatty alcohol alkoxylate, preferably selected from: Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof.

The microorganism, which is preferably a yeast cell, used in the present methods may already be engineered or selected for producing the hydrophobic compound at a high titer. The present methods may further increase the titer. In some embodiments, the titer of the hydrophobic compound is increased by at least 5% compared to the titer obtained in a fermentation performed under similar conditions in the absence of extractant or in the presence of extractant in an amount lower than its cloud concentration in an aqueous solution, such as by at least 10%, such as by at least 15%, such as by at least 20%, such as by at least 25%, such as by at least 30%, such as by at least 35%, such as by at least 40%, such as by at least 45%, such as by at least 46%, such as by at least 47%, such as by at least 48%, such as by at least 49%, such as by at least 50%, such as by at least 51%, such as by at least 52%, such as by at least 53%, such as by at least 54%, such as by at least 55% or more. The term "similar conditions" here refers to a fermentation of the same microorganism or yeast cell, which is performed under the same conditions but either in the absence of extractant or in the presence of extractant in an amount lower than its cloud concentration in an aqueous solution at the cultivation temperature or at room temperature. In some embodiments, the hydrophobic compound is a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate and/or a fatty aldehyde. In some embodiments, the hydrophobic compound is a terpene such as a terpenoid.

Thus, when a microorganism such as a yeast cell produces fatty alcohols, the methods may further comprise the step of recovering said fatty alcohols, for example as described above, and chemically converting at least part of the fatty alcohols to the corresponding fatty acyl acetates. This can be done by performing an acetylation reaction as is known in the art, for example as described in Fritz et al., 1959, or Mattson et al., 1964. The methods may additionally or alternatively comprise the step of chemically converting at least part of the fatty alcohols to the corresponding fatty aldehydes. This can be done by performing an oxidation reaction as is known in the art, for example as described in Steves et al., 2013. The resulting fatty acyl acetates and/or fatty aldehydes may then be recovered.

Acetylation may be carried out with acetic anhydride using pyridine as catalyst. The resulting fatty acetates are then extracted from the reaction mix with an organic solvent and the solvent is removed by evaporation.

Oxidation may be carried out using known procedures for the oxidation of primary alcohols including, but not limited to, those published by Hoover et al., 2011, using Tetrakisacetonitrile copper(I) triflate/TEMPO catalyst system, Omura et al. (1978), Corey et al. (1972), Ratcliffe et al. (1970), Ley et al. (1994), or Anelli et al. (1987). The resulting fatty aldehydes are then extracted from the reaction mix with an organic solvent and the solvent may be removed by evaporation and the aldehydes are purified using distillation or column chromatography.

Method for increasing the secretion of a hydrophobic compound in a fermentation

Herein are disclosed methods for increasing the secretion of a hydrophobic compound in a fermentation. The methods comprise the step of culturing a microorganism capable of producing said hydrophobic compound in a culture medium under conditions allowing production of said hydrophobic compound, wherein the culture medium comprises an extractant in an amount equal to or greater than its cloud concentration measured in an aqueous solution. The methods thus preferably comprise culturing a yeast cell in a culture medium under conditions allowing production of said hydrophobic compound, wherein the culturing step is performed at a cultivation temperature, wherein the culture

medium comprises an extractant in an amount equal to or greater than its cloud concentration measured in an aqueous solution at the cultivation temperature, wherein the extractant is a non-ionic ethoxylated surfactant, whereby the secretion of the hydrophobic compound from the yeast cell is increased compared to a fermentation performed

5 under the same conditions but either in the absence of extractant or in the presence of extractant in an amount lower than its cloud concentration in an aqueous solution at the cultivation temperature. The extractant is a non-ionic surfactant, preferably a fatty alcohol alkoxylate, preferably selected from: Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), De-

10 hypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof, or a non-ionic ethoxylated surfactant such as an antifoaming agent, for example a polyethoxylated surfactant selected from: Agnique BP420 (CAS number 68002-96-0), a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent

15 comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof. The method may also further comprise a step of recovering the hydrophobic compound from the fermentation broth. The methods thus result in an increased secretion compared to a fermentation

20 performed under similar conditions in the absence of extractant or in the presence of extractant in an amount lower than its cloud concentration in an aqueous solution. The term "similar conditions" here refers to a fermentation of the same microorganism or yeast cell, which is performed under the same conditions but either in the absence of extractant or in the presence of extractant in an amount lower than its cloud concentration

25 in an aqueous solution at the cultivation temperature or at room temperature.

The present methods are particularly useful for increasing the secretion of hydrophobic compounds produced by fermentation of a microorganism capable of producing these compounds, preferably a yeast cell, or for example any of the microorganisms described in the above section "Microorganism". The hydrophobic compound may be any

30 compound described in the above section "Hydrophobic compound", in particular a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate, a fatty aldehyde and a terpene such as a terpenoid. The presence of a non-ionic surfactant, in particular a non-ionic ethoxylated surfactant such as an antifoaming agent, preferably a fatty alcohol alkox-

35 ylate or a polyethoxylated surfactant, in the culture medium, results in an increase in the secretion of the hydrophobic compound from the microorganism compared to the

secretion observed in a fermentation performed in similar conditions but with an amount of non-ionic surfactant which is lower than its cloud concentration.

5 In some embodiments, the hydrophobic compound is a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate or a fatty aldehyde as described herein. In other embodiments, the hydrophobic compound is a terpene such as a terpenoid as described herein. In some embodiments, the hydrophobic compound is a mixture of hydrophobic compounds, such as a mixture of fatty alcohols, fatty acyl acetates, fatty aldehydes and/or terpenes such as terpenoids as described herein. In particular embodiments, the hydrophobic compound is a desaturated fatty alcohol, a desaturated fatty acyl acetate or
10 a desaturated fatty aldehyde as described herein.

The non-ionic surfactant is a non-ionic ethoxylated surfactant which may be an anti-foaming agent. The antifoaming agent is preferably a polyethoxylated non-ionic surfactant, such as a polyethylene polypropylene glycol, a mixtures of polyether dispersions,
15 an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents, or a combination thereof.

20 In some embodiments, the non-ionic surfactant is a non-ionic ethoxylated surfactant, preferably selected from a fatty alcohol alkoxylate and a polyethoxylated surfactant, and is added in an amount greater than its cloud concentration measured in an aqueous solution. In some embodiments, the non-ionic ethoxylated surfactant is a polyethoxylated surfactant and is added in an amount greater than its cloud concentration
25 measured in an aqueous solution. The cloud concentration may be determined in the cultivation medium, for example at room temperature or at the cultivation temperature.

In some embodiments, the non-ionic surfactant is a non-ionic ethoxylated surfactant present in an amount greater than its cloud concentration by at least 50%, such as at
30 least 100%, such as at least 150%, such as at least 200%, such as at least 250%, such as at least 300%, such as at least 350%, such as at least 400%, such as at least 500%, such as at least 750%, such as at least 1000%, or more. The cloud concentration may be determined in the cultivation medium, for example at room temperature or at the cultivation temperature.

35

In some embodiments, the non-ionic surfactant is a non-ionic ethoxylated surfactant preferably selected from a fatty alcohol alkoxylate and a polyethoxylated surfactant and is present in an amount greater than its cloud concentration by at least 50%, such as at least 100%, such as at least 150%, such as at least 200%, such as at least 250%, such as at least 300%, such as at least 350%, such as at least 400%, such as at least 500%, such as at least 750%, such as at least 1000%, or more. In some embodiments the polyethoxylated surfactant is selected from: Agnique BP420 (CAS number 68002-96-0), a polyethylene polypropylene glycol, a mixture of polyether dispersions, an anti-foaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof. In some embodiments, the fatty alcohol alkoxylate is selected from: Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof. The cloud concentration may be determined in the cultivation medium, for example at room temperature or at the cultivation temperature.

In some embodiments, the amount of non-ionic ethoxylated surfactant is at least 2-fold its cloud concentration, such as at least 3-fold its cloud concentration, such as at least 4-fold its cloud concentration, such as at least 5-fold its cloud concentration, such as at least 6-fold its cloud concentration, such as at least 7-fold its cloud concentration, such as at least 8-fold its cloud concentration, such as at least 9-fold its cloud concentration, such as at least 10-fold its cloud concentration, such as at least 12.5-fold its cloud concentration, such as at least 15-fold its cloud concentration, such as at least 17.5-fold its cloud concentration, such as at least 20-fold its cloud concentration, such as at least 25-fold its cloud concentration, such as at least 30-fold its cloud concentration. The cloud concentration may be determined in the cultivation medium, for example at room temperature or at the cultivation temperature.

In some embodiments, the non-ionic surfactant is a polyethoxylated surfactant. In some embodiments the amount of polyethoxylated surfactant (extractant) is at least 2-fold its cloud concentration, such as at least 3-fold its cloud concentration, such as at least 4-fold its cloud concentration, such as at least 5-fold its cloud concentration, such as at least 6-fold its cloud concentration, such as at least 7-fold its cloud concentration, such as at least 8-fold its cloud concentration, such as at least 9-fold its cloud concentration,

such as at least 10-fold its cloud concentration, such as at least 12.5-fold its cloud concentration, such as at least 15-fold its cloud concentration, such as at least 17.5-fold its cloud concentration, such as at least 20-fold its cloud concentration, such as at least 25-fold its cloud concentration, such as at least 30-fold its cloud concentration. In some
5 embodiments the polyethoxylated surfactant is selected from: Agnique BP420 (CAS number 68002-96-0), a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof.
10 The cloud concentration may be determined in the cultivation medium, for example at room temperature or at the cultivation temperature.

In some embodiments, the non-ionic surfactant is a fatty alcohol alkoxylate. In some
15 embodiments the amount of fatty alcohol alkoxylate (extractant) is at least 2-fold its cloud concentration, such as at least 3-fold its cloud concentration, such as at least 4-fold its cloud concentration, such as at least 5-fold its cloud concentration, such as at least 6-fold its cloud concentration, such as at least 7-fold its cloud concentration, such as at least 8-fold its cloud concentration, such as at least 9-fold its cloud concentration, such as at least 10-fold its cloud concentration, such as at least 12.5-fold its cloud concentration, such as at least 15-fold its cloud concentration, such as at least 17.5-fold its cloud concentration, such as at least 20-fold its cloud concentration, such as at least 25-fold its cloud concentration, such as at least 30-fold its cloud concentration. In some
20 embodiments the fatty alcohol alkoxylate is selected from: Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof. The cloud concentration may be determined in the cultivation medium, for example at room temperature or at the cultivation temperature.

30 In some embodiments, the culture medium comprises at least 1% vol/vol extractant, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%,
35 such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at

- least 30% vol/vol extractant, wherein the extractant is a non-ionic ethoxylated surfactant such as a fatty alcohol alkoxylate or a polyethoxylated surfactant. In some embodiments the polyethoxylated surfactant is selected from: Agnique BP420 (CAS number 68002-96-0), a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof. In some embodiments the fatty alcohol alkoxylate is selected from: Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof. The cloud concentration may be determined in the cultivation medium, for example at room temperature or at the cultivation temperature.
- In some embodiments, the non-ionic surfactant is an antifoaming agent. In some embodiments the antifoaming agent is an ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agent or an ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agent, for example, C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate (CAS number 68002-96-0). The cloud concentration of C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate (CAS number 68002-96-0) is about 1% vol/vol at room temperature. Accordingly, when this antifoaming agent is used, the culture medium preferably comprises at least 1% vol/vol of C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate, or more.
- In some embodiments, the antifoaming agent is a polyethylene polypropylene glycol, for example Kolliphor® P407 (CAS number 9003-11-6), also termed poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol). The cloud concentration of Kolliphor® P407 is 10% at a temperature above 100°C. Accordingly, when a polyethylene polypropylene glycol such as Kolliphor® P407 is used, the culture medium preferably comprises at least 10% vol/vol of polyethylene polypropylene glycol such as Kolliphor® P407, such as at least 11% vol/vol, such as at least 12% vol/vol, such as at

least 13% vol/vol, such as at least 14% vol/vol, such as at least 15% vol/vol, such as at least 16% vol/vol, such as at least 17% vol/vol, such as at least 18% vol/vol, such as at least 19% vol/vol, such as at least 20% vol/vol, such as at least 25% vol/vol, such as at least 30% vol/vol, such as at least 35% vol/vol of polyethylene polypropylene glycol
5 such as Kolliphor® P407, or more.

In some embodiments, the non-ionic surfactant is an antifoaming agent. In some embodiments the antifoaming agent is Agnique BP420 (CAS number 68002-96-0). The cloud concentration of Agnique BP420 (CAS number 68002-96-0) is about 1% vol/vol
10 at room temperature. Accordingly, when this antifoaming agent is used, the culture medium preferably comprises at least 1% vol/vol of Agnique BP420 (CAS number 68002-96-0), such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at
15 least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol Agnique BP420 (CAS number 68002-96-0), or more.

In some embodiments, the antifoaming agent is a mixture of polyether dispersions,
20 such as antifoam 204 (product number A6426 or A8311 from Sigma Aldrich). The cloud concentration of antifoam 204 is 1% in an aqueous solution at a temperature of 18.0 to 21.0°C. Accordingly, when a mixture of polyether dispersions such as antifoam 204 is used, the culture medium preferably comprises at least 1% vol/vol of a mixture of polyether dispersions such as antifoam 204, such as at least 1.5%, such as at least 2%,
25 such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol of a mixture of polyether dispersions such as antifoam 204, or more.
30

In some embodiments, the antifoaming agent is an antifoaming agent comprising polyethylene glycol monostearate or simethicone. Simethicone comprises polyethylene glycol monostearate, which, without being bound by theory, appears to be the compound
35 important for the ability of simethicone to act as an extractant. Polyethylene glycol monostearate has a cloud point of 1% in an aqueous solution at 5°C. Accordingly,

when simethicone or a surfactant comprising polyethylene glycol monostearate is used, the culture medium preferably comprises at least 1% vol/vol of polyethylene glycol monostearate or simethicone, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol polyethylene glycol monostearate or simethicone, or more.

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In some embodiments, the extractant is a fatty alcohol alkoxylate, preferably selected from: Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof. These fatty alcohol alkoxylates have a cloud point of 1% in an aqueous solution at room temperature. Accordingly, when a fatty alcohol alkoxylate, preferably selected from: Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof, is used, the culture medium preferably comprises at least 1% vol/vol of said fatty alcohol alkoxylate, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol fatty alcohol alkoxylate, preferably selected from: Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof.

The microorganism used in the present methods may already be engineered or selected for producing the hydrophobic compound. Preferably the microorganism is a yeast cell. The present methods may further help increase the secretion of the hydrophobic compound. In some embodiments, the secretion of the hydrophobic compound

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from the cell is increased by at least 5% compared to the secretion observed in a fermentation performed under similar conditions in the absence of extractant or in the presence of extractant in an amount lower than its cloud concentration in an aqueous solution, such as by at least 5%, such as by at least 7.5%, such as by at least 10%,
5 such as by at least 12.5%, such as by at least 15%, such as by at least 20%, such as by at least 25%, such as by at least 30%, such as by at least 35%, such as by at least 36%, such as by at least 37%, such as by at least 38%, such as by at least 39%, such as by at least 40%, such as by at least or more. The term "similar conditions" here refers to a fermentation of the same microorganism or yeast cell, which is performed under the same conditions but either in the absence of extractant or in the presence of
10 extractant in an amount lower than its cloud concentration in an aqueous solution at the cultivation temperature or at room temperature.

In some embodiments, the hydrophobic compound is a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate and/or a fatty aldehyde. In some embodiments, the hydrophobic
15 compound is a terpene such as a terpenoid.

Thus, when a microorganism, in particular a yeast, produces fatty alcohols, the methods may further comprise the step of recovering said fatty alcohols, for example as described above, and chemically converting at least part of the fatty alcohols to the corresponding fatty acyl acetates. This can be done by performing an acetylation reaction as
20 is known in the art, for example as described in Fritz et al., 1959, or Mattson et al., 1964. The methods may additionally or alternatively comprise the step of chemically converting at least part of the fatty alcohols to the corresponding fatty aldehydes. This
25 can be done by performing an oxidation reaction as is known in the art, for example as described in Steves et al., 2013. The resulting fatty acyl acetates and/or fatty aldehydes may then be recovered.

For example, acetylation may be carried out with acetic anhydride using pyridine as
30 catalyst. The resulting fatty acetates are then extracted from the reaction mix with an organic solvent and the solvent is removed by evaporation.

Oxidation may for example be carried out using known procedures for the oxidation of primary alcohols including, but not limited to, those published by Hoover et al., 2011,
35 using Tetrakisacetone nitrile copper(I) triflate/TEMPO catalyst system, Omura et al. (1978), Corey et al. (1972), Ratcliffe et al. (1970), Ley et al. (1994), or Anelli et al.

(1987). The resulting fatty aldehydes are then extracted from the reaction mix with an organic solvent and the solvent is removed by evaporation and the aldehydes are purified using distillation or column chromatography.

5 *Product phase comprising the hydrophobic compound***

The fermentation itself may be performed as is known in the art. In some embodiments, the fermentation is performed in a bioreactor. The fermentation is conducted under conditions that allow the microorganism present in the fermentation to produce the hydrophobic compound of interest. Such conditions which are suitable for production of a hydrophobic compound such as a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate, a fatty aldehyde and/or a terpene such as a terpenoid by a yeast cell are readily available to the skilled person. Suitable microorganisms, in particular yeast cells, are known in the art, e.g. such yeast cells are described in e.g. WO 2016/207339, WO 2018/109163, WO 2018/109167, international application PCT/EP2020/053306 and EP application 19218703.7, and have also been described herein.

The addition of an extractant, i.e. a non-ionic surfactant, in particular a non-ionic ethoxylated surfactant, preferably selected from a fatty alcohol alkoxylate or a polyethoxylated surfactant, for example any of the non-ionic surfactants, non-ionic ethoxylated surfactants, antifoaming agents or polyethoxylated surfactants described herein, results in the generation of an emulsion in the fermentation broth, where the hydrophobic compound produced by the microorganism is present in the emulsion. Similarly, the addition of such surfactants in a concentration equal to or greater than their cloud concentration measured in an aqueous solution, for example at the cultivation temperature or at room temperature, in a fermentation where the microorganism is a yeast cell, likewise results in the generation of an emulsion in the fermentation broth, which contains the hydrophobic compound. Any of the present methods thus may also comprise a step of breaking the emulsion to recover a product phase comprising the extractant and the hydrophobic compound. Once the emulsion is broken, the fermentation broth is separated in three phases: a water phase, comprising mainly water and aqueous compounds, a phase comprising cells and cellular debris, and a product phase mainly comprising the extractant and the hydrophobic compound. Thus a composition is obtained consisting of three phases.

Thus in some embodiments, the method is for producing a hydrophobic compound and comprises the step of providing a microorganism, preferably a yeast cell, capable of producing said hydrophobic compound, and culturing said microorganism in a culture medium under conditions allowing production of said hydrophobic compound, wherein

5 the culture medium comprises a non-ionic surfactant, more particularly a non-ionic ethoxylated surfactant, preferably selected from a fatty alcohol alkoxylate preferably selected from Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac®

10 LF300 or Dehypon® 2574, and combinations thereof, and a polyethoxylated surfactant, such as a polyethoxylated surfactant or an antifoaming agent selected from: Agnique BP420 (CAS number 68002-96-0), a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents

15 or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof, in an amount equal to or greater than its cloud concentration measured in an aqueous solution, preferably at the cultivation temperature or at room temperature, whereby a composition consisting of three phases is obtained in the fermentation broth, and the method further comprises the step of recovering the product phase.

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In some embodiments, the method is for increasing the titer of a hydrophobic compound in a fermentation as described herein above and comprises the step of providing a microorganism, preferably a yeast cell, capable of producing said hydrophobic compound, and culturing said microorganism or yeast cell in a culture medium under conditions allowing production of said hydrophobic compound, wherein the culture medium

25 comprises a non-ionic surfactant, more particularly a non-ionic ethoxylated surfactant, preferably selected from a fatty alcohol alkoxylate, preferably selected from Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon®

30 2574, and combinations thereof, and a polyethoxylated surfactant, such as a polyethoxylated surfactant or an antifoaming agent selected from: Agnique BP420 (CAS number 68002-96-0), a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone

35 and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof, in

an amount equal to or greater than its cloud concentration measured in an aqueous solution, preferably at the cultivation temperature or at room temperature, whereby a composition consisting of three phases is obtained in the fermentation broth, and the method further comprises the step of recovering the product phase.

5

In some embodiments, the method is for increasing secretion of a hydrophobic compound in a fermentation as described herein above and comprises the step of providing a microorganism, preferably a yeast cell, capable of producing said hydrophobic compound, and culturing said microorganism or yeast cell in a culture medium under conditions allowing production of said hydrophobic compound, wherein the culture medium comprises a non-ionic surfactant, more particularly a non-ionic ethoxylated surfactant, preferably selected from a fatty alcohol alkoxylate, preferably selected from Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and combinations thereof, and a polyethoxylated surfactant, such as an anti-foaming agent selected from: Agnique BP420 (CAS number 68002-96-0), a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof, in an amount equal to or greater than its cloud concentration measured in an aqueous solution, preferably at the cultivation temperature or at room temperature, whereby a composition consisting of three phases is obtained in the fermentation broth, and the method further comprises the step of recovering the product phase.

In preferred embodiments, most of the hydrophobic compound of the fermentation broth is present in the product phase. For example, at least 50% of the hydrophobic compound is present in the product phase, such as at least 55%, such as at least 60%, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the hydrophobic compound is present in the product phase. In some embodiments, the product phase comprises at least 50% of the hydrophobic compound initially present in the fermentation broth, such as at least 55%, such as at least 60%, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such

as at least 85%, such as at least 90%, such as at least 95%, such as at least 96%,
such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the
hydrophobic compound initially present in the fermentation broth. In some embodi-
ments, the hydrophobic compound is a fatty alcohol, a fatty alcohol ester, a fatty acyl
5 acetate and/or a fatty aldehyde. In some embodiments, the hydrophobic compound is a
terpene such as a terpenoid.

The step of breaking the emulsion may be performed as is known in the art, for exam-
ple by submitting the emulsion to a step of phase separation as is known in the art. In
10 some embodiments, the step of phase separation is a step of centrifugation, for exam-
ple 5 minutes at 10 000 g. In some embodiments, the centrifugation is performed for 1
minute or more, such as for 2 minutes or more, such as for 3 minutes or more, such as
for 4 minutes or more, such as for 5 minutes or more, such as for 6 minutes or more,
such as for 7 minutes or more, such as for 8 minutes or more, such as for 9 minutes or
15 more, such as for 10 minutes or more. In some embodiments, the centrifugation is per-
formed at 3 000 g or more, such as at 4 000 g or more, such as at 5 000 g or more,
such as at 6 000 g or more, such as at 7 000 g or more, such as at 8 000 g or more,
such as at 9 000 g or more, such as at 10 000 g or more, such as at 11 000 g or more,
such as at 12 000 g or more, such as at 13 000 g or more, such as at 14 000 g or
20 more, such as at 15 000 g or more, such as at 17 500 g or more, such as at 20 000 g
or more.

Following the step of breaking the emulsion, the product phase comprising the extract-
ant and the hydrophobic compound may be recovered from the composition. The
25 method may in such embodiments further comprise the step of separating the hydro-
phobic compound from the extractant. This can be performed by methods known in the
art, such as by distillation, for example distillation under reduced pressure, or by col-
umn purification, or any other suitable method. The extractant may be recirculated to
the fermentor or bioreactor.

30 In some embodiments, the method involves culturing a microorganism, preferably a
yeast cell, capable of producing a fatty alcohol, such as a desaturated fatty alcohol or a
mixture of (saturated and/or desaturated) fatty alcohols. The desaturated fatty alcohol
may be recovered as described above. In such embodiments, the method may further
35 comprise a step of recovering the produced fatty alcohol and chemically converting at
least part thereof to the corresponding fatty acyl acetate and/or to the corresponding

fatty aldehyde. The term "corresponding" here refers to a compound, fatty acyl acetate or fatty aldehyde, having the same carbon chain length as the fatty alcohol it is obtained from.

5 Thus, when a microorganism, preferably a yeast cell, produces fatty alcohols, the methods may further comprise the step of recovering said fatty alcohols, for example as described above, and chemically converting at least part of the fatty alcohols to the corresponding fatty acyl acetates. This can be done by performing an acetylation reaction as is known in the art, for example as described in Fritz et al., 1959, or Mattson et
10 al., 1964. The methods may additionally or alternatively comprise the step of chemically converting at least part of the fatty alcohols to the corresponding fatty aldehydes. This can be done by performing an oxidation reaction as is known in the art, for example as described in Steves et al., 2013. The resulting fatty acyl acetates and/or fatty aldehydes may then be recovered.

15 Acetylation for example may be carried out with acetic anhydride using pyridine as catalyst. The resulting fatty acetates are then extracted from the reaction mix with an organic solvent and the solvent is removed by evaporation.

20 Oxidation may for example be carried out according to Stahl protocol using Tetrakisacetonitrile copper(I) triflate/TEMPO catalyst system. The resulting fatty aldehydes are then extracted from the reaction mix with an organic solvent and the solvent may be removed by evaporation.

25 *Hydrophobic compound obtainable by the present methods*

The present disclosure also provides a hydrophobic compound obtainable by the methods described herein.

30 In particular, a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate, a fatty aldehyde and a terpene such as a terpenoid obtainable by the present methods are disclosed.

In some embodiments, the hydrophobic compound obtained by the present methods is a fatty alcohol. The fatty alcohol may be saturated or desaturated. Biological production of fatty alcohols from microorganisms such as yeast cells, in particular microorganisms
35 and yeast cells engineered to produce fatty alcohols of interest, may yield a mixture of

fatty alcohols comprising odd-chain fatty alcohols – in contrast to what is observed in chemical synthesis processes, where only even-chain fatty alcohols are obtained. Thus, in some embodiments, the hydrophobic compound obtained by the present methods comprises or consists of a mixture of fatty alcohols which comprises odd-chain fatty alcohols in addition to even-chain fatty alcohols. The term “odd-chain” fatty alcohols refers to fatty alcohols having a carbon chain length which is an odd number of carbon atoms, such as 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 carbon atoms. The term “even-chain” fatty alcohols refers to fatty alcohols having a carbon chain length which is an even number of carbon atoms, such as 8, 10, 12, 14, 16, 18, 20 or 22 carbon atoms.

The present method allows recovering the different fatty alcohols produced by the microorganism, preferably a yeast cell, in a single process. When expressing insect desaturases and reductases, the resulting mix of fatty alcohols produced by the microorganism will typically have a similar composition as the one produced in the pheromone glands of the insects. This allows for the production of pheromone mixes suitable for various insects instead of producing individual pheromone components in separate processes that then need to be mixed in appropriate proportions. Nevertheless, as shown in example 5, the resulting mixture of fatty alcohols may contain by-products characteristic of biological production. Thus in some embodiments where production of a desired desaturated fatty alcohol is performed, the produced fatty alcohols comprise at least 1%, such as at least 2%, such as at least 3%, such as at least 4%, such as at least 5%, such as at least 10%, such as at least 15%, such as at least 20% of a desaturated fatty alcohol having a desaturation at another position than the desired fatty alcohol and/or at least 1%, such as at least 2%, such as at least 3%, such as at least 4%, such as at least 5%, such as at least 10%, such as at least 15%, such as at least 20% of the corresponding saturated fatty alcohol. If the mix of fatty alcohols recovered from the fermentation broth is chemically oxidized into aldehydes or acetylated into acetates, then corresponding mixes of aldehydes and acetates are produced.

In some embodiments, the hydrophobic compound obtained by the present methods is a fatty alcohol ester, which may be saturated or desaturated. In some embodiments, the hydrophobic compound obtained by the present methods comprises or consists of a mixture of fatty alcohol esters which comprises odd-chain fatty alcohol esters in addition to even-chain fatty alcohol esters. The term “odd-chain” alcohol esters refers to

fatty alcohol esters having a carbon chain length which is an odd number of carbon atoms, such as 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 carbon atoms. The term “even-chain” fatty alcohol esters refers to fatty alcohol esters having a carbon chain length which is an even number of carbon atoms, such as 8, 10, 12, 14, 16, 18, 20 or 22 carbon atoms.

In some embodiments, the hydrophobic compound obtained by the present methods is a fatty aldehyde, which may be saturated or desaturated. In some embodiments, the hydrophobic compound obtained by the present methods comprises or consists of a mixture of fatty aldehydes which comprises odd-chain fatty aldehydes in addition to even-chain fatty aldehydes. The term “odd-chain” fatty aldehydes refers to fatty aldehydes having a carbon chain length which is an odd number of carbon atoms, such as 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 carbon atoms. The term “even-chain” fatty aldehydes refers to fatty aldehydes having a carbon chain length which is an even number of carbon atoms, such as 8, 10, 12, 14, 16, 18, 20 or 22 carbon atoms.

In some embodiments, the hydrophobic compound obtained by the present methods is a fatty acyl acetate, which may be saturated or desaturated. In some embodiments, the hydrophobic compound obtained by the present methods comprises or consists of a mixture of fatty acyl acetates which comprises odd-chain fatty acyl acetates in addition to even-chain fatty acyl acetates. The term “odd-chain” fatty acyl acetates refers to fatty acyl acetates having a carbon chain length which is an odd number of carbon atoms, such as 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 carbon atoms. The term “even-chain” fatty acyl acetates refers to fatty acyl acetates having a carbon chain length which is an even number of carbon atoms, such as 8, 10, 12, 14, 16, 18, 20 or 22 carbon atoms.

In some embodiments, the hydrophobic compound obtained by the present methods is a terpene such as a terpenoid, for example as described herein above.

Pheromone composition

Also disclosed herein is a pheromone composition comprising a desaturated fatty alcohol, a desaturated fatty acyl acetate and/or a desaturated fatty aldehyde. For example, a pheromone composition may comprise (Z)-11-hexadecenol, (Z)-11-hexadecenal and/or (Z)-11-hexadecen-1-yl acetate. For example, the pheromone composition may comprise codlemone (E8,E10-dodecadien-1-ol), E8,E10-dodecadienyl acetate and/or

E8,E10-dodecadienal. At least one of the desaturated fatty alcohol, the desaturated fatty acyl acetate and the desaturated fatty aldehyde is preferably obtainable by the methods disclosed herein above.

- 5 In some embodiments of the present disclosure, the pheromone composition comprises (*Z*)-11-hexadecenol, (*Z*)-11-hexadecenal and (*Z*)-11-hexadecen-1-yl acetate, where at least one of the (*Z*)-11-hexadecenol, (*Z*)-11-hexadecenal or (*Z*)-11-hexadecen-1-yl acetate and is obtainable by the methods disclosed herein above. In other em-
10 bodiments, the pheromone composition comprises codlemone (*E8,E10*-dodecadien-1-ol), *E8,E10*-dodecadienyl acetate and/or *E8,E10*-dodecadienal.

- Accordingly, the present methods may further comprise the step of formulating the re-
covered desaturated fatty alcohol, desaturated fatty acyl acetate or desaturated fatty
aldehyde into a pheromone composition. The present pheromone compositions may be
15 used as integrated pest management products, which can be used in a method of mon-
itoring the presence of pest or in a method of disrupting the mating of pest.

- Pheromone compositions as disclosed herein may be used as biopesticides. Such
compositions can be sprayed or dispensed on a culture, in a field or in an orchard.
20 They can also, as is known in the art, be soaked e.g. onto a rubber septa, or mixed
with other components. This can result in mating disruption, thereby preventing pest re-
production, or it can be used in combination with a trapping device to entrap the pests.
Non-limiting examples of pests against which the present pheromone compositions can
be used are: cotton bollworm (*Helicoverpa armigera*), striped stemborer (*Chilo sup-*
25 *pressalis*), diamond back moth (*Plutella xylostella*), cabbage moth (*Mamestra brassi-*
cae), large cabbage-heart caterpillar (*Crocidolomia binotalis*), European corn stalk
borer (*Sesamia nonagrioides*), currant clearwing (*Synanthedon tipuliformis*) and arti-
choke plume moth (*Platyptilia carduidactyla*). Accordingly, use of the present composi-
tions on a culture can lead to increased crop yield, with substantially no environmental
30 impact.

- The relative amounts of the different compounds in the present pheromone composi-
tions may vary depending on the nature of the crop and/or of the pest to be controlled;
geographical variations may also exist. Determining the optimal relative amounts may
35 thus require routine optimisation.

Examples of compositions used as repellents can be found in Kehat & Dunkelblum, 1993, for *H. armigera*, in Alfaro et al., 2009, for *C. suppressalis*, in Eizaguirre et al., 2002, for *S. nonagrioides*; in Wu et al., 2012, for *P. xylostella*; in Bari et al., 2003, for *P. carduidactyla*

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In some embodiments of the present disclosure, the pheromone composition may further comprise one or more additional compounds such as a liquid or solid carrier or substrate. For example, suitable carriers or substrate include vegetable oils, refined mineral oils or fractions thereof, rubbers, plastics, silica, diatomaceous earth, wax matrix and cellulose powder.

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The pheromone composition may be formulated as is known in the art. For example, it may be under the form of a solution, a gel, a powder. The pheromone composition may be formulated so that it can be easily dispensed, as is known in the art.

15 Examples

All strains are described in Table 6.

Example 1 – in situ extraction of fatty alcohols produced by fermentation

Engineered *Yarrowia lipolytica* strains ST8327 and ST8762 are capable of producing fatty alcohols (saturated and unsaturated). Strain ST8327 has been engineered to produce (Z)11-hexadecen-1-ol. It also produces smaller amounts of (Z)9-hexadecen-1-ol and hexadecanol. The strain expresses the $\Delta 11$ desaturase from *Amyelois transitella* (SEQ ID NO: 1) and fatty acyl-CoA reductase from *Helicoverpa armigera* (SEQ ID NO: 5). Strain ST8762 has been engineered to produce (Z)9-tetradecen-1-ol. The strain expresses the $\Delta 9$ desaturase from *Drosophila melanogaster* (SEQ ID NO: 16) and fatty acyl-CoA reductase from *Helicoverpa armigera* (SEQ ID NO: 5). Both strains have additional modifications that decrease degradation of fatty alcohols and improve fatty acid biosynthesis. A strain was inoculated from a YPD agar plate (10 g/L yeast extract, 10 g/L peptone, 20 g/L glucose, 15 g/L agar agar) to an initial OD₆₀₀ of 0.1-0.2 into 2.5 mL YPG medium (10 g/L yeast extract, 10 g/L peptone, 40 g/L glycerol) in 24 well-plate (EnzyScreen). The plate was incubated at 28°C and 300 rpm for 22 hours. The well-plate was centrifuged at 3,500 g for 5 min at 4°C, the medium was removed and the cells were resuspended in 1.25 mL production medium (50 g/L glycerol, 5 g/L yeast extract, 4 g/L KH₂PO₄, 1.5 g/L MgSO₄, 0.2 g/L NaCl, 0.265 g/L CaCl₂·2H₂O, 2 mL/L trace elements solution: 4.5 g/L CaCl₂·2H₂O, 4.5 g/L ZnSO₄·7H₂O, 3 g/L FeSO₄·7H₂O, 1 g/L

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H₃BO₃, 1 g/L MnCl₂·4H₂O, 0.4 g/L N Na₂MoO₄·2H₂O, 0.3 g/L CoCl₂·6H₂O, 0.1 g/L CuSO₄·5H₂O, 0.1 g/L KI, 15 g/L EDTA). At the same time, 95 µL of Antifoam A (ethoxylated and propoxylated C16-18 alcohols, CAS No. 68002-96-0), corresponding to ~7 v/v% (i.e. above the recommended dose of 0.1 v/v% for foam management and above the cloud concentration), was added. The plate was incubated at 28°C and 300 rpm for 28 hours. Each experiment was performed in biological triplicates.

The intracellular and extracellular concentrations of fatty alcohols were assessed as follows. 1000 µL of culture broth was transferred to a 4 mL gas-tight glass extraction vial. The sample was centrifuged at 3,500 g for 5 min at room temperature. The supernatant was transferred into a new glass vial with 990 µL of hexane and 10 µL of internal standard (IS) solution (20 mg/L of methyl Z10-heptadecenoate in ethyl acetate). The vial was vortexed for 10 s and centrifuged as before. 250 µL of the upper hexane phase was transferred to a GC vial for GC-MS analysis of the extracellular fatty alcohol concentration. The pellet remaining after the removal of the supernatant from the centrifuged culture broth was resuspended in 990 µL of solvent mixture (EtOAc and EtOH) and 10 µL of IS solution as above. The sample was incubated for 1 h with periodic mixing. 300 µL water was added and the vials were centrifuged at 3,500 g for 5 min at room temperature. 250 µL of the upper organic phase was transferred to a GC vial for GC-MS analysis of the intracellular fatty alcohols. GC-MS analyses were performed on an Agilent 7820A GC coupled to a mass selective detector Agilent 5977B. The GC was equipped with an DB Fatwax column (30 m×0.25 mm×0.25 µm), and helium was used as carrier gas. The MS was operated in electron impact mode (70eV), scanning between m/z 30 and 400, and the injector was configured in split mode 20:1 at 220°C. Oven temperature was set to 80°C for 1 min, then increased at a rate of 20°C/min to 210°C, followed by a hold at 210°C for 7 min, and then increased at a rate of 20 C/min to 230°C. Compounds were identified by comparison of retention times and mass spectra of the reference compounds. Compounds were quantified by the ion 55.1 m/z. Data were analysed by the Agilent Masshunter software. The concentrations of fatty alcohols were calculated based on standard calibration curves prepared with reference standards.

Results are shown in Table 1, the standard deviations were calculated from biological triplicates. The addition of the antifoaming agent has two beneficial effects: first, the fatty alcohol total titer was increased; second, the extracellular fraction of the total fatty

alcohols produced was increased (from 2-8% to 70-73%). These effects simplify the downstream processing and improve the overall economics of the process.

- 5 The example thus demonstrates that adding an extractant in an amount greater than its cloud concentration results in an increase in the titer of both a saturated fatty alcohol and of an unsaturated fatty alcohol, as well as in an increase in extracellular concentrations of the same. This is independent of the genotype of the strains, as it is observed in two different strains, and confirmed in other strains (see example 12).

10 Table 1. *In situ* extraction of fatty alcohols produced by fermentation.

Strain	ST8327		ST8762	
Antifoam A addition	no	Yes (7 v/v%)	no	Yes (7 v/v%)
Unsaturated fatty alcohol	Z11-hexadecen-1-ol		Z9-tetradecen-1-ol	
Saturated fatty alcohol	Hexadecanol		Tetradecanol	
Total concentration of unsaturated fatty alcohol (mg/L)	801.1±82.0	1520.1±210.0	101.5±10.9	163.3±11.6
Extracellular concentration of unsaturated fatty alcohol (mg/L) (fraction of total)	18.6±22.9 (2.3%)	1057.7±126.7 (69.6%)	4.9±2.3 (4.9%)	119.5±11.1 (73.2%)
Total concentration of saturated fatty alcohol (mg/L)	380.6±26.4	862.7±136.2	385.0±23.1	414.2±29.1
Extracellular concentration of saturated fatty alcohol	8.5±8.8 (2.2%)	629.1±83.9 (72.9%)	29.6±5.6 (7.7%)	303.3±27.5 (73.2%)

(mg/L) (fraction of total)				
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Example 2 – *in situ* extraction and recovery of fatty alcohols produced by fermentation

Here we investigated different amounts of antifoam added and the influence on the recovery of fatty alcohols in a separate phase. Substances like Antifoam A commonly used as antifoaming agents in microbial fermentations are known emulsifiers. The cloud concentration of Antifoam A was experimentally determined to be ~1 v/v% anti-foam in an aqueous solution. The dose recommended for foam management by the manufacturer is 0.1 v/v%.

The experiments were performed with engineered *Y. lipolytica* strain ST8881 following the procedures as in Example 1. The strain ST8881 is similar to ST8327, but has several additional genetic modifications that further enhance the fatty acid biosynthesis. Antifoam A was added at 0, 0.4, 2, or 5 % v/v concentrations. Results are shown in Table 2 and Figure 1.

When antifoam A is added in 0.4 v/v%, below its cloud concentration with aqueous systems, Antifoam A acts as an emulsifier in the fermentation culture (Fig. 1B). In this case the secretion of the target hydrophobic compound was 14.5%, the majority of the product remaining intracellular. Applying centrifugation for 5 min at 16,000 g at room temperature resulted in separation of the solid cellular fraction from the liquid phase. However, centrifugation for 5 min at 16,000 g at room temperature did not result in successful emulsion break, implying the need for complicated recovery of the hydrophobic target compound using organic solvents and cell disruption.

When antifoam A is added in 2 and 5 v/v%, above its cloud concentration with aqueous systems, it constitutes a separate immiscible light phase (Fig. 1C and 1D). This separate immiscible oily phase apparently acts as an *in situ* extractant, and resulted in 66.5% and 78.0% secretion of the target hydrophobic compound. Applying centrifugation for 5 min at 16,000 g at room temperature successfully separated the three present phases, resulting in isolation of the hydrophobic target compound in the oily phase without applying costly cell disruption techniques and extraction with organic solvents for product recovery.

Table 2. *In situ* extraction and recovery of fatty alcohols produced by fermentation.

Antifoam A concentration v/v%	0	0.4	2	5
Concentration of extracellular Z11-hexadecen-1-ol (mg/L)	0.4±0.6	213.6±5.4	965.2±142.5	1249.6±245.4
% secretion (calculated as fraction of extracellular concentration in relation to total concentration)	0.02%	14.5%	66.5%	78.0%
Phase separation after centrifugation	2 phases (water, cells) Fig. 1A	2 phases (water, cells) Fig. 1B	3 phases (anti-foam/fatty alcohols, water, cells) Fig. 1C	3 phases (anti-foam/fatty alcohols, water, cells) Fig. 1D

Example 3 – *in situ* extraction and recovery of fatty alcohols produced by fermentation using various antifoaming agents

Here we investigated the effect of different antifoaming oils and agents on the recovery of fatty alcohols in a separate phase. The experiments were performed with engineered *Y. lipolytica* strain ST8881 following the procedures as in Example 1.

- The following commonly used antifoaming oils and agents were tested at 3 v/v%:
- corn oil,
 - oleic acid,
 - Antifoam A,
 - Kolliphor® P407 (a poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol)),
 - A-204 (a mixture of organic polyether dispersions),
 - Simethicone (a silicone emulsion), and

- dodecane.

The suppliers are indicated in Table 3. The control experiment was performed without addition of any antifoaming oil or agent. Antifoaming oils and agents are added at 3 vol/vol% to the culture broth, which is a significantly lower amount than the concentration of organic phase in classic biphasic fermentations as known in the art (above 5-10 v/v%).

The *in situ* extraction performance of commonly used antifoaming oils and agents is shown in Table 3. Cultures with Corn oil, Antifoam A, Kolliphor® P407, A-204 and Simethicone showed increased concentration of fatty alcohols compared to control cultivations. Cultures with Corn oil, Antifoam A, Kolliphor® P407, A-204 and Simethicone presented also higher secretion rates of fatty alcohols compared to control cultivations.

In the case of oleic acid, Antifoam A, Kolliphor® P407, A-204 and Simethicone, applying centrifugation for 5 min at 10,000 g at 30°C resulted in separation of the solid cellular fraction from the liquid phase (Fig. 2C, Fig. 2D, Fig. 2E, Fig. 2F and Fig. 2G, respectively). In the case of corn oil and dodecane, the solid cellular fraction constituted a dispersion with the organic upper phase (Fig. 2B and Fig. 2H, respectively).

In the case of Antifoam A, Kolliphor® P407, A-204 and Simethicone, centrifugation for 5 min at 10,000 g at 30°C resulted in successful water-oil emulsion break.

Table 3. *In situ* extraction and recovery of fatty alcohols produced by fermentation using antifoaming agents

Anti-foam-ing agent	Pro-vider	CAS No	Total concentration of Z11-hexa-decen-1-ol (mg/L)	Extracellular concentration of Z11.hexa-decen-1-ol (% secretion)	Phase separation after centri-fugation 10,000 x g for 5 min at 30°C
Control without	-	-	2190.0±320.0	0 (0%)	Phase 1: Water Phase 2: cells Fig. 2A

anti-foam addition					
Corn oil	Roquette	8001-30-7	2699.6±42.3	646.6±437.1 (24%)	Phase 1: suspension of oil and cells Phase 2: water Fig. 2B
Oleic acid	Merck	112-80-1	1008.4±648.6	0 (0%)	Phase 1: emulsion of oil and water Phase 2: cells Fig. 2C
Anti-foam A	Bekchem	68002-96-0	3406.6±276.3	1354.8±365.1 (39.8%)	Phase 1: organic Phase 2: water Phase 3: cells Fig. 2D
Kolli-phor® P407	Merck	9003-11-6	2546.5±96.4	177.7±34.1 (6.9%)	Phase 1: organic Phase 2: water Phase 3: cells Fig. 2E
A-204	Sigma		2946.2±459.5	723.5±1040.99 (24.6%)	Phase 1: organic Phase 2: water Phase 3: cells Fig. 2F
Sime-thi-cone	Dow	9004-67-5 63231-67-4 9004-99-3	1919.0±153.9	222.5±58.0 (11.6%)	Phase 1: organic Phase 2: water Phase 3: cells Fig. 2G
Do-dec-ane	Merck	112-40-3	720.0±95.6	0 (0%)	Phase 1: organic with cells Phase 2: water Phase 3: cells Fig. 2H

Example 4 – *in situ* extraction and recovery of other lipophilic/hydrophobic compounds

5 Microorganisms capable of producing various lipophilic compounds, e.g. engineered to produce free fatty acids, fatty acyl acetates, fatty aldehydes and terpenes such as terpenoids, are cultivated in the presence of antifoaming agents such as a polyethoxylated surfactant at concentrations equal to or higher than their cloud concentration.

10 The resulting fermentation broth is subjected to centrifugation and the light phase containing non-ionic surfactant and the product is separated by centrifugation. The light phase is further subjected to distillation, possibly under vacuum, in order to separate the product from the non-ionic surfactant and other non-volatile impurities. The distilled product can be for example a mix of fatty alcohols. The fatty alcohols mix can be acetylated into the corresponding fatty alcohol acetates or oxidized into the corresponding
15 fatty aldehydes.

Acetylation is carried out with acetic anhydride using pyridine as catalyst. The resulting fatty alcohol acetates are then extracted from the reaction mix with an organic solvent and the solvent is removed by evaporation. Oxidation is carried out according to Stahl
20 protocol using Tetrakisacetonitrile copper(I) triflate/TEMPO catalyst system. The resulting fatty aldehydes are then extracted from the reaction mix with an organic solvent and the solvent is removed by evaporation. The resulting fatty alcohol acetates or fatty aldehydes are formulated and used for plant protection from insects.

25 **Example 5 – *biological activity of pheromone preparations obtained by fermentation***

(Z)11-hexadecen-1-ol (Z11-16:OH) produced by fermentation in *Yarrowia lipolytica*, for example, will typically co-occur with (Z)9-hexadecen-1-ol (Z9-16:OH), which is produced due to the action of the native *Yarrowia lipolytica* desaturase *OLE1*. In engineered strains, Z9-16:OH was also produced in the amounts of 5-20% of the amount of
30 Z11-16:OH. Saturated fatty alcohols of carbon chain 16 were also produced as by-products, when reductase acts directly on the saturated substrate hexadecenyl-CoA.

When the mix of fatty alcohols recovered from the fermentation broth is chemically oxidized into aldehydes or acetylated into acetates, then corresponding mixes of aldehydes and acetates are produced.

- 5 In an exemplary sample preparation, the composition was as follows: 65% Z11-16:Ald, 4% Z9-16:Ald, 10% 16:Ald.

Fermented Z11-16:Ald can be used for controlling cotton bollworm *Helicoverpa armigera* by mating disruption. The pheromone glands of *H. armigera* contain Z11-16:Ald,
10 Z9-16:Ald, and 16:Ald.

The ratio between Z11-16:Ald and Z9-16:Ald varies from 99:1 to 90:10 (<http://www.pherobase.com/database/species/species-Helicoverpa-armigera.php>). Z9-16:Ald is a minor component of *H. armigera* pheromone mix and it was reported to enhance the activity of an artificial pheromone formulation, when it was added to Z11-16:Ald (Kehat et al., 1990). n-Hexadecanal 16:Ald is also present in *H. armigera* pheromone glands at 4-20%, but it is neutral in regard to behavioral response.

Fermented Z11-16:Ald can be used for controlling the Asiatic rice borer *Chilo suppressalis* by mating disruption. The pheromone glands of *C. suppressalis* also contain Z11-16:Ald, Z9-16:Ald, and 16:Ald. The ratio between Z11-16:Ald and Z9-16:Ald is 10:1 (<http://www.pherobase.com/database/species/species-Chilo-suppressalis.php>). Z9-16:Ald is synergistic to the two primary pheromone components Z11-16:Ald and Z13-18:Ald (Tatsuki et al., 1983). n-Hexadecanal 16:Ald is a neutral component and does
25 not elicit a behavioral response.

Fermented Z11-16:Ald can be used for controlling the Yellow rice stemborer *Scirpophaga incertulas* by mating disruption. The pheromone glands of *S. incertulas* contain Z11-16:Ald, Z9-16:Ald, and 16:Ald. The ratio between Z11-16:Ald and Z9-16:Ald is 4:1 to 2:1 (<http://www.pherobase.com/database/species/species-Scirpophaga-incertulas.php>).

Hence the two key by-products that were produced by engineered yeasts are present in amounts similar to the natural range of pheromone composition in insects. Z9-16
35 compounds are usually biologically active and beneficial for the behavioral activity.

Example 6 – Strain construction

- Strains ST3705 (Example 2 and Table 4 of WO 2016/207339) and ST5290 (Example 4 of WO 2018/109167) are *Saccharomyces cerevisiae* strains engineered to produce (Z)11-hexadecen-1-ol and Z9-tetradecenyl acetate, respectively. Strain ST3705 expresses the Δ 11 desaturase from *Amyelopsis transitella* and fatty acyl-CoA reductase from *Helicoverpa armigera*. Strain ST5290 expresses Δ 9 desaturase from *Drosophila melanogaster*, fatty acyl-CoA reductase from *Helicoverpa armigera* and acetyltransferase ATF1 from *Saccharomyces cerevisiae*.
- Strain ST4840 is a *Yarrowia lipolytica* wild-type strain. *Y. lipolytica* strain ST6629 is a *Yarrowia lipolytica* strain and has been described previously in WO 2018/109167 (Example 9 of WO 2018/109167). In this strain, the open-reading frame of genes *HFD4* (YALI0B01298g), *HFD3* (YALI0A17875), *HFD2* (YALI0E15400) and *HFD1* (YALI0F23793g), as well as nucleotides –1130 to –100 upstream of the coding sequence of *GPAT* (YALI0C00209g) were deleted. A premature Stop-codon and frame-shift was introduced in *PEX10* (YALI0C01023g) and *FAO1* (YALI0B14014g) resulting in non-functional genes.
- In *Y. lipolytica* strain ST9426 was engineered to improve the mevalonate pathway flux and used as a terpenoids platform strain. In this strain, the open reading frame of genes *KU70* (YALI0C08701g) and *PEX10* (YALI0C01023g), as well as nucleotides –529 to –50 upstream of the coding sequence of squalene synthase (*SQS1*, YALI0A10076g) were deleted. Furthermore, genes isopentenyl-diphosphate delta-isomerase (*IDI1*, YALI0F04015g), farnesyl diphosphate synthase (*ERG20*, YALI0E05753g), 3-hydroxy-3-methylglutaryl coenzyme A reductase (*HMG1*, YALI0E04807g) and geranylgeranyl pyrophosphate synthase (*GGPPS*, SEQ ID:46) were also overexpressed.
- Y. lipolytica* strains ST7982, ST8327, ST9253, ST10229, ST10230, ST10231, ST9423, ST9424, and ST10151 were constructed as follows.
- All heterologous genes were synthesized by GeneArt (Life Technologies) in codon optimized versions for *Y. lipolytica*. All genes were amplified by PCR using Phusion U Hot Start DNA Polymerase (ThermoFisher) to obtain the fragments for cloning into yeast expression vectors. The primers and the resulting DNA fragments (BioBricks) are listed

in Table 4. The PCR products were separated on a 1%-agarose gel containing Red-Safe™ (iNtRON Biotechnology). PCR products of the correct size were excised from the gel and purified using the Nucleospin Gel and PCR Clean-up kit (Macherey-Nagel).

5

Integrative yeast vectors with USER cassette were linearized with FastDigest SfaAI (ThermoFisher) for 2 hours at 37°C and then nicked with Nb.BsmI (New England Biolabs) for 1 hour at 65°C. The resulting vectors containing sticky ends were separated by gel electrophoresis, excised from the gel, and gel-purified using the Nucleospin Gel and PCR Clean-up kit (Macherey-Nagel). The DNA fragments were cloned into the so prepared vectors by USER-cloning as described in (Holkenbrink et al., 2018). The reaction was transformed into chemically competent *E. coli* DHalpha cells and the cells were plated on Lysogeny Broth (LB) agar plates with 100 mg/L ampicillin. The plates were incubated overnight at 37°C and the resulting colonies were screened by colony PCR. The plasmids were purified from overnight *E. coli* liquid cultures and the correct cloning was confirmed by sequencing. The constructed vectors are listed in Table 5.

Yeast strains were constructed by transformation of DNA vectors as described in Holkenbrink et al., 2018. Integrative vectors were linearized with FastDigest NotI prior to transformation. When needed, helper vectors to promote the integration into specific genomic regions were co-transformed with the integrative plasmid or DNA repair fragments (Tables 4 and 5). Strains were selected on yeast peptone dextrose (YPD) agar with appropriate antibiotics selection. Correct genotype was confirmed by colony PCR and when needed by sequencing. The resulting strains are listed in Table 6.

25

Table 4. DNA fragments (BioBricks) obtained by PCR using the indicated template and primers.

DNA fragment name	Description	Fw_primer (5'->3') Hybridises at positions	Rv_primer (5'->3') Hybridises at positions	Template DNA
BB2311	5' end of FAS2 (l1220F)	Yali0B 2566672..2566691	Yali0B 2567146..2567159	<i>Y. lipolytica</i> genomic DNA

	repair fragment			
BB2312	3' end of FAS2 (I1220F) repair fragment	Yali0B 2567171..2567190	Yali0B 2567645..2567662	<i>Y. lipolytica</i> genomic DNA
BB2313	FAS2 (I1220F)	BB2311 1..20	BB2312 475..492	BB2311, BB2312
BB1006	Linearized vector PL3405	1097..1129	1696..1727	PL3405*
BB1005	Hygromy- cin re- sistance cassette	2894..2923	3906..3935	PL4132*
BB1135	Vector backbone	2306..2336	5129..5152;1..13	PL6681*
BB8388	Region from <i>Y. lipolytica</i> genome	Yali0F 2011922..2011937	Yali0F 2012405..2012421	<i>Y. lipolytica</i> genomic DNA
BB1631	<i>Y. lipolyt- ica</i> PEX20 and LIP2 termina- tor	516..546	1789..1815	PL6371*
BB8389	Region from <i>Y. lipolytica</i> genome	Yali0F 2012722..2012743	Yali0F 2013206..2013221	<i>Y. lipolytica</i> genomic DNA
BB2608	<i>Y. lipolyt- ica</i> LIP2	Yali0A 2187638..2187643	Yali0A 2188554..2188574	<i>Y. lipolytica</i> genomic DNA

	termina- tor			
BB8049	Desatu- rase from <i>Amyelois transitella</i> expressed under Y. <i>lipolytica</i> TEF pro- moter and LIP2 ter- minator	BB2093 1..14	BB2608 2188554..2188574	BB2093, BB8047, BB2608
BB8048	<i>Y. lipolyt- ica</i> TEF promoter	Yali0C 1244246..1244265	Yali0C 1243743..1243761	<i>Y. lipolytica</i> genomic DNA
BB8047	Desatu- rase from <i>Amyelois transitella</i>	4..23	963..981	SEQ ID NO: 1
BB8169	Linearized vector PL6677	1814..1833	840..857	PL6677*
BB8269	Linearized vector PL8006	1739..1756	765..782	PL8006
BB8167	Fatty acyl reductase from <i>H. armigera</i> expressed under Y. <i>lipolytica</i> TEF pro- moter and	867..891	3703..3723	PL8236

	LIP2 terminator			
BB8168	Fatty acyl reductase from <i>H. armigera</i> expressed under <i>Y.lipolytica</i> TEF promoter and LIP2 terminator	878..897	3705..3723	PL8236
BB8212	Fatty acyl reductase from <i>H. armigera</i> expressed under <i>Y. lipolytica</i> TEF promoter and LIP2 terminator	878..891	3703..3723	PL8236
BB8213	Fatty acyl reductase from <i>H. armigera</i> expressed under <i>Y. lipolytica</i> TEF promoter	878..897	2753..2773	PL8236

BB2719	Tef promoter of <i>Y. lipolytica</i>	Yali0C 1244252..1244265	Yali0C 1243743..1243761	<i>Y. lipolytica</i> genomic DNA
BB2693	Desaturase from <i>L. brotana</i>	4..22	997..1014	SEQ ID NO: 43
BB1688	Tef promoter of <i>Y. lipolytica</i>	Yali0C 1244254..1244265	Yali0C 1243743..1243762	<i>Y. lipolytica</i> genomic DNA
BB1740	Fatty acyl reductase from <i>H. armigera</i>	4..21	1348..1368	SEQ ID NO: 5
BB1635	tRNA from <i>Y. lipolytica</i>	3154..3183	3342..3371	PL4589
BB1636	region from <i>Y. lipolytica</i> genome	3392..3424	3458..3498	PL4589
BB7970	FAS1 from <i>Y. lipolytica</i>	Yali0B 2006887..2006907	Yali0B 2000650..2000673	<i>Y. lipolytica</i> genomic DNA
BB2209	TEF promoter of <i>Y. lipolytica</i>	Yali0C 1244252..1244265	Yali0C 1243743..1243761	<i>Y. lipolytica</i> genomic DNA
BB2093	TEF promoter of <i>Y. lipolytica</i>	Yali0C 1244252..1244265	Yali0C 1243743..1243761	<i>Y. lipolytica</i> genomic DNA

BB8969	Desaturase of <i>Drosophila grimshawi</i>	4..18	1137..1149	SEQ ID NO: 44
BB8971	Desaturase of <i>Drosophila virilis</i>	4..18	1131..1146	SEQ ID NO: 45
BB8662	FAS2 (I1220F)	Yali0B 3170..3189	Yali0B 4143..4160	Genomic DNA of strain ST7982
BB7983	Region from <i>Y. lipolytica</i> genome	Yali0B 1644153..1644173	Yali0B 1644632..1644647	<i>Y. lipolytica</i> genomic DNA
BB7984	Region from <i>Y. lipolytica</i> genome	Yali0B 1644658..1644677	Yali0B 1645125..1645143	<i>Y. lipolytica</i> genomic DNA
BB7985	Region from <i>Y. lipolytica</i> genome	BB7983 1..21	BB7984 468..486	BB7983, BB7984
BB9296	Desaturase of <i>Drosophila melanogaster</i>	4..19	1071..1086	SEQ ID NO: 16
BB8386	Region from <i>Y. lipolytica</i> genome	YALI0F 3823053..3823069	YALI0F 3823536.. 3823552	<i>Y. lipolytica</i> genomic DNA
BB8387	Region from <i>Y. lipolytica</i> genome	YALI0F 3823853..3823869	YALI0F 3824332..3824352	<i>Y. lipolytica</i> genomic DNA

	<i>lipolytica</i> genome			
BB8663	Region from <i>Y. lipolytica</i> genome	YALI0C 406284..406298	YALI0C 405783..405802	<i>Y. lipolytica</i> genomic DNA
BB8664	Region from <i>Y. lipolytica</i> genome	YALI0C 405647..405665	YALI0C 405163..405177	<i>Y. lipolytica</i> genomic DNA
BB2722	<i>Y. lipolytica</i> EXP promoter	YALI0C 1663140..1663158	YALI0C 1664123..1664141	<i>Y. lipolytica</i> genomic DNA
BB8644	<i>Y. lipolytica</i> GPD promoter	YALI0C 825834..825853	YALI0C 826740..826766	<i>Y. lipolytica</i> genomic DNA
BB8836	HMG1	YALI0E 539630..539649	YALI0E 542627..542629	<i>Y. lipolytica</i> genomic DNA
BB8837	ERG20	YALI0E 642319..642337	YALI0E 641303..641324	<i>Y. lipolytica</i> genomic DNA
BB8838	IDI1	YALI0F 601747..601765	YALI0F 600953..600970	<i>Y. lipolytica</i> genomic DNA
BB8847	geranyl- geranyl pyrophos- phate syn- thase of <i>Synecho- coccus</i> sp.	1..17	878..894	SEQ ID NO: 46
BB9273	β -farne- sene syn- thase of <i>Artemisia annua</i>	4..20	1710..1725	SEQ ID NO: 47

Table 5. List of vectors used

Expression vector	Parent vector	DNA fragments cloned into parent vector (crRNA sequence, if applicable)
PL4589*		
PL3431		BB1006, BB1005
PL8264		BB1135, BB8388, BB1631, BB8389
PL9002	PL3405	BB8955
PL7981	PL6371*	BB8049, BB8048, BB8047
PL8037	PL6684*	BB8049, BB8048, BB8047
PL8033	PL3431	BB1635, BB1636, (YALI0D; 2193232.. 2193213)
PL8071	PL6679*	BB8212, BB8213
PL8034	PL3431	BB1635, BB1636, (YALI0E; 1722566.. 1722585)
PL8053		BB8169, BB8167, BB8168
PL7983	PL3405*	BB1635, BB1636, (YALI0B; 1644658.. 1644677)
PL8158	PL3431	BB1635, BB1636 (YALI0A; 1556748.. 1556767)
PL8150		BB8269, BB8167, BB8168
PL8071	PL6679	BB8212, BB8213
PL6638	PL3405	BB1635, BB1636 (YALI0E; 2882052.. 2882071)
PL6631	PL3405	BB1635, BB1636, (YALI0D; 2193232.. 2193213)
PL7912	PL6684	BB2719, BB2693
PL8680	PL6371	BB2209, BB2693
PL6630	PL3405	BB1635, BB1636 (YALI0C; 568875.. 568856)
PL8655	PL8264	BB2209, BB7970
PL8646	PL3431	BB1635, BB1636, (YALI0F; 2012605.. 2012624)
PL8236	PL6679	BB1688, BB1740

PL8655	PL8264	BB7970, BB2209
PL9023	PL9002	BB2093, BB8969
PL9025	PL9002	BB2093, BB8971
PL7088	PL3405	BB1635, BB1636, YALI0B; 2567145.. 2567164)
PL9003	PL3405	BB2093, BB9296
PL8863	PL3431	BB1635, BB1636 (YALI0A; 1017584.. 1017603)
PL8263		BB1135, BB8386, BB1631, BB8387
PL8622		BB1135, BB8663, BB1631, BB8664
PL8864	PL8263	BB8838, BB2722, BB8644, BB8837
PL8540	PL3405	BB1635, BB1636 (YALI0F; 3823780.. 3823799)
PL8865	PL8622	BB8836, BB2722, BB8644, BB8847
PL8625	PL3405	BB1635, BB1636 (YALI0C; 405763.. 405782)
PL5239	PL3405	BB1635, BB1636 (YALI0C; 140578.. 140597)
PL9389	PL6371*	BB2093, BB9273
PL8032	PL3431	BB1635, BB1636 (YALI0C, 568856.. 568875)

Table 6. Yeast strains.

Strain name	Intrinsic genome edits	Overexpressed genes	Parent strain (integrated vector)
ST3705		Atrd11 HarFAR	
ST5290		Dmd9 HarFAR ScATF1	
ST6629	Δ hfd1-4 Δ fao1 Δ pex10 Δ -1130- 100_PrGPAT		
ST7982	B19382*		ST6629 (PL7088, BB2313)
ST8200		Atrd11	ST6629 (PL7981, PL6630)

ST8201		Atrd11	ST8200 (PL8037,PL8033)
ST8223		Atrd11 HarFAR	ST8201 (PL6638,PL8071)
ST8246		Atrd11 HarFAR	ST8223 (PL8034,PL8053)
ST8264	B12342*	Atrd11 HarFAR	ST8246 (PL7983,BB7985)
ST8327	B12342*	Atrd11 HarFAR	ST8264 (PL8158,PL8150)
ST8225	B19382*	HarFAR	ST7982 (PL6638,PL8071)
ST8373	B19382*	HarFAR LboPPTQ	ST8225 (PL7912,PL6631)
ST9136	B19382*	HarFAR LboPPTQ	ST8373 (PL8680,PL6630)
ST9253	B19382*	HarFAR LboPPTQ YIFAS1	ST9136 (PL8655,PL8646)
ST10229	B19382*	HarFAR YIFAS1 Dgd9	ST8225 (PL9023)
ST10230	B19382*	HarFAR YIFAS1 Dvd9	ST8225 (PL9025)
ST10231	B19382*	HarFAR YIFAS1 Dmd9	ST8225 (PL9003)
ST6029	$\Delta ku70$		ST4840 (PL6364, Marella et al., 2020)
ST9423	SQS_Pr50bp		ST6029 (YALI0A; 1017515..1017559; 1018039..1018083,PL8863)
ST9424		IDI1 ERG20	ST9423 (PL8864,PL8540)
ST9425		HMG1 SynGGPPs7	ST9424 (PL8865,PL8625)

ST9426	Δ pex10		ST9425 (YALI0C; 139673..139717; 140852..140896, PL5239)
ST10151		AaBFS	ST9426 (PL9389,PL8032)

Example 7 - Selection of surfactants: ethoxylates/ethoxylated non-ionic surfactants

Selected non-ionic ethoxylated surfactants with trade name, manufacturer, chemical name, CAS No., cloud point ($^{\circ}$ C, by provider), cloud concentration (v/v%, in aqueous systems at room temperature, experimentally measured), recommended dose for foam management (% , by provider) were tested (Table 7).

Simple model mixture experiments were performed using 1 mL production medium (50 g/L glycerol, 5 g/L yeast extract, 4 g/L KH_2PO_4 , 1.5 g/L MgSO_4 , 0.2 g/L NaCl, 0.265 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 2 mL/L trace elements solution: 4.5 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 4.5 g/L $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 3 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1 g/L H_3BO_3 , 1 g/L $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.4 g/L N $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.3 g/L $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1 g/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.1 g/L KI, 15 g/L EDTA) supplemented with 0 v/v%, 0.1 v/v% (below the measured cloud concentration, close to recommended dose for foam management), and 3 v/v% (above the measured cloud concentration) surfactants in 2 mL spin tubes. The tubes were vortexed for 5 s to mimic mixing conditions during the fermentation process. Spin tests were carried out at room temperature at 15,000 g for 5 min in a benchtop centrifuge.

The spin tests revealed that when the surfactants were applied at the recommended low doses for foam management in a fermentation process (0.1 v/v%, below their cloud concentrations), no phase separation was detected (only one homogeneous aqueous phase). However, when the surfactants were used in a concentration higher than their cloud concentrations, the hydrophobic oily and the hydrophilic aqueous phase could be easily separated by the applied centrifugal force during the spin tests. Applying a surfactant above its recommended dose for foam management, and above its cloud concentration enables increased production, secretion by in-situ extraction of hydrophobic pheromone products of a fermentation. In addition, by simple mechanical separation, the easy and economic recovery of hydrophobic pheromone products of a fermentation is facilitated.

Table 7. Measured cloud concentration: measured in aqueous systems (production media – example A) at room temperature (v/v%). Foam management dose: Dose recommended dose for foam management (%) by provider. OW: visible oil-water phase separation only above cloud concentration; OW*: visible oil-water phase separation only above cloud concentration (water phase cloudy – some miscibility of surfactant with water); OW**: visible oil-water phase separation only above cloud concentration, some cloudiness in the interface of the two separated phases.

Surfactant trade name	Manufacturer	Chemical name	CAS No.	Cloud Point °C -by provider	Measured cloud concentration	Foam management dose (%)	Spin test
Anti-foam A	Bekchem	ethoxylated and propoxylated C16-18 alcohols	68002-96-0	23-27 (butyldiglycol)	approx. 1	Up to 0.1	OW
Plurafac® LF300	BASF	Oxirane, methyl-, polymer with oxirane, monoisotridecyl ether, block	196823-11-7	22 (1% aqueous)	approx. 1		OW*
Agnique BP420	BASF	ethoxylated and propoxylated	68002-96-0	26 (butyldiglycol)	approx. 1		OW

		C16-18 alcohols					
Plu- rafac® LF1300	BASF	ethox- ylated and propox- ylated C16-18 alcohols	68002- 96-0	26 (bu- tyldi- glycol)	ap- prox. 1		OW
Plu- rafac® SLF180	BASF	Ethox- ylated alkyl alcohol	No CAS, Ref. No 02- 211963 0747- 33-0000	16-21 (1% aque- ous)	approx. 1	0.1 – 0.5	OW*
De- hypon® 2574	BASF	Fatty al- cohol, ethox- ylated and propox- ylated	68154- 97-2	40 (bu- tyldi- glycol)	approx. 1		OW
Imben- tin SG/251	KLK Oleo	Alcohols C8-18, ethox- ylated, propox- ylated	68002- 96-0	21-26 (butyldi- glycol)	approx. 1	0.1	OW**
A-204	Sigma	mixture of or- ganic poly- ether	- (product number A6426 and A8311,	18-21 (1% aque- ous)	approx. 1	0.005- 0.01	OW

		disper- sions	MDL number MFCD0 013052 3)				
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Example 8 - in situ extraction and recovery of fatty alcohols produced by fermentation

- Here we investigated different amounts of antifoam added and the influence on the recovery of fatty alcohols in a separate phase. Substances like Antifoam A (Bekchem)
- 5 commonly used as antifoaming agents in microbial fermentations are known emulsifiers. The cloud concentration of Antifoam A was experimentally determined to be ~1 v/v% antifoam in an aqueous solution. The dose recommended for foam management by the manufacturer is 0.1 v/v%.
- 10 The experiments were performed with engineered *Y. lipolytica* strain ST8327 following the procedures as in Example 1. Antifoam A was added at 0, 0.4, 2, or 5 % v/v concentrations. Results are shown in Table 8.
- When antifoam A is added in 0.4 v/v%, below its cloud concentration measured in an aqueous solution, Antifoam A acts as an emulsifier in the fermentation culture, similar
- 15 to what was observed in Example 2. In this case the secretion of the target hydrophobic compound was 20-25%, the majority of the product remaining intracellular. Applying centrifugation for 5 min at 16,000 g at room temperature resulted in separation of the solid cellular fraction from the liquid phase. However, centrifugation for 5 min at 16,000
- 20 g at room temperature did not result in successful emulsion break, implying the need for complicated recovery of the hydrophobic target compound using organic solvents and cell disruption.
- When antifoam A is added in 2 and 5 v/v%, above its cloud concentration measured in an aqueous solution, it constitutes a separate immiscible light phase (as also seen in
- 25 Fig. 1C and 1D). This separate immiscible oily phase apparently acts as an *in situ* extractant, and resulted in 73-74% and 67% secretion of the target hydrophobic compound. Applying centrifugation for 5 min at 16,000 g at room temperature successfully separated the three present phases, resulting in isolation of the hydrophobic target

compound in the oily phase without applying costly cell disruption techniques and extraction with organic solvents for product recovery.

Table 8. *In situ* extraction and recovery of fatty alcohols produced by fermentation.

Antifoam A concentration v/v%	0	0.4	2	5
Concentration of extracellular Z11-hexadecen-1-ol (mg/L)/ Z9-hexadecen-1-ol (mg/L)	35.3 ± 8.2/ 0.3 ± 0.5	219.6 ± 81.1/ 40.9 ± 16.5	664.3 ± 73.9/ 136.6 ± 21.6*	1149 ± 167.9/ 271.8 ± 33.4
% secretion (calculated as fraction of extracellular concentration in relation to total concentration)	3.5%/0.2%	24.5%/19.8 %	74.3%/72.8 %	67.1%/66.8%
Phase separation after centrifugation	2 phases (water, cells) Fig. 1A	2 phases (water, cells) Fig. 1B	3 phases (anti-foam/fatty alcohols, water, cells) Fig. 1C	3 phases (anti-foam/fatty alcohols, water, cells) Fig. 1D

5 *Technical duplicates only

Example 9 - Increased production, secretion, and recovery of fatty alcohols by the yeast Saccharomyces cerevisiae

Here the increased production, secretion, and recovery of fatty alcohols by the engineered yeast *Saccharomyces cerevisiae* was demonstrated.

10

- Strain ST3705 was inoculated from a YPD agar plate (10 g/L yeast extract, 10 g/L peptone, 20 g/L glucose, 15 g/L agar agar) to an initial OD₆₀₀ of 0.1-0.2 into 2.5 mL YPD medium (10 g/L yeast extract, 10 g/L peptone, 40 g/L glucose) in 24 well-plate (Enzy-Screen). The plate was incubated at 28°C and 300 rpm for 22 hours. The well-plate
- 5 was centrifuged at 3,500 g for 5 min at 4°C, the medium was removed and the cells were resuspended in 1.25 mL production medium (50 g/L glucose, 20 mg/L uracil, 5 g/L yeast extract, 4 g/L KH₂PO₄, 1.5 g/L MgSO₄, 0.2 g/L NaCl, 0.265 g/L CaCl₂·2H₂O, 2 mL/L trace elements solution: 4.5 g/L CaCl₂·2H₂O, 4.5 g/L ZnSO₄·7H₂O, 3 g/L FeSO₄·7H₂O, 1 g/L H₃BO₃, 1 g/L MnCl₂·4H₂O, 0.4 g/L N Na₂MoO₄·2H₂O, 0.3 g/L
- 10 CoC₁₂·6H₂O, 0.1 g/L CuSO₄·5H₂O, 0.1 g/L KI, 15 g/L EDTA). At the same time, control 1, 0 v/v% surfactant, control 2, 0.1 v/v% surfactant (approximate recommended dose by manufacturer for foam management) and 3 v/v% (above the cloud concentration of the surfactant) was added of the following surfactants: Antifoam A (Bekchem; ethoxylated and propoxylated C16-18 alcohols, CAS No. 68002-96-0); Agnique BP420
- 15 (BASF; ethoxylated and propoxylated C16-18 alcohols, CAS No. 68002-96-0); Plurafac® LF1300 (BASF; ethoxylated and propoxylated C16-18 alcohols, CAS No. 68002-96-0); Dehypon® 2574 (BASF; Fatty alcohol, ethoxylated and propoxylated, CAS-nummer: 68154-97-2). The plate was incubated at 28°C and 300 rpm for 28 hours. Each experiment was performed in biological triplicates.
- 20
- The intracellular and extracellular concentrations of fatty alcohols were assessed as follows. 1000 µL of appropriately diluted culture broth was transferred to a 4 mL gas-tight glass extraction vial. The sample was centrifuged at 3,500 g for 5 min at room temperature. The supernatant was transferred into a new glass vial with 1000 µL of
- 25 hexane and 10 µL of internal standard (IS) solution (20 mg/L of methyl nonadecanoate in ethyl acetate). The vial was vortexed for 10 s and centrifuged as before. 250 µL of the upper hexane phase was transferred to a GC vial for GC-MS analysis of the extracellular fatty alcohol concentration. The pellet remaining after the removal of the supernatant from the centrifuged culture broth was resuspended in 1000 µL of solvent mixture (EtOAc and EtOH) and 10 µL of IS solution as above. The sample was incubated
- 30 for 1 h with periodic mixing. 300 µL water was added and the vials were centrifuged at 3,500 g for 5 min at room temperature. 250 µL of the upper organic phase was transferred to a GC vial for GC-MS analysis of the intracellular fatty alcohols.
- 35
- GC-MS analyses were performed on an Agilent 7820A GC coupled to a mass selective detector Agilent 5977B. The GC was equipped with an DB Fatwax column (30 m×0.25

mm×0.25 µm), and helium was used as carrier gas. The MS was operated in electron impact mode (70eV), scanning between m/z 30 and 400, and the injector was configured in split mode 20:1 at 220°C. Oven temperature was set to 80°C for 1 min, then increased at a rate of 20°C /min to 210°C, followed by a hold at 210°C for 7 min, and then increased at a rate of 20 C/min to 230°C. Compounds were identified by comparison of retention times and mass spectra of the reference compounds. Compounds were quantified by the ion 55.1 m/z. Data were analyzed by the Agilent Masshunter software. The concentrations of fatty alcohols were calculated based on standard calibration curves prepared with reference standards.

Results are shown in Table 9. Significant amount of extracellular of fatty alcohols was obtained when the surfactant was added in high concentrations, i.e. above its cloud concentration. In addition, the total production of fatty alcohols also increased when the surfactant was added in high concentrations (3 v/v%). When the surfactant was dosed above its cloud concentration, it constituted a separate immiscible hydrophobic phase together with the in-situ extracted fatty alcohols. Therefore, phase separation and product recovery could be facilitated by simple mechanical phase separation, without the use of organic solvents or costly separation methods.

Table 9

ST3705						
Unsaturated fatty alcohol Z11-hexadecen-1-ol						
Surfactant	0 v/v% surfactant		0.1 v/v% surfactant		3 v/v% surfactant	
	Total concentration (mg/L)	Extracellular concentration (mg/L) (fraction of total)	Total concentration (mg/L)	Extracellular concentration (mg/L) (fraction of total)	Total concentration (mg/L)	Extracellular concentration (mg/L) (fraction of total)
Antifoam A	23.2±43.4	0.0±0.0 (0%)	23.3±13.5	1.7±0.6 (7%)	10.2±1.0	3.5±0.8 (34%)
Agnique BP420			9.5±3.2	0.3±0.4 (0%)	16.0±4.4	8.7±3.7 (54%)

Plurafac® LF1300			9.5±3.2	0.3±0.4 (0%)	16.0±4.4	8.7±3.7 (54%)
De- hypon® 2574			9.0±0.2	0.0±0.0 (0%)	20.2±1.7	10.5±1.5 (52%)
Saturated fatty alcohol Hexadecanol						
Antifoam A	11.6±6.6	2.7±0.5 (23%)	56.1±13.3	47.0±11.4 (84%)	190±15	179.7±8.5 (94%)
Agnique BP420			24.8±17.1	11.5±10.8 (46%)	116±35	104.6 ± 27.1 (90%)
Plurafac® LF1300			24.8±17.1	11.5±10.8 (46%)	116±35	104.6 ± 27.1 (90%)
De- hypon® 2574			4.3±0.5	0.0±0.0 (0%)	15±2	14.0±2.0 (94%)

*Example 10 - Increased production, secretion, and recovery of fatty alcohol acetate esters by the yeast *Saccharomyces cerevisiae**

Here the increased production, secretion, and recovery of fatty alcohol acetate esters by the engineered yeast *Saccharomyces cerevisiae* was demonstrated.

The experiments were performed following the procedures using strain ST5290 as in Example 9, with the modification of supplementing the production medium with an additional 76 mg/L histidine, 0.5 g/L myristic acid methyl ester. For acetate esters of fatty alcohols, GC-MS analyses were performed following the procedures as in Example 9. Apart from acetate esters of fatty alcohols, untargeted screen of data for other esters revealed no significant production of other ester compounds.

The results are shown in Table 10. Significant production and secretion of fatty alcohol acetate esters were observed when the cultivation was supplemented with surfactant above its cloud concentration (at 3 v/v%). When the surfactant was dosed above its cloud concentration, it constituted a separate immiscible hydrophobic phase together

with the in-situ extracted fatty alcohols. Therefore, phase separation and product recovery could be facilitated by simple mechanical phase separation, without the use of organic solvents or costly separation methods.

5 Table 10

ST5290						
Acetate ester of unsaturated fatty alcohol Z9-tetradecen-1-yl acetate						
Surfac- tant	0 v/v% surfactant		0.1 v/v% surfactant		3 v/v% surfactant	
	Total con- centration (mg/L)	Extracel- lular con- centration (mg/L) (fraction of total)	Total con- centration (mg/L)	Extracel- lular con- centration (mg/L) (fraction of total)	Total con- centration (mg/L)	Extracel- lular con- centration (mg/L) (fraction of total)
Antifoam A	0±0	0±0 (0%)	0±0	0±0 (0%)	4.5±1.8	4.4±1.8 (99%)
Agnique BP420			0±0	0±0 (0%)	1.5±1.4	1.5±1.4 (100%)
Plurafac® LF1300			0±0	0±0 (0%)	0±0	1.5±1.4 (100%)
De- hypon® 2574			0.5±0.5	0±0 (0%)	0.5±0.4	0.5±0.4 (100%)
Acetate ester of saturated fatty alcohol tetradecanyl acetate						
Antifoam A	2.1±1.9	0±0 (0%)	5.7±3.2	1.8±2.2 (32%)	28.8±8.0	25.2±4.7 (88%)
Agnique BP420			1.7±2.4	0.5±0.5 (27%)	22.5±16.4	19.6±13.2 (87%)
Plurafac® LF1300			1.7±2.4	0.5±0.5 (27%)	22.5±16.4	19.6±13.2 (87%)
De- hypon® 2574			1.8±0.6	0±0 (0%)	0.2±0.2	0.2±0.2 (100%)

*Example 11 - Increased production, secretion, and recovery of fatty alcohols by the yeast *Yarrowia lipolytica**

Here the increased production, secretion, and recovery of fatty alcohols by the engineered yeast *Yarrowia lipolytica* was demonstrated.

5

Using strains ST8327 and ST9253, the experiments were following the procedures as in Example 9, with the modification of using YPG medium (10 g/L yeast extract, 10 g/L peptone, 40 g/L glycerol), and production medium (50 g/L glycerol, 5 g/L yeast extract, 4 g/L KH_2PO_4 , 1.5 g/L MgSO_4 , 0.2 g/L NaCl , 0.265 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 2 mL/L trace elements solution: 4.5 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 4.5 g/L $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 3 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1 g/L H_3BO_3 , 1 g/L $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.4 g/L $\text{N Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.3 g/L $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1 g/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.1 g/L KI , 15 g/L EDTA).

15

For strain ST8327, GC-MS analyses were performed as in example 9. For strain ST9253, oven temperature was set to 80°C for 1 min, then increased at a rate of 20°C/min to 150°C, and then increased at a rate of 1°C/min to 200°C, and then increased at a rate of 20°C/min to 230°C.

20

Results are shown in Table 11 and Table 12. A significant increase in total titer and secretion was observed when surfactants were supplemented above their cloud concentrations (3 v/v%). When the surfactant was dosed above its cloud concentration, it constituted a separate immiscible hydrophobic phase together with the in-situ extracted fatty alcohols. Therefore, phase separation and product recovery could be facilitated by simple mechanical phase separation, without the use of organic solvents or costly separation methods.

25

Table 11

ST8327						
Unsaturated fatty alcohol Z11-hexadecen-1-ol						
Surfactant	0 v/v% surfactant		0.1 v/v% surfactant		3 v/v% surfactant	
	Total concentration (mg/L)	Extracellular concentration (mg/L) (fraction of total)	Total concentration (mg/L)	Extracellular concentration (mg/L) (fraction of total)	Total concentration (mg/L)	Extracellular concentration (mg/L) (fraction of total)

Antifoam A	761±47	17±7 (2%)	914±68	41±43 (5%)	1721±314	1371±224 (80%)
Agnique BP420			1141±266	53±38 (5%)	1052±224	521±36 (50%)
Plurafac® LF1300			1141±266	53±38 (5%)	1052±224	521±36 (50%)
De-hypon® 2574			827±275	304±164 (37%)	1217±73	709±50 (58%)
Unsaturated fatty alcohol Z9-hexadecen-1-ol						
Antifoam A	77±3	0±0 (0%)	105±13	1±2 (1%)	163±28	133±17 (81%)
Agnique BP420			128±24	2±3 (2%)	119±27	58±3 (48%)
Plurafac® LF1300			128±24	2±3 (2%)	119±27	58±3 (48%)
De-hypon® 2574			72±25	26±15 (36%)	129±19	71±15 (55%)
Saturated fatty alcohol hexadecanol						
Antifoam A	456±149	9±4 (2%)	560±91	24±26 (4%)	1155±214	890±126 (77%)
Agnique BP420			751±149	48±43 (6%)	740±202	363±11 (49%)
Plurafac® LF1300			751±149	48±43 (6%)	740±202	363±11 (49%)
De-hypon® 2574			764±298	262±144 (34%)	560±33	342±24 (61%)

Table 12

ST9253			
Unsaturated fatty alcohol Z11-tetradecen-1-ol			
	0 v/v% surfactant	0.1 v/v% surfactant	3 v/v% surfactant

Surfac- tant	Total con- centration (mg/L)	Extracel- lular con- centration (mg/L) (fraction of total)	Total con- centration (mg/L)	Extracel- lular con- centration (mg/L) (fraction of total)	Total con- centration (mg/L)	Extracel- lular con- centration (mg/L) (fraction of total)
Antifoam A	18.8 ± 0.8	3.1 ± 0.1 (16%)	17.9 ± 0.9	3.8±0.2 (22%)	35.3±2.8	30.6±2.0 (87%)
Agnique BP420			21.0±12.5	10.7±6.5 (51%)	28.5±6.1	23.7±5.4 (83%)
Plurafac® LF1300			21.0±12.5	10.7±6.5 (51%)	28.5±6.1	23.7±5.4 (83%)
De- hypon® 2574			15.5±2.7	6.0±1.1 (39%)	23.8±2.2	15.6±0.9 (66%)
Unsaturated fatty alcohol E11-tetradecen-1-ol						
Antifoam A	108.7 ± 4.6	10.3 ± 0.5 (10%)	110.3 ± 7.1	17.1 ± 3.1 (16%)	350.7 ± 31.3	324.4 ± 23.3 (93%)
Agnique BP420			150.2 ± 107.1	81.1 ± 59.4 (54%)	254.8 ± 65.5	228.1 ± 58.7 (90%)
Plurafac® LF1300			150.2 ± 107.1	81.1 ± 59.4 (54%)	254.8 ± 65.5	228.1 ± 58.7 (90%)
De- hypon® 2574			112.4 ± 16.4	44.0 ± 7.9 (39%)	209.7 ± 10.1	153.4 ± 1.1 (73%)

*Example 12 - Increased production, secretion and recovery of fatty alcohols by the yeast *Yarrowia lipolytica**

Here the increased production, secretion, and recovery of fatty alcohols by the engineered yeast *Yarrowia lipolytica* was demonstrated.

5

- The experiments were performed following the procedures as in Example 11 using strains ST10229, ST10230 and ST10231, with the modification of supplementing both YPG and production media with 150 mg/L nourseothricin. When the cells were resuspended in production medium, control 1, 0 v/v% Antifoam A, control 2, 0.1 v/v% Antifoam A (approximate recommended dose by manufacturer for foam management) and 3 v/v% (above the cloud concentration of the surfactant) Antifoam A (Bekchem; ethoxylated and propoxylated C16-18 alcohols, CAS No. 68002-96-0) was added. GC-MS analyses were performed as in example 9.
- The results are shown in Table 13. Significant production and secretion of fatty alcohols were observed when the cultivation was supplemented with surfactant above its cloud concentration (at 3 v/v%). When the surfactant was dosed above its cloud concentration, it constituted a separate immiscible hydrophobic phase together with the in-situ extracted fatty alcohols. Therefore, phase separation and product recovery could be facilitated by simple mechanical phase separation, without the use of organic solvents or costly separation methods.

Table 13

Unsaturated fatty alcohol Z9-tetradecen-1-ol						
Saturated fatty alcohols tetradecanol						
ST10229						
Surfactant	0 v/v% surfactant		0.1 v/v% surfactant		3 v/v% surfactant	
	Total concentration of unsaturated/saturated fatty alcohol (mg/L)	Extracellular concentration of unsaturated/saturated fatty alcohol (mg/L) (fraction of total)	Total concentration of unsaturated/saturated fatty alcohol (mg/L)	Extracellular concentration of unsaturated/saturated fatty alcohol (mg/L) (fraction of total)	Total concentration of unsaturated/saturated fatty alcohol (mg/L)	Extracellular concentration of unsaturated/saturated fatty alcohol (mg/L) (fraction of total)
Antifoam A	80.5 ± 37.3/	0 ± 0 (0%)/	79.6 ± 12.9/	0 ± 0 (0 %)/	194.4 ± 22.5/	177.8 ± 33.8

	127.6 ± 64.3	0 ± 0 (0%)	111.9 ± 17.8	0 ± 0 (0%)	215.9 ± 24.9	(91%)/ 198.1 ± 37.7 (92%)
ST10230						
Antifoam A	106.7 ± 7.3/ 189.7 ± 15.0	0 ± 0 (0 %)/ 0 ± 0 (0%)	105.7 ± 7.0/ 169.1 ± 13.1	1.0 ± 0.5 (1 %)/ 0 ± 0 (0%)	176.9 ± 14.3/ 218.0 ± 18.3	145.7 ± 2.2 (81%)/ 179.6 ± 4.2 (82%)
ST10231						
Antifoam A	22.2 ± 3.2/ 490.8 ± 19.0	0 ± 0 (0 %)/ 5.2 ± 7.1 (1%)	44.4 ± 5.6/ 445.0 ± 30.9	0 ± 0 (0 %)/ 5.7 ± 2.1 (1.2%)	97.7 ± 0.1/ 443.2 ± 3.8*	69.3 ± 4.1 (70%)/ 307 ± 18.7 (69%)*

* only technical duplicates available

Example 13 - Increased secretion and recovery of fatty aldehydes by the yeast Yarrowia lipolytica

5

Here the increased secretion and recovery of fatty aldehydes by the engineered yeast *Yarrowia lipolytica* was demonstrated.

10 Using strain ST8327, the experiments were performed following the procedures as in Example 10. GC-MS analyses were performed as in Example 9.

15 Result are shown in Table 14. Significant secretion was observed when surfactants were supplemented above their cloud concentrations (3 v/v%). When the surfactant was dosed above its cloud concentration, it constituted a separate immiscible hydrophobic phase together with the in-situ extracted fatty alcohols. Therefore, phase separation and product recovery were facilitated by simple mechanical phase separation, without the use of organic solvents or costly separation methods.

Table 14

ST8327			
Unsaturated fatty aldehyde Z11-hexadecen-1-al			
Surfac- tant	0 v/v% surfactant	0.1 v/v% surfactant	3 v/v% surfactant
	Extracellular concen- tration (mg/L) (fraction of to- tal)	Extracellular concen- tration (mg/L) (fraction of to- tal)	Extracellular concen- tration (mg/L) (fraction of to- tal)
Antifoam A	2.9±0.3 (23%)	0.8±1.1 (6%)	15.0±2.9 (80%)
Agnique BP420		0.4±0.5 (2%)	5.0±0.6 (43%)
Plurafac® LF1300		0.4±0.5 (2%)	5.0±0.6 (43%)
De- hypon® 2574		2.9±1.9 (36%)	3.9±0.3 (57%)
Saturated fatty aldehyde hexadecanal			
Antifoam A	0.0±1.3 (0%)	6.8±11.8 (22%)	37.6±9.3 (82%)
Agnique BP420		0.0±0.0 (0%)	7.3±0.7 (35%)
Plurafac® LF1300		0.0±0.0 (0%)	7.3±0.7 (35%)
De- hypon® 2574		8.3±1.4 (46%)	8.3±1.4 (44%)

Example 14 - Purification of fatty alcohols from the recovered mixture of fatty alcohols and surfactants

- 5 The purification of fatty alcohols from the hydrophobic mixture of fatty alcohols and surfactants was demonstrated by distillation.

Model mixtures were prepared of different commercially available ethoxylated surfactants (Antifoam A from Bekchem, Plurafac® LF 1300, Dehypon® 2574) and a technical grade fatty alcohol mixture. The model mixtures were subjected to vacuum distillation in a laboratory scale distillation setup equipped with Vigreux column. The experiments were carried out applying 5 mbar vacuum, 200-210°C final pot and 170-180°C final receiver temperature. The light phase was collected and analyzed for mass and composition.

Appropriate amounts of samples were transferred to 50 mL volumetric flasks and weighed on an analytical balance. The samples were dissolved in ethyl acetate in the volumetric flasks and were well mixed. 1 mL of the diluted aliquots were transferred to GC vials and 10 µL of internal standard (IS) solution (20 mg/L of methyl nonadecanoate in ethyl acetate) was added to each GC vial. The vials were vortexed for 10 s before analysis. GC and data analysis were performed as described in Example 11.

Results are shown in Table 15. The composition of the collected light phase from a model mixture distillation experiment demonstrated the enrichment of the target fatty alcohol compounds in the relevant fraction. Distillation is a cost-effective solution to purify the target compounds from the recovered hydrophobic surfactant-fatty alcohol, fatty alcohol ester and fatty aldehyde mixtures presented in Examples 9-11 and 13.

Table 15

Surfactant	Start mass model mixture (g)	Composition start Z11-16:OH (% of mass)	Composition start 16:OH (% of mass)	Composition start Z9-16:OH (5 of mass)
Antifoam A	60.1	21.9	1.4	0.5
Plurafac® LF 1300	60.0	21.3	1.3	0.5
Dehypon® 2574	60.0	21.6	1.4	0.5

Surfactant	Light phase mass (g)	Composition light phase	Composition light phase	Composition light phase Z9-
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		Z11-16:OH (% of mass)	16:OH (% of mass)	16:OH (% of mass)
Antifoam A	8.7	54.7	4.1	1.7
Plurafac® LF 1300	7.7	55.2	4.0	2.1
Dehypon® 2574	6.8	49.3	3.5	1.8

Example 15 – Increased secretion and recovery of isoprenoids by the yeast Yarrowia lipolytica

Here the increased secretion, and recovery of isoprenoids by the engineered yeast
5 *Yarrowia lipolytica* was demonstrated.

Engineered *Yarrowia lipolytica* strains ST10151 is capable of producing β -farnesene. The strain expresses the β -farnesene synthase from *Artemisia annua* (SEQ ID NO: 47). The strain has additional modifications that increase mevalonate (MVA) pathway
10 flux.

The experiments were performed following the procedures as in Example 11 using strain ST10151. When the cells were resuspended in production medium, 0 v/v% , 0.1 v/v% or 3 v/v% surfactant was added of the following surfactants: Antifoam A (Bek-
15 chem) or A-204 (Sigma). The plates were incubated at 28°C and 300 rpm for 28 hours. Each experiment was performed in biological triplicates.

The extracellular and intracellular samples for GC-MS analysis were analysed following the procedures as in Example 9, except that Patchouli alcohol (2 g/L of Patchouli alco-
20 hol in ethyl acetate) was used as internal standard. The GC-FID analysis for β -farnesene was performed using Agilent GC 7890B with a flame ionization detector (FID) and equipped with a fused-silica capillary column (BP5, 30 m x 0.32 mm ID, 0.25 μ m, Ag-
ilent Technologies). Hydrogen at a constant flow rate of 2.0 mL/min was used as the carrier gas. The GC oven temperature started at 50°C for 1.5 min and then increased
25 to 170°C at 30°C/min and hold for 1.5 min. Then from 170 to 300°C at 15°C/min and hold for 4.5 min. The injector and detector ports were both kept at 300°C and the injector operated in a split mode of 20:1. For quantification of β -farnesene calibration standards containing β -farnesene with a concentration range of 0.01 mg/ml to 1 mg/ml were

prepared. 10 µl of 2 g/l of Patchouli alcohol in ethyl acetate was added to 990 µl standard and a calibration curve was obtained. Data analysis was performed with MasHunter Quantitative Analysis Version 10.1.

- 5 Result are shown in Table 16. Significant production and secretion of isoprenoids were observed when surfactants (Antifoam A and A-204) were supplied above their cloud concentrations (3 v/v%). When the surfactants were dosed above their cloud concentration, it constituted a separate immiscible hydrophobic phase together with the in-situ extracted isoprenoids. Therefore, phase separation and product recovery was facilitated by simple mechanical phase separation, without the use of organic solvents or
- 10 costly separation methods.

Table 16

Surfactant	0 v/v% surfactant		0.1 v/v% surfactant		3 v/v% surfactant	
	Total concentration (mg/L)	Extracellular concentration (mg/L) (fraction of total)	Total concentration (mg/L)	Extracellular concentration (mg/L) (fraction of total)	Total concentration (mg/L)	Extracellular concentration (mg/L) (fraction of total)
Antifoam A	3.9±0.1	0.0±0.0 (0%)	4.4±0.1	0.0±0.0 (0%)	27.7±0.2	25.7±2.0 (92.8%)
A-204			6.7±1.3	0.0±0.0 (0%)	35.6±5.8	17.7±5.0 (69.2%)

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Sequence overview

Sequence ID NO:		Description
1	Atr_Δ11	<i>Amyelois transitella</i> Δ11-desaturase

2	Sl_Δ11	<i>Spodoptera littoralis</i> Δ11-desaturase
3	As_Δ11	<i>Agrotis segetum</i> Δ11-desaturase
4	Tni_Δ11	<i>Trichoplusia ni</i> Δ11-desaturase
5	Har_FAR	<i>Helicoverpa armigera</i> fatty acyl-CoA reductase
6	Hs_FAR	<i>Heliothis subflexa</i> fatty acyl-CoA reductase
7	Has_FAR	<i>Helicoverpa assulta</i> fatty acyl-CoA reductase
8	ScFAA1	<i>Saccharomyces cerevisiae</i> fatty acyl synthetase
9	YIFAA	<i>Yarrowia lipolytica</i> fatty acyl synthetase
10	ScATF1	<i>Saccharomyces cerevisiae</i> acetyltransferase
11	Fao1	<i>Yarrowia lipolytica</i> fatty alcohol oxidase
12	Hfd1	<i>Yarrowia lipolytica</i> fatty aldehyde dehydrogenase 1
13	Hfd4	<i>Yarrowia lipolytica</i> fatty aldehyde dehydrogenase 4
14	Pex10	<i>Yarrowia lipolytica</i> peroxisome biogenesis factor 10
15	GPAT	<i>Yarrowia lipolytica</i> glycerol-3-phosphate acyltransferase
16	DmeΔ9	<i>Drosophila melanogaster</i> Δ9 desaturase
17	Ban_FAR	<i>Bicyclus anynana</i> fatty acyl reductase
18	SlΔ9	<i>Spodoptera litura</i> Δ9 desaturase
19	Yli_POX1 (XP_504703)	<i>Yarrowia lipolytica</i> Peroxisomal oxidase 1
20	Yli_POX2 (XP_505264)	<i>Yarrowia lipolytica</i> Peroxisomal oxidase 2

21	Yli_POX3 (XP_503244)	<i>Yarrowia lipolytica</i> Peroxisomal oxidase 3
22	Yli_POX4 (XP_504475)	<i>Yarrowia lipolytica</i> Peroxisomal oxidase 4
23	Yli_POX5 (XP_502199)	<i>Yarrowia lipolytica</i> Peroxisomal oxidase 5
24	Yli_POX6 (XP_503632)	<i>Yarrowia lipolytica</i> Peroxisomal oxidase 6
25	Ase_POX	<i>Agrotis segetum</i> Peroxisomal oxidase
26	Ath_POX1	<i>Arabidopsis thaliana</i> Peroxisomal oxidase 1
27	Ath_POX2	<i>Arabidopsis thaliana</i> Peroxisomal oxidase 2
28	Ani_POX	<i>Aspergillus nidulans</i> Peroxisomal oxidase
29	Cma_POX	<i>Cucurbita maxima</i> Peroxisomal oxidase
30	Hsa_POX1-2	<i>Homo sapiens</i> Peroxisomal oxidase
31	Pur_POX	<i>Paenarthrobacter ureafaciens</i> Peroxisomal oxidase
32	Rno_POX2	<i>Rattus norvegicus</i> Peroxisomal oxidase
33	Sce_OLE1	<i>Saccharomyces cerevisiae</i> Δ_{Z9} -desaturase
34	Yli_OLE1 (XP_501496)	<i>Yarrowia lipolytica</i> Δ_{Z9} -desaturase
35	Cro_Z11	<i>Choristoneura rosaceana</i> Δ_{Z11-14} -desaturase
36	Onu_11	<i>Ostrinia nubilalis</i> Δ_{Z11-14} -desaturase
37	Tpi_D13	<i>Thaumetopoea pityocampa</i> Δ_{Z11-14} -desaturase
38	Dpu_E9-14	<i>Dendrophilus punctatus</i> Δ_{E9-14} -desaturase

39	Gmo_CPRQ	<i>Grapholita molesta</i> $\Delta_{Z/E10}$ -14-desaturase
40	Epo_E11	<i>Epiphyas postvittana</i> desaturase
41	Sls_ZE11	<i>Spodoptera littoralis</i> desaturase
42	Cpa_E11	<i>Choristoneura parallela</i> desaturase
43	LboPPTQ	<i>Lobesia brotana</i> desaturase
44	Dgd9	<i>Drosophila grimshawi</i> desaturase
45	Dvd9	<i>Drosophila virilis</i> desaturase
46	SynGGPPs7	<i>Synechococcus</i> sp. geranylgeranyl diphosphate synthase
47	Aa_BFS	<i>Artemisia annua</i> β -farnesene synthase
48	Cpo_CPRQ	<i>Cydia pomonella</i> CPO_CPRQ desaturase (AHW98354)
49	Cpo_NPVE	<i>Cydia pomonella</i> desaturase
50	Cpo_SPTQ	<i>Cydia pomonella</i> desaturase

Items

1. A method for producing a hydrophobic compound such as a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate, a fatty aldehyde and/or a terpene such as a terpenoid in a fermentation, said method comprising the step of providing a microorganism, preferably a yeast cell, capable of producing said hydrophobic compound and culturing said microorganism in a culture medium under conditions allowing production of said hydrophobic compound, wherein the culture medium comprises an extractant in an amount equal to or greater than its cloud concentration in an aqueous solution, wherein the extractant a non-ionic surfactant such as an antifoaming agent, preferably a polyethoxylated surfactant selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof, the method optionally further comprising the step of recovering the hydrophobic compound.

2. A method for increasing the titer of a hydrophobic compound such as a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate, a fatty aldehyde and/or a terpene such as a terpenoid in a fermentation, said method comprising culturing a microorganism, preferably a yeast cell, capable of producing said hydrophobic compound in a culture medium under conditions allowing production of said hydrophobic compound, wherein the culture medium comprises an extractant in an amount equal to or greater than its cloud concentration in an aqueous solution, wherein the extractant is a non-ionic surfactant such as an antifoaming agent, preferably a polyethoxylated surfactant selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof, whereby the titer of the hydrophobic compound is increased compared to a fermentation performed under similar conditions in the absence of extractant or in the presence of extractant in an amount lower than its cloud concentration in an aqueous solution.
3. A method for increasing the secretion of a hydrophobic compound such as a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate, a fatty aldehyde and/or a terpene such as a terpenoid from a microorganism, preferably a yeast cell, capable of producing said hydrophobic compound in a fermentation, said method comprising culturing said microorganism in a culture medium under conditions allowing production of said hydrophobic compound, wherein the culture medium comprises an extractant in an amount equal to or greater than its cloud concentration in an aqueous solution, wherein the extractant is a non-ionic surfactant such as an antifoaming agent, preferably a polyethoxylated surfactant selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof, whereby the secretion of the hydrophobic compound from the microorganism is increased compared to a fermentation performed under similar conditions in the absence of extractant or in the presence of extractant in an amount lower than its cloud concentration in an aqueous solution.

4. The method according to any one of the preceding items, wherein the extractant is a non-ionic ethoxylated surfactant.
5. The method according to any one of the preceding items, wherein the extractant is a fatty alcohol alkoxylate or a polyethoxylated surfactant, preferably selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate, simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents or ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof.
6. The method according to any one of the preceding items, wherein the fatty alcohols are saturated fatty alcohols, desaturated fatty alcohols, or a mixture thereof.
7. The method according to any one of the preceding items, wherein the fatty acyl acetates are saturated fatty acyl acetates, desaturated fatty acyl acetates, or a mixture thereof.
8. The method according to any one of the preceding items, wherein the fatty aldehydes are saturated fatty aldehydes, desaturated fatty aldehydes, or a mixture thereof.
9. The method according to any one of the preceding items, wherein the fatty alcohol esters are saturated fatty alcohol esters, desaturated fatty alcohol esters, or a mixture thereof.
10. The method according to any one of the preceding items, wherein the fatty alcohols, fatty alcohol esters, fatty acyl acetates and/or fatty aldehydes have a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22.
11. The method according to any one of the preceding items, wherein the terpene is a hemiterpene, a monoterpene, a sesquiterpene, a disesterterpene, a triterpene, a sesquaraterpene, a tetraterpene, or a polyterpene.

12. The method according to any one of the preceding items, wherein the terpene is a terpenoid, such as a hemiterpenoid, a monoterpenoid, a sesquiterpenoid, a disesterterpenoid, a triterpenoid, a sesquaraterpenoid, a tetraterpenoid or a polyterpenoid.
- 5
13. The method according to any one of the preceding items, wherein the extractant is an ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agent or an ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agent, preferably having a cloud concentration of 1% vol/vol in an aqueous solution.
- 10
14. The method according to any one of the preceding items, wherein the extractant is selected from C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate (CAS number 68002-96-0), Agnique BP420 (CAS number 68002-96-0), a polyethylene polypropylene glycol, antifoam 204, a surfactant comprising polyethylene glycol monostearate, and a fatty alcohol alkoxylate, such as Kolliphor® P407 (CAS number 9003-11-6), simethicone, Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), or Imbentin SG/251 (CAS number 68002-96-0).
- 15
- 20
15. The method according to any one of the preceding items, wherein the culture medium comprises at least 1% vol/vol extractant, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol extractant.
- 25
- 30
16. The method according to any one of the preceding items, wherein the non-ionic surfactant is an antifoaming agent.
- 35
17. The method according to any one of the preceding items, wherein the non-ionic surfactant is an ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agent or an ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agent, such as C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate (CAS number 68002-96-0), and

- wherein the culture medium comprises at least 1% vol/vol of C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate, or more.
18. The method according to any one of the preceding items, wherein the non-ionic surfactant is a polyethylene polypropylene glycol, for example Kolliphor® P407 (CAS number 9003-11-6), and wherein the culture medium comprises at least 10% vol/vol of polyethylene polypropylene glycol such as Kolliphor® P407, such as at least 11% vol/vol, such as at least 12% vol/vol, such as at least 13% vol/vol, such as at least 14% vol/vol, such as at least 15% vol/vol, such as at least 16% vol/vol, such as at least 17% vol/vol, such as at least 18% vol/vol, such as at least 19% vol/vol, such as at least 20% vol/vol, such as at least 25% vol/vol, such as at least 30% vol/vol, such as at least 35% vol/vol of polyethylene polypropylene glycol such as Kolliphor® P407, or more.
19. The method according to any one of the preceding items, wherein the non-ionic surfactant is Agnique BP420 (CAS number 68002-96-0), and wherein the culture medium comprises at least 10% vol/vol of Agnique BP420 (CAS number 68002-96-0), such as at least 11% vol/vol, such as at least 12% vol/vol, such as at least 13% vol/vol, such as at least 14% vol/vol, such as at least 15% vol/vol, such as at least 16% vol/vol, such as at least 17% vol/vol, such as at least 18% vol/vol, such as at least 19% vol/vol, such as at least 20% vol/vol, such as at least 25% vol/vol, such as at least 30% vol/vol, such as at least 35% vol/vol of polyethylene polypropylene glycol such as Agnique BP420 (CAS number 68002-96-0), or more.
20. The method according to any one of the preceding items, wherein the non-ionic surfactant is a mixture of polyether dispersions, such as antifoam 204, and wherein the culture medium comprises at least 1% vol/vol of a mixture of polyether dispersions such as antifoam 204, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as

- at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol of a mixture of polyether dispersions such as antifoam 204, or more.
- 5
21. The method according to any one of the preceding items, wherein the non-ionic surfactant is simethicone, and wherein the culture medium comprises at least
- 10 1% vol/vol of simethicone, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least
- 15 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol simethicone, or more.
22. The method according to any one of the preceding claims, wherein the non-ionic surfactant is a fatty alcohol alkoxylate selected from Plurafac® LF300
- 20 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and wherein the culture medium comprises at least
- 25 1% vol/vol of Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), Imbentin SG/251 (CAS number 68002-96-0), such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such
- 30 as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), Im-
- 35 bentin SG/251 (CAS number 68002-96-0), or more.

23. The method according to any one of the preceding items, wherein the culture medium comprises the extractant in an amount greater than its cloud concentration by at least 50%, such as at least 100%, such as at least 150%, such as at least 200%, such as at least 250%, such as at least 300%, such as at least 350%, such as at least 400%, such as at least 500%, such as at least 750%, such as at least 1000%, or more.
24. The method according to any one of the preceding items, wherein the culture medium comprises the extractant in an amount at least 2-fold its cloud concentration, such as at least 3-fold its cloud concentration, such as at least 4-fold its cloud concentration, such as at least 5-fold its cloud concentration, such as at least 6-fold its cloud concentration, such as at least 7-fold its cloud concentration, such as at least 8-fold its cloud concentration, such as at least 9-fold its cloud concentration, such as at least 10-fold its cloud concentration, such as at least 12.5-fold its cloud concentration, such as at least 15-fold its cloud concentration, such as at least 17.5-fold its cloud concentration, such as at least 20-fold its cloud concentration, such as at least 25-fold its cloud concentration, such as at least 30-fold its cloud concentration.
25. The method according to any one of the preceding items, wherein the hydrophobic compound produced by the microorganism is present in an emulsion in the fermentation broth, the method further comprising a step of breaking said emulsion, thereby obtaining a composition comprising a product phase comprising the extractant and the hydrophobic compound.
26. The method according to item 25, wherein the step of breaking the emulsion comprises or consists of a step of phase separation, such as a step of centrifugation, of the fermentation broth, thereby obtaining a composition consisting of three phases: a water phase, a phase comprising cells and cellular debris, and the product phase comprising the extractant and the hydrophobic compound.
27. The method according to item 26, wherein the product phase comprises at least 50% of the hydrophobic compound initially present in the fermentation broth, such as at least 55%, such as at least 60%, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 95% or more.

28. The method according to any one of items 26 to 27, further comprising recovering the product phase comprising the extractant and the hydrophobic compound from the composition, and optionally further separating the hydrophobic compound from the extractant, wherein the separation preferably is a distillation such as a distillation under reduced pressure, or a column purification.
29. The method according to any one of the preceding items, wherein the hydrophobic compound is one or more fatty alcohols, and wherein the method further comprises the steps of:
- recovering said one or more fatty alcohols, preferably by a distillation step such as a distillation under reduced pressure, or by a column purification, thereby obtaining a mixture of fatty alcohols,
 - chemically converting at least part of the fatty alcohols of said mixture to the corresponding fatty acyl acetates and/or to the corresponding fatty aldehydes.
30. The method according to item 29, wherein at least part of the fatty alcohols are converted to the corresponding fatty acyl acetates by acetylation.
31. The method according to any one of items 29 to 30, wherein at least part of the fatty alcohols are converted to the corresponding fatty aldehydes by oxidation.
32. The method according to any one of items 29 to 31, further comprising the step of recovering said corresponding fatty acyl acetates and/or said corresponding fatty aldehydes.
33. The method according to any one of the preceding items, wherein the extractant is recovered from the fermentation broth and optionally recycled to the fermentation broth.
34. The method according to any one of the preceding items, wherein the titer of the hydrophobic compound is increased by at least 5% compared to the titer obtained in a fermentation performed under similar or identical conditions but either in the absence of extractant or in the presence of extractant in an amount lower than its cloud concentration in an aqueous solution, such as by at least 10%, such as by at least 15%, such as by at least 20%, such as by at least

25%, such as by at least 30%, such as by at least 35%, such as by at least 40%, such as by at least 45%, such as by at least 46%, such as by at least 47%, such as by at least 48%, such as by at least 49%, such as by at least 50%, such as by at least 51%, such as by at least 52%, such as by at least 53%, such as by at least 54%, such as by at least 55% or more, preferably wherein the non-ionic surfactant is selected from: Agnique BP420 (CAS number 68002-96-0), a polyethylene polypropylene glycol, a mixture of polyether disper-
 5 sions, an antifoaming agent comprising polyethylene glycol monostearate, si-
 methicone, ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming
 10 agents and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agents and combinations thereof.

35. The method according to any one of the preceding items, wherein the secretion of the hydrophobic compound is increased by at least 5% compared to a fer-
 15 mentation performed under similar or identical conditions but either in the ab-
 sence of extractant or in the presence of extractant in an amount lower than its
 cloud concentration measured in an aqueous solution, such as by at least 7.5%,
 such as by at least 10%, such as by at least 12.5%, such as by at least 15%,
 such as by at least 20%, such as by at least 25%, such as by at least 30%,
 20 such as by at least 35%, such as by at least 36%, such as by at least 37%,
 such as by at least 38%, such as by at least 39%, such as by at least 40%,
 such as by at least or more, preferably wherein the non-ionic surfactant is se-
 lected from: Agnique BP420 (CAS number 68002-96-0), a polyethylene poly-
 propylene glycol, a mixture of polyether dispersions, an antifoaming agent com-
 25 prising polyethylene glycol monostearate, simethicone, ethoxylated and propox-
 ylated C₁₆-C₁₈ alcohol-based antifoaming agents and ethoxylated and propox-
 ylated C₁₆-C₁₈ alcohol-based agents and combinations thereof.

36. The method according to any one of the preceding items, wherein the hydro-
 30 phobic compound is a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate
 and/or a fatty aldehyde and the extractant is an ethoxylated and propoxylated
 C₁₆-C₁₈ alcohol-based agent or antifoaming agent, preferably wherein the me-
 dium comprises more than 1% vol/vol of ethoxylated and propoxylated C₁₆-C₁₈
 alcohol-based agent or antifoaming agent.

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37. The method according to item 36, wherein the titer of fatty alcohol, the titer of fatty acyl acetate and/or the titer of fatty aldehyde is increased by at least 5% compared to the titer obtained in a fermentation performed under similar or identical conditions but either in the absence of ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agent or antifoaming agent or in the presence of ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agent or antifoaming agent in an amount lower than its cloud concentration in an aqueous solution, such as by at least 10%, such as by at least 15%, such as by at least 20%, such as by at least 25%, such as by at least 30%, such as by at least 35%, such as by at least 40%, such as by at least 45%, such as by at least 46%, such as by at least 47%, such as by at least 48%, such as by at least 49%, such as by at least 50%, such as by at least 51%, such as by at least 52%, such as by at least 53%, such as by at least 54%, such as by at least 55% or more.
38. The method according to any one of items 36 to 37, wherein the secretion of the fatty alcohol, the fatty acyl acetate and/or the fatty aldehyde is increased by at least 5% compared to a fermentation performed under similar or identical conditions but either in the absence of ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agent or antifoaming agent or in the presence of ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based agent or antifoaming agent in an amount lower than its cloud concentration in an aqueous solution, such as by at least 7.5%, such as by at least 10%, such as by at least 12.5%, such as by at least 15%, such as by at least 20%, such as by at least 25%, such as by at least 30%, such as by at least 35%, such as by at least 36%, such as by at least 37%, such as by at least 38%, such as by at least 39%, such as by at least 40%, such as by at least or more.
39. The method according to any one of the preceding items, wherein the hydrophobic compound is a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate and/or a fatty aldehyde and the extractant is a polyethylene polypropylene glycol, preferably wherein the medium comprises more than 1% vol/vol of polyethylene polypropylene glycol.
40. The method according to item 39, wherein the titer of fatty alcohol, the titer of fatty acyl acetate and/or the titer of fatty aldehyde is increased by at least 5% compared to the titer obtained in a fermentation performed under similar or

- identical conditions but either in the absence of polyethylene polypropylene glycol or in the presence of polyethylene polypropylene glycol in an amount lower than its cloud concentration in an aqueous solution, such as by at least 10%, such as by at least 15%, such as by at least 20%, such as by at least 25%,
 5 such as by at least 30%, such as by at least 35%, such as by at least 40%, such as by at least 45%, such as by at least 46%, such as by at least 47%, such as by at least 48%, such as by at least 49%, such as by at least 50%, such as by at least 51%, such as by at least 52%, such as by at least 53%, such as by at least 54%, such as by at least 55% or more.
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41. The method according to any one of items 39 to 40, wherein the secretion of the fatty alcohol, the fatty acyl acetate and/or the fatty aldehyde is increased by at least 5% compared to a fermentation performed under similar or identical conditions but either in the absence of polyethylene polypropylene glycol or in
 15 the presence of polyethylene polypropylene glycol in an amount lower than its cloud concentration in an aqueous solution, such as by at least 7.5%, such as by at least 10%, such as by at least 12.5%, such as by at least 15%, such as by at least 20%, such as by at least 25%, such as by at least 30%, such as by at least 35%, such as by at least 36%, such as by at least 37%, such as by at
 20 least 38%, such as by at least 39%, such as by at least 40%, such as by at least or more.
42. The method according to any one of the preceding items, wherein the hydrophobic compound is a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate
 25 and/or a fatty aldehyde and the extractant is a mixture of polyether dispersions, preferably wherein the medium comprises more than 1% vol/vol of a mixture of polyether dispersions.
43. The method according to item 42, wherein the titer of fatty alcohol, the titer of fatty acyl acetate and/or the titer of fatty aldehyde is increased by at least 5% compared to the titer obtained in a fermentation performed under similar or identical conditions but either in the absence of the mixture of polyether disper-
 30 sions or in the presence of the mixture of polyether dispersions in an amount lower than its cloud concentration in an aqueous solution, such as by at least 10%, such as by at least 15%, such as by at least 20%, such as by at least 25%, such as by at least 30%, such as by at least 35%, such as by at least
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40%, such as by at least 45%, such as by at least 46%, such as by at least 47%, such as by at least 48%, such as by at least 49%, such as by at least 50%, such as by at least 51%, such as by at least 52%, such as by at least 53%, such as by at least 54%, such as by at least 55% or more.

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44. The method according to any one of items 42 to 43, wherein the secretion of the fatty alcohol, the fatty acyl acetate and/or the fatty aldehyde is increased by at least 5% compared to a fermentation performed under similar or identical conditions but either in the absence of mixture of polyether dispersions or in the presence of the mixture of polyether dispersions in an amount lower than its cloud concentration in an aqueous solution, such as by at least 7.5%, such as by at least 10%, such as by at least 12.5%, such as by at least 15%, such as by at least 20%, such as by at least 25%, such as by at least 30%, such as by at least 35%, such as by at least 36%, such as by at least 37%, such as by at least 38%, such as by at least 39%, such as by at least 40%, such as by at least or more.

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45. The method according to any one of the preceding items, wherein the hydrophobic compound is a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate and/or a fatty aldehyde and the extractant is simethicone, preferably wherein the medium comprises more than 1% vol/vol of simethicone.

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46. The method according to item 45, wherein the titer of fatty alcohol, the titer of fatty acyl acetate and/or the titer of fatty aldehyde is increased by at least 5% compared to the titer obtained in a fermentation performed under similar or identical conditions but either in the absence of simethicone or in the presence of simethicone in an amount lower than its cloud concentration in an aqueous solution, such as by at least 10%, such as by at least 15%, such as by at least 20%, such as by at least 25%, such as by at least 30%, such as by at least 35%, such as by at least 40%, such as by at least 45%, such as by at least 46%, such as by at least 47%, such as by at least 48%, such as by at least 49%, such as by at least 50%, such as by at least 51%, such as by at least 52%, such as by at least 53%, such as by at least 54%, such as by at least 55% or more.

47. The method according to any one of items 45 to 46, wherein the secretion of the fatty alcohol, the fatty acyl acetate and/or the fatty aldehyde is increased by at least 5% compared to a fermentation performed under similar or identical conditions but either in the absence of simethicone or in the presence of the antifoaming agent comprising simethicone in an amount lower than its cloud concentration in an aqueous solution, such as by at least 7.5%, such as by at least 10%, such as by at least 12.5%, such as by at least 15%, such as by at least 20%, such as by at least 25%, such as by at least 30%, such as by at least 35%, such as by at least 36%, such as by at least 37%, such as by at least 38%, such as by at least 39%, such as by at least 40%, such as by at least or more.
48. The method according to any one of the preceding items, wherein the hydrophobic compound is a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate and/or a fatty aldehyde and the extractant is a C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate (CAS number 68002-96-0), Agnique BP420 (CAS number 68002-96-0), a polyethylene polypropylene glycol, antifoam 204, a surfactant comprising polyethylene glycol monostearate, and a fatty alcohol alkoxylate, such as Kolliphor® P407 (CAS number 9003-11-6), simethicone, Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), or Imbentin SG/251 (CAS number 68002-96-0), preferably wherein the medium comprises more than 1% vol/vol of the extractant.
49. The method according to item 48, wherein the titer of fatty alcohol, the titer of fatty acyl acetate and/or the titer of fatty aldehyde is increased by at least 5% compared to the titer obtained in a fermentation performed under similar or identical conditions but either in the absence of the extractant or in the presence of the extractant in an amount lower than its cloud concentration in an aqueous solution, such as by at least 10%, such as by at least 15%, such as by at least 20%, such as by at least 25%, such as by at least 30%, such as by at least 35%, such as by at least 40%, such as by at least 45%, such as by at least 46%, such as by at least 47%, such as by at least 48%, such as by at least 49%, such as by at least 50%, such as by at least 51%, such as by at least 52%, such as by at least 53%, such as by at least 54%, such as by at least 55% or more.

50. The method according to any one of items 48 to 49, wherein the secretion of the fatty alcohol, the fatty acyl acetate and/or the fatty aldehyde is increased by at least 5% compared to a fermentation performed under similar or identical conditions but either in the absence of the extractant or in the presence of the extractant in an amount lower than its cloud concentration in an aqueous solution, such as by at least 7.5%, such as by at least 10%, such as by at least 12.5%, such as by at least 15%, such as by at least 20%, such as by at least 25%, such as by at least 30%, such as by at least 35%, such as by at least 36%, such as by at least 37%, such as by at least 38%, such as by at least 39%, such as by at least 40%, such as by at least or more.
51. The method according to any one of the preceding items, wherein the microorganism is a yeast such as a yeast of the genus *Saccharomyces*, *Pichia*, *Yarrowia*, *Kluyveromyces*, *Candida*, *Rhodotorula*, *Rhodospiridium*, *Cryptococcus*, *Trichosporon* and *Lipomyces*, preferably the genus is *Saccharomyces* or *Yarrowia*.
52. The method according to any one of the preceding items, wherein the microorganism is a yeast of a species selected from *Saccharomyces cerevisiae*, *Pichia pastoris*, *Kluyveromyces marxianus*, *Cryptococcus albidus*, *Lipomyces lipofera*, *Lipomyces starkeyi*, *Rhodospiridium toruloides*, *Rhodotorula glutinis*, *Trichosporon pullulan* and *Yarrowia lipolytica*, preferably the yeast cell is a *Saccharomyces cerevisiae* cell or a *Yarrowia lipolytica* cell.
53. The method according to any one of the preceding items, wherein the hydrophobic compound is (Z)-11-hexadecen-1-ol and the microorganism is a yeast cell capable of producing (Z)-11-hexadecen-1-ol with a titer of at least 0.2 mg/L, preferably wherein said yeast cell is a *Saccharomyces cerevisiae* cell, said yeast cell expressing:
- a $\Delta 11$ -desaturase selected from the group consisting of the *Amyelois transitella* $\Delta 11$ -desaturase (Atr_ $\Delta 11$; SEQ ID NO: 1), the *Spodoptera littoralis* $\Delta 11$ -desaturase (Sl_ $\Delta 11$; SEQ ID NO: 2), the *Agrotis segetum* $\Delta 11$ -desaturase (As_ $\Delta 11$; SEQ ID NO: 3), the desaturase from *Lobesia botrana* (Lbo_PPTQ; SEQ ID NO: 43), the desaturase from *Drosophila grimshawi* (Dgd9; SEQ ID NO: 44), the desaturase from *Drosophila virilis* (Dvd9; SEQ ID NO: 45) and the

- 5 *Trichoplusia ni* Δ 11-desaturase (Tni_ Δ 11; SEQ ID NO: 4) or a functional thereof having at least 65% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 95%, such as 100% homology to Atr_ Δ 11 (SEQ ID NO: 1), Sl_ Δ 11 (SEQ ID NO: 2), As_ Δ 11 (SEQ ID NO: 3), Lbop_PPTQ (SEQ ID NO: 43), Dgd9 (SEQ ID NO: 44), Dvd9 (SEQ ID NO: 45) or Tni_ Δ 11 (SEQ ID NO: 4), and
- 10 - an alcohol-forming fatty acyl-CoA reductase (FAR) selected from the group consisting of Har_FAR (SEQ ID NO: 5), Hs_FAR (SEQ ID NO: 6), and Has_FAR (SEQ ID NO: 7), or a variant thereof having at least 80% homology, such as at least 85%, such as at least 90%, such as at least 95%, such as 100% homology to Har_FAR (SEQ ID NO: 5), Hs_FAR (SEQ ID NO: 6), or Has_FAR (SEQ ID NO: 7);
- 15 whereby
- the Δ 11-desaturase is capable of converting at least part of said hexadecanoyl-CoA to (Z)11-hexadecenoyl-CoA; and
- the FAR is capable of converting at least part of said (Z)11-hexadecenoyl-CoA to (Z)-11-hexadecenol.
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54. The method according to item 53, wherein the yeast cell further expresses a fatty acyl synthetase (FAA), such as Sc_FAA1 (SEQ ID NO: 8) or Yl_FAA (SEQ ID NO: 9) or a variant thereof having at least 75% homology, such as at least 80% homology, such as at least 85% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology, such as 100% homology to Sc_FAA1 (SEQ ID NO: 8) or Yl_FAA (SEQ ID NO: 9).
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55. The method according to any one of items 53 to 54, further comprising the step of converting at least part of the (Z)-11-hexadecen-1-ol into (Z)-11-hexadecen-1-yl acetate by chemical conversion or by expression of an acetyltransferase such as a heterologous acetyltransferase (AcT) from said yeast cell or by over-expression of a native acetyltransferase from said yeast cell, wherein said
- 35 acetyltransferase is capable of converting at least part of the (Z)-11-hexadecen-

- 1-ol into (Z)11-hexadecen-1-yl acetate, preferably wherein the acetyltransferase is Sc_Atf1 (SEQ ID NO: 10) or a variant thereof having at least 75% homology, such as at least 80% homology, such as at least 85% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology, such as 100% homology to Sc_Atf1 (SEQ ID NO: 10).
56. The method according to any one of items 1 to 55, wherein the hydrophobic compound is a desaturated fatty alcohol and the microorganism is an oleaginous yeast cell such as a *Yarrowia* cell, for example a *Yarrowia lipolytica* cell, capable of producing said desaturated fatty alcohol, said oleaginous yeast cell:
- expressing at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and
 - expressing at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol; and
 - having a mutation resulting in reduced activity of Fao1 (SEQ ID NO: 11) and a mutation resulting in reduced activity of at least one of Hfd1 (SEQ ID NO: 12), Hfd4 (SEQ ID NO: 13), Pex10 (SEQ ID NO: 14) and GPAT (SEQ ID NO: 15) or having a mutation resulting in reduced activity of at least one protein having at least 90% homology to Fao1 (SEQ ID NO: 11) and a mutation resulting in reduced activity of at least one of Hfd1 (SEQ ID NO: 12), Hfd4 (SEQ ID NO: 13), Pex10 (SEQ ID NO: 14) and GPAT (SEQ ID NO: 15), such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Fao1 (SEQ ID NO: 11) and at least one of Hfd1 (SEQ ID NO: 12), Hfd4 (SEQ ID NO: 13), Pex10 (SEQ ID NO: 14) and GPAT (SEQ ID NO: 15).
57. The method according to item 56, wherein the at least one heterologous desaturase is selected from the group consisting of a $\Delta 3$ desaturase, a $\Delta 5$ desaturase, a $\Delta 6$ desaturase, a $\Delta 7$ desaturase, a $\Delta 8$ desaturase, a $\Delta 9$ desaturase, a $\Delta 10$ desaturase, a $\Delta 11$ desaturase, a $\Delta 12$ desaturase, a $\Delta 13$ desaturase and a

5 Δ 14 desaturase, preferably wherein the desaturase is derived from an insect, such as from the *Lepidoptera* order, preferably the desaturase is a Δ 11 desaturase having at least 60% homology to the Δ 11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 1, a Δ 9 desaturase having at least 60% homology to the Δ 9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 16, a desaturase having at least 60% homology to the desaturase from *Lobesia botrana* as set forth in SEQ ID NO: 43, a desaturase having at least 60% homology to the desaturase from *Drosophila grimshawi* as set forth in SEQ ID NO: 44 or a desaturase having at least 60% homology to the desaturase from *Drosophila virilis* as set forth in SEQ ID NO: 45.

15 58. The method according to any one of items 56 to 57, wherein the fatty acyl reductase (FAR) is selected from:

- i) a FAR having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 5;
- ii) a FAR having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 7;
- iii) a FAR having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 6; and
- 20 iv) a FAR having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 17,

preferably the FAR has at least 80% homology to the FAR from *Helicoverpa armigera* or to the FAR from *Heliothis subflexa*.

25 59. The method according to any one of items 56 to 58, further comprising the step of converting at least part of the desaturated fatty alcohol to a fatty acyl acetate by chemical conversion or by expression of an acetyltransferase such as a heterologous acetyltransferase (AcT) from said oleaginous yeast cell or by overexpression of a native acetyltransferase from said oleaginous yeast cell, wherein

30 said acetyltransferase is capable of converting at least part of the desaturated fatty alcohol to a fatty acyl acetate, preferably wherein the acetyltransferase is Sc_Atf1 (SEQ ID NO: 10) or a variant thereof having at least 75% homology, such as at least 80% homology, such as at least 85% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least

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97% homology, such as at least 98% homology, such as at least 99% homology, such as 100% homology to Sc_At1f1 (SEQ ID NO: 10).

- 5 60. The method according to any one of items 1 to 52, wherein the hydrophobic compound is a desaturated fatty alcohol and the microorganism is a yeast cell such as a *Yarrowia* cell, for example a *Yarrowia lipolytica* cell, capable of producing said desaturated fatty alcohol, said yeast cell expressing:
- at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA having a carbon chain length of 14; and
 - 10 - at least one heterologous fatty acyl-CoA reductase (FAR), capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol.
- 15 61. The method according to item 60, wherein the at least one heterologous desaturase is derived from an organism selected from *Pelargonium hortorum*, *Ricinus communis*, *Drosophila melanogaster*, *Spodoptera litura* and *Tribolium castaneum*, preferably the desaturase is derived from *Drosophila melanogaster*, preferably wherein the at least one heterologous desaturase is selected from the group consisting of:
- 20 i) a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 16;
 - ii) a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Spodoptera litura* as set forth in SEQ ID NO: 18;
 - iii) a desaturase having at least 60% homology to the desaturase from
 - 25 *Lobesia botrana* as set forth in SEQ ID NO: 43;
 - iv) a desaturase having at least 60% homology to the desaturase from *Drosophila grimshawi* as set forth in SEQ ID NO: 44; and
 - v) a desaturase having at least 60% homology to the desaturase from *Drosophila virilis* as set forth in SEQ ID NO: 45,
 - 30 vi) a $\Delta 11$ desaturase having at least 60% homology to the $\Delta 11$ desaturase from *Choristoneura parallela* as set forth in SEQ ID NO: 42,
 - vii) a $\Delta 11$ desaturase having at least 60% homology to the $\Delta 11$ desaturase from *Choristoneura rosaceana* as set forth in SEQ ID NO: 35.
- 35 62. The method according to any one of items 60 to 61, wherein the fatty acyl-CoA reductase (FAR) is selected from:

- i) a FAR having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 5;
 - ii) a FAR having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 7;
 - 5 iii) a FAR having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 6; and
 - iv) a FAR having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 17,
- preferably the FAR is a FAR having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 5.

63. The method according to any one of items 60 to 62, further comprising the step of converting at least part of the desaturated fatty alcohol to a fatty acyl acetate by chemical conversion or by expression of an acetyltransferase such as a heterologous acetyltransferase (AcT) from said oleaginous yeast cell or by overexpression of a native acetyltransferase from said oleaginous yeast cell, wherein said acetyltransferase is capable of converting at least part of the desaturated fatty alcohol to a fatty acyl acetate, preferably wherein the acetyltransferase is Sc_At1 (SEQ ID NO: 10) or a variant thereof having at least 75% homology, such as at least 80% homology, such as at least 85% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology, such as 100% homology to Sc_At1 (SEQ ID NO: 10).

64. The method according to any one of items 1 to 52, wherein the hydrophobic compound is a desaturated fatty alcohol and the microorganism is a yeast cell capable of producing said desaturated fatty alcohol, said yeast cell:

- having one or more mutations resulting in reduced activity of one or more native acyl-CoA oxidases; and
- expressing at least one first group of enzymes comprising at least one acyl-CoA oxidase capable of oxidising a fatty acyl-CoA, wherein the first group of enzymes is capable of shortening a fatty acyl-CoA of a first carbon chain length X to a shortened fatty acyl-CoA having a second carbon chain length X', wherein $X' \leq X-2$; and

- expressing at least one heterologous desaturase capable of introducing at least one double bond in said fatty acyl-CoA and/or in said shortened fatty acyl-CoA; and
 - expressing at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol.
65. The method according to item 64, wherein the native acyl-CoA oxidase and/or the heterologous acyl-CoA oxidase is a peroxisomal acyl-CoA oxidase.
66. The method according to any one of items 64 to 65, wherein the at least one acyl-CoA oxidase of the first group of enzymes is a native acyl-CoA oxidase or a heterologous acyl-CoA oxidase, which is optionally overexpressed compared to a reference yeast strain not expressing said at least one first group of enzymes, preferably the at least one acyl-CoA oxidase of the first group of enzymes is a heterologous acyl-CoA oxidase, optionally wherein the at least one first group of enzymes comprises an acyl-CoA oxidase derived from an organism of a genus selected from *Yarrowia*, *Agrotis*, *Arabidopsis*, *Aspergillus*, *Cucurbita*, *Homo*, *Paenarthrobacter* and *Rattus*, preferably the at least one first group of enzymes comprises an acyl-CoA oxidase derived from *Yarrowia lipolytica*, *Agrotis segetum*, *Arabidopsis thaliana*, *Aspergillus nidulans*, *Cucurbita maxima*, *Homo sapiens*, *Paenarthrobacter ureafaciens* or *Rattus norvegicus*, preferably the at least one acyl-CoA oxidase of the first group of enzymes is an acyl-CoA oxidase selected from the group consisting of Yli_POX1 (SEQ ID NO: 19), Yli_POX2 (SEQ ID NO: 20), Yli_POX3 (SEQ ID NO: 21), Yli_POX4 (SEQ ID NO: 22), Yli_POX5 (SEQ ID NO: 23), Yli_POX6 (SEQ ID NO: 24), Ase_POX (SEQ ID NO: 25), Ath_POX1 (SEQ ID NO: 26), Ath_POX2 (SEQ ID NO: 27), Ani_POX (SEQ ID NO: 28), Cma_POX (SEQ ID NO: 29), Hsa_POX1-2 (SEQ ID NO: 30), Pur_POX (SEQ ID NO: 31), and Rno_POX2 (SEQ ID NO: 32), or a functional variant thereof having at least 60% homology thereto, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%,

such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology thereto.

- 5 67. The method according to any one of items 64 to 66, wherein the at least one heterologous desaturase is selected from the group consisting of a $\Delta 3$ desaturase, a $\Delta 5$ desaturase, a $\Delta 6$ desaturase, a $\Delta 7$ desaturase, a $\Delta 8$ desaturase, a $\Delta 9$ desaturase, a $\Delta 10$ desaturase, a $\Delta 11$ desaturase, a $\Delta 12$ desaturase, a $\Delta 13$ desaturase and a $\Delta 14$ desaturase, and/or wherein the desaturase is derived from a yeast such as *Saccharomyces* or *Yarrowia*, such as *Saccharomyces cerevisiae* or *Yarrowia lipolytica*, or from an insect, such as from the *Diptera*, the *Coleoptera*, or the *Lepidoptera* order, such as of the genus *Amyelois*, *Choristoneura*, *Drosophila*, *Ostrinia*, *Thaumetopoea*, *Dendrophilus*, *Grapholita*, *Cydia*, *Epiphyas*, or *Spodoptera*, such as *Drosophila melanogaster*, *Amyelois transitella*, *Choristoneura rosaceana*, *Ostrinia nubilalis*, *Thaumetopoea pityocampa*, *Dendrophilus punctatus*, *Grapholita molesta*, *Cydia pomonella*, *Epiphyas postvittana*, *Spodoptera littoralis* or *Choristoneura parallela*.
- 10 68. The method according to any one of items 64 to 67, wherein the desaturase is a Δ_{Z9} -desaturase such as Sce_OLE1 (SEQ ID NO: 33), Yli_OLE1 (SEQ ID NO: 34) or Dme_D9 (SEQ ID NO: 16), a Δ_{Z11} -desaturase such as Atr_D11 (SEQ ID NO: 1), Cro_Z11 (SEQ ID NO: 35), Onu_11 (SEQ ID NO: 36), Tpi_D13 (SEQ ID NO: 37), a Δ_{E9} -desaturase such as Dpu_E9-14 (SEQ ID NO: 38), a $\Delta_{Z/E10}$ -desaturase such as Gmo_CPRQ (SEQ ID NO: 39), or a desaturase such as Epo_E11 (SEQ ID NO: 40), Sis_ZE11 (SEQ ID NO: 41), Lbo_PPTQ (SEQ ID NO: 43), Dgd9 (SEQ ID NO: 44), Dvd9 (SEQ ID NO: 45) or Cpa_E11 (SEQ ID NO: 42), or a functional variant thereof having at least 60% homology thereto, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology thereto.
- 20 69. The method according to any one of items 64 to 68, wherein the fatty acyl-CoA reductase is derived from an insect such as an insect of the *Lepidoptera* order,
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- such as of the genus *Helicoverpa*, *Heliothis* or *Bicyclus*, preferably the fatty acyl-CoA reductase is a fatty acyl-CoA reductase native to *Helicoverpa armigera*, *Helicoverpa assulta*, *Heliothis subflexa*, *Bicyclus anynana*, or a functional variant thereof, preferably the fatty acyl-CoA reductase is selected from the group consisting of a fatty acyl-CoA reductase having at least 80% homology to Har_FAR (SEQ ID NO: 5), Has_FAR (SEQ ID NO: 7), Ban_FAR (SEQ ID NO: 17) or Hs_FAR (SEQ ID NO: 6).
70. The method according to any one of items 64 to 69, further comprising the step of converting at least part of the desaturated fatty alcohol to a fatty acyl acetate by chemical conversion or by expression of an acetyltransferase such as a heterologous acetyltransferase (AcT) from said yeast cell or by overexpression of a native acetyltransferase from said yeast cell, wherein said acetyltransferase is capable of converting at least part of the desaturated fatty alcohol to a desaturated fatty acyl acetate, preferably wherein the acetyltransferase is Sc_Atf1 (SEQ ID NO: 10) or a functional variant thereof having at least 75% homology, such as at least 80% homology, such as at least 85% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology, such as 100% homology to Sc_Atf1 (SEQ ID NO: 10).
71. The method according to any one of items 64 to 70, further comprising the step of converting at least part of the desaturated fatty alcohol to a desaturated fatty aldehyde by expression of at least one alcohol dehydrogenase and/or at least one fatty alcohol oxidase from said yeast cell.
72. A hydrophobic compound obtainable by the method according to any one of the preceding items.
73. The hydrophobic compound according to item 72, wherein the hydrophobic compound is a fatty alcohol, a fatty alcohol ester, a fatty acyl acetate or a fatty aldehyde, preferably as defined in any one of items 1 to 71.

74. The hydrophobic compound according to any one of items 72 to 73, wherein the hydrophobic compound is one or more fatty alcohols, wherein at least one of said fatty alcohols has a carbon chain length of 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23, preferably of 9, 11, 13, 15, 17, 19, 21, or 23.
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75. The hydrophobic compound according to any one of items 72 to 74, wherein the hydrophobic compound is one or more fatty acyl acetates, wherein at least one of said fatty acyl acetates has a carbon chain length of 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23, preferably of 9, 11, 13, 15, 17, 19, 21, or 23.
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76. The hydrophobic compound according to any one of items 72 to 75, wherein the hydrophobic compound is one or more fatty aldehydes, wherein at least one of said fatty aldehydes has a carbon chain length of 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23, preferably of 9, 11, 13, 15, 17, 19, 21, or 23.
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77. A method of monitoring the presence of pest or disrupting the mating of pest, said method comprising the steps of:
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- i) producing a hydrophobic compound by the method of any of items 1 to 71, wherein the hydrophobic compound is a desaturated fatty alcohol, a desaturated fatty acyl acetate and/or a desaturated fatty aldehyde,
 - ii) formulating said desaturated fatty alcohol, desaturated fatty acyl acetate and/or desaturated fatty aldehyde as a pheromone composition, and
 - iii) employing said pheromone composition as an integrated pest management composition.
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