PROCESSES FOR OBTAINING MICROBIAL OIL FROM MICROBIAL CELLS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of the filing date of United States Provisional Patent Application No. 61/918,953 filed December 20, 2013, the disclosure of which is hereby incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0002] Disclosed herein are processes for obtaining a microbial oil comprising one or more polyunsaturated fatty acids (PUFAs) from one or more microbial cells by lysing the cells to form a lysed cell composition and then recovering the oil from the lysed cell composition. Further disclosed herein is microbial oil comprising one or more PUFAs that is recovered from microbial cells by at least one process described herein.

[0003] Microbial oil containing one or more PUFAs is produced by microorganisms, such as, for example, algae and fungi.

[0004] A typical process for obtaining PUFA containing oil from microbial cells involves growing microorganisms that are capable of producing the desired oil in a fermentor, pond or bioreactor to produce a microbial cell biomass; separating the biomass from the fermentation medium in which the biomass was grown; drying the microbial cell biomass, using a water-immiscible organic solvent (e.g., hexane) to extract the oil from the dried cells; and removing the organic solvent (e.g., hexane) from the oil. This process can further involve diluting the fermentation medium containing the cell biomass with water followed by centrifugation to separate the biomass from the diluted fermentation medium.

[0005] Another process for obtaining PUFA containing oil from microbial cells involves growing microorganisms that are capable of producing the desired oil in a fermentor, pond or bioreactor to produce a microbial cell biomass; releasing the PUFA containing oil into the fermentation medium in which the cells were grown by using mechanical force (e.g., homogenization), enzymatic treatment, or chemical treatment to disrupt the cell walls; and recovering the oil from the resulting composition comprising PUFA containing oil, cell debris, and liquid using an water miscible organic solvent, e.g., isopropyl alcohol. The oil can be separated mechanically from the composition and the alcohol must be removed from both the oil and the aqueous biomass waste stream.

[0006] The industrial scale employment of either of the above processes for obtaining PUFA containing oils from microbial cells requires the use of a large amount of volatile and flammable organic solvent, which creates hazardous operating conditions and requires the use of expensive explosion-proof equipment. Additionally, the use of an organic solvent generates an organic solvent waste stream that requires implementation of an expensive solvent recovery process to address the strict environmental limits on volatile organic compound (VOC) emissions, which in turn results in the need for more manpower and costly equipment.

[0007] Further, the use of heat in the above processes to dry the cells and/or remove the solvent from the recovered oil can degrade the PUFA containing oils and increase energy usage, which can further increase processing costs. Degradation occurs when PUFA containing oils are exposed to oxygen such as when the integrity of the microbial cell walls is disrupted and/or the microbial cells are exposed to heat.

[0008] A solvent-free process for obtaining PUFA containing oil from microbial cells involves growing microorganisms that are capable of producing the desired oil in a fermentor, pond or bioreactor to produce a microbial cell biomass; releasing the PUFA containing oil into the fermentation medium in which the cells were grown by using mechanical force (e.g.,

homogenization), enzymatic treatment, or chemical treatment to disrupt the cell walls; and recovering crude oil from the resulting composition comprising PUFA containing oil, cell debris, and liquid by raising the pH, adding a salt, heating, and/or agitating the resulting composition. This solvent-free process for obtaining PUFA containing oil from cells, however, can require long oil recovery times, large amounts of salt, and/or many steps, which can all increase processing costs.

[0009] As a result, there remains a need for a process for obtaining high quality PUFA containing oils from microbial cells that does not use a volatile organic solvent, can be performed using readily available equipment, requires a minimum number of steps, has shorter oil recovery times, and can provide a high yield of high quality PUFA containing oil.

[0010] Disclosed herein is a process for obtaining a microbial oil comprising one or more polyunsaturated fatty acids (PUFAs) from one or more microbial cells comprising (a) lysing the cells comprising the microbial oil to form a lysed cell composition; (b) demulsifying the lysed cell composition comprising adding an emulsifier prior to, during, or after (a) to form a demulsified lysed cell composition; (c) separating the oil from the demulsified lysed cell composition; and (d) recovering the oil.

[0011] Disclosed herein is a process for obtaining a microbial oil comprising one or more polyunsaturated fatty acids (PUFAs) from one or more microbial cells comprising (a) lysing the cells comprising the microbial oil to form a lysed cell composition; (b) demulsifying the lysed cell composition comprising adding an emulsifier prior to, during, or after (a) to form a demulsified lysed cell composition; (c) separating the oil from the demulsified lysed cell composition; and (d) recovering the oil, where at least one of (a) or (b) further comprises heating the composition to a temperature of at least 70° C.

[0012] Disclosed herein is a process for obtaining a microbial oil comprising one or more polyunsaturated fatty acids (PUFAs) from one or more microbial cells comprising (a) lysing the

cells comprising the microbial oil to form a lysed cell composition; (b) demulsifying the lysed cell composition comprising adding an emulsifier prior to, during, or after (a) and raising the pH of the lysed cell composition to 8 or above to form a demulsified lysed cell composition; (c) separating the oil from the demulsified lysed cell composition; and (d) recovering the oil.

[0013] Disclosed herein is a process for obtaining a microbial oil comprising one or more polyunsaturated fatty acids (PUFAs) from one or more microbial cells comprising (a) lysing the cells comprising the microbial oil to form a lysed cell composition; (b) demulsifying the lysed cell composition comprising adding an ionic emulsifier prior to, during, or after (a) to form a demulsified lysed cell composition; (c) separating the oil from the demulsified lysed cell composition; and (d) recovering the oil.

[0014] Disclosed herein is a process for obtaining a microbial oil comprising one or more polyunsaturated fatty acids (PUFAs) from one or more microbial cells comprising (a) lysing the cells comprising the microbial oil to form a lysed cell composition; (b) demulsifying the lysed cell composition comprising adding an ionic emulsifier prior to, during, or after (a) to form a demulsified lysed cell composition; (c) separating the oil from the demulsified lysed cell composition; and (d) recovering the oil, where at least one of (a) or (b) further comprises heating the composition to a temperature of at least 70° C.

[0015] Disclosed herein is a process for obtaining a microbial oil comprising one or more polyunsaturated fatty acids (PUFAs) from one or more microbial cells comprising (a) lysing the cells comprising the microbial oil to form a lysed cell composition; (b) demulsifying the lysed cell composition comprising adding an ionic emulsifier prior to, during, or after (a) and raising the pH of the lysed cell composition to 8 or above to form a demulsified lysed cell composition; (c) separating the oil from the demulsified lysed cell composition; and (d) recovering the oil.

[0016] Disclosed herein is a microbial oil obtained by any of the processes described herein.

[0017] The features and advantages of the invention may be more readily understood by those of ordinary skill in the art upon reading the following detailed description. It is to be appreciated that certain features of the invention that are, for clarity reasons, described above and below in the context of separate embodiments, may also be combined so as to form a sub-combinations thereof.

[0018] Embodiments identified herein as exemplary are intended to be illustrative and not limiting.

[0019] The term “about” is intended to capture variations above and below the stated number that may achieve substantially the same results as the stated number.

[0020] Fatty acids are classified based on the length and saturation characteristics of the carbon chain. Fatty acids present in a microbial oil can have from 4 to 28 carbon atoms and are termed short chain, medium chain, or long chain fatty acids based on the number of carbons present in the chain. Fatty acids are termed saturated fatty acids when no double bonds are present between the carbon atoms, and are termed unsaturated fatty acids when double bonds are present. Unsaturated long chain fatty acids are monounsaturated when only one double bond is present and are polyunsaturated when more than one double bond is present.

[0021] The microbial oil described herein refers to oil that comprises one or more PUFAs and is obtained from microbial cells.

[0022] Polyunsaturated fatty acids (PUFAs) are classified based on the position of the first double bond from the methyl end of the fatty acid; omega-3 (n-3) fatty acids contain a first double bond at the third carbon, while omega-6 (n-6) fatty acids contain a first double bond at the sixth carbon. For example, docosahexaenoic acid (DHA) is an omega-3 long chain polyunsaturated fatty acid (LC-PUFA) with a chain length of 22 carbons and 6 double bonds, often designated as "22:6n-3." In one embodiment, the PUFA is selected from an omega-3 fatty acid, an omega-6 fatty acid, and mixtures thereof. In another embodiment, the PUFA is selected

from LC-PUFAs. In a still further embodiment, the PUFA is selected from docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), arachidonic acid (ARA), gamma-linolenic acid (GLA), dihomo-gamma-linolenic acid (DGLA), stearidonic acid (SDA), and mixtures thereof. In another embodiment, the PUFA is selected from DHA, ARA, and mixtures thereof. In a further embodiment, the PUFA is DHA. In yet a further embodiment, the PUFA is ARA.

[0023] LC-PUFAs are fatty acids that contain at least 3 double bonds and have a chain length of 18 or more carbons or 20 or more carbons. LC-PUFAs of the omega-6 series include, but are not limited to, di-homo-gammalinoleic acid (C20:3n-6), arachidonic acid (C20:4n-6) ("ARA"), docosatetraenoic acid or adrenic acid (C22:4n-6), and docosapentaenoic acid (C22:5n-6) ("DPA n-6"). The LC-PUFAs of the omega-3 series include, but are not limited to, eicosatrienoic acid (C20:3n-3), eicosatetraenoic acid (C20:4n-3), eicosapentaenoic acid (C20:5n-3) ("EPA"), docosapentaenoic acid (C22:5n-3), and docosahexaenoic acid (C22:6n-3). The LC-PUFAs also include fatty acids with greater than 22 carbons and 4 or more double bonds including, but not limited to, C24:6(n-3) and C28:8(n-3).

[0024] The PUFAs can be in the form of a free fatty acid, salt, fatty acid ester (e.g. methyl or ethyl ester), monoacylglycerol (MAG), diacylglycerol (DAG), triacylglycerol (TAG), and/or phospholipid (PL).

[0025] Highly unsaturated fatty acids (HUFAs) are omega-3 and/or omega-6 polyunsaturated fatty acids that contain 4 or more unsaturated carbon-carbon bonds.

[0026] As used herein, a "cell" refers to an oil-containing biomaterial, such as biomaterial derived from oleaginous microorganisms. Oil produced by a microorganism or obtained from a microbial cell is referred to as “microbial oil”. Oil produced by algae and/or fungi is also referred to as algal and/or fungal oil, respectively.

[0027] As used herein, a "microbial cell" or "microorganism" refers to organisms such as algae, bacteria, fungi, yeast, protist, and combinations thereof, e.g., unicellular organisms. In some embodiments, a microbial cell is a eukaryotic cell. A microbial cell includes, but is not limited to, golden algae (e.g., microorganisms of the kingdom *Stramenopiles*); green algae; diatoms; dinoflagellates (e.g., microorganisms of the order *Dinophyceae* including members of the genus *Crypthecodinium* such as, for example, *Crypthecodinium cohnii* or *C. cohnii*); microalgae of the order Thraustochytriales; yeast (Ascomycetes or Basidiomycetes); and fungi of the genera *Mucor*, *Mortierella*, including but not limited to *Mortierella alpina* and *Mortierella sect. schmuckeri*, and *Pythium*, including but not limited to *Pythium insidiosum*.

[0028] In one embodiment, the microbial cells are from the genus *Mortierella*, genus *Crypthecodinium*, or order Thraustochytriales. In a still further embodiment, the microbial cells are from *Crypthecodinium Cohnii*. In yet an even further embodiment, the microbial cells are selected from *Crypthecodinium Cohnii*, *Mortierella alpina,* genus *Thraustochytrium*, genus *Schizochytrium*, and mixtures thereof.

[0029] In a still further embodiment, the microbial cells include, but are not limited to, microorganisms belonging to the genus *Mortierella*, genus *Conidiobolus*, genus *Pythium*, genus *Phytophthora*, genus *Penicillium*, genus *Cladosporium*, genus *Mucor*, genus *Fusarium*, genus *Aspergillus*, genus *Rhodotorula*, genus *Entomophthora*, genus *Echinosporangium*, and genus *Saprolegnia*. In another embodiment, ARA is obtained from microbial cells from the genus *Mortierella*, which includes, but is not limited to, *Mortierella elongata, Mortierella exigua, Mortierella hygrophila, Mortierella alpina, Mortierella schmuckeri,* and *Mortierella minutissima*. In a further embodiment, ARA is obtained from microbial cells from *Mortierella elongata* IFO8570, *Mortierella exigua* IFO8571, *Mortierella hygrophila* IFO5941, *Mortierella alpina* IFO8568, ATCC16266, ATCC32221, ATCC42430, CBS219.35, CBS224.37, CBS250.53,

CBS343.66, CBS527.72, CBS529.72, CBS608.70, and CBS754.68, and mutants thereof. In a still further embodiment, the microbial cells are from *Mortierella alpina*.

[0030] In an even further, the microbial cells are from microalgae of the order Thraustochytriales, which includes, but is not limited to, the genera *Thraustochytrium* (species include *arudimentale*, *aureum*, *benthicola*, *globosum*, *kinnei*, *motivum*, *multirudimentale*, *pachydermum*, *proliferum*, *roseum*, *striatum*); the genera *Schizochytrium* (species include *aggregatum*, *limnaceum*, *mangrovei*, *minutum*, *octosporum*); the genera *Ulkenia* (species include *amoeboidea*, *kerguelensis*, *minuta*, *profunda*, *radiate*, *sailens*, *sarkariana*, *schizochytrops*, *visurgensis*, *yorkensis*); the genera *Aurantiacochytrium*; the genera *Oblongichytrium*; the genera *Sicyoidochytium*; the genera *Parientichytrium*; the genera *Botryochytrium*; and combinations thereof. Species described within Ulkenia will be considered to be members of the genus *Schizochytrium*. In another embodiment, the microbial cells are from the order Thraustochytriales. In yet another embodiment, the microbial cells are from *Thraustochytrium.* In still a further embodiment, the microbial cells are from *Schizochytrium.* In a still further embodiment, the microbial cells are chosen from genus *Thraustochytrium*, *Schizochytrium*, or mixtures thereof.

[0031] In one embodiment, the process comprises lysing microbial cells comprising a microbial oil to form a lysed cell composition. The terms “lyse” and "lysing" refer to a process whereby the wall and/or membrane of the microbial cell is ruptured. In one embodiment, the microbial cell is lysed by being subjected to at least one treatment selected from mechanical, chemical, enzymatic, physical, and combinations thereof. In another embodiment, the process comprises lysing the microbial cells comprising the microbial oil to form a lysed cell composition, wherein the lysing is selected from mechanical, chemical, enzymatic, physical, and combinations thereof.

[0032] In some embodiments, prior to lysing the cell, the cell can be washed and/or pasteurized. In some embodiments, washing the cells includes using an aqueous solution, such as water, to remove any extracellular water-soluble or water-dispersible compounds. In some embodiments, the cell can be washed once, twice, thrice, or more. In some embodiments, pasteurizing the cell includes heating the cell to inactivate any undesirable enzymes, for example any enzymes that might degrade the oil or reduce the yield of PUFAs. In some embodiments, the cell is washed and then pasteurized before being lysed. In some embodiments, the cells that are being lysed are contained in a fermentation broth.

[0033] In some embodiments, the process comprises lysing unwashed microbial cells comprising a microbial oil to form a lysed cell composition. In some embodiments, a fermentation broth comprising microbial cells comprising microbial oil is first washed with, for example, water and then the cells lysed to form a lysed cell composition. In other embodiments, the process comprises lysing unwashed cells in a fermentation medium to form a lysed cell composition.

[0034] Mechanical treatment includes, but is not limited to, homogenization, ultrasound, cold-pressing, milling, and combinations thereof. In some embodiments, the process comprises lysing the cells by homogenization. In some embodiments, the process comprises lysing the cell with a homogenizer.

[0035] Homogenization includes, but is not limited to, processes that utilize a French cell press, sonicator, homogenizer, microfluidizer, ball mill, rod mill, pebble mill, bead mill, high pressure grinding roll, vertical shaft impactor, industrial blender, high shear mixer, paddle mixer, polytron homogenizer, industrial homogenizer (e.g., Niro Soavi VHP Homogenizer and APV Rannie and APV Gaulin homogenizers), Industrial high shear fluid processors (e.g., Microfluidics high shear fluid processor), cell lysing/bead mill homogenizers (e.g., Dyno-Mill and Buhler), and combinations thereof. In some embodiments, the cells flow through a

homogenizer that is optionally heated. In some embodiments, suitable homogenization can include 1 to 3 passes through a homogenizer at either high and/or low pressures.

[0036] In some embodiments, the pressure during homogenization is 150 bar to 1,400 bar; 150 bar to 1,200 bar; 150 bar to 900 bar; 150 bar to 300 bar; 300 bar to 1,400 bar; 300 bar to 1,200 bar; 300 bar to 900 bar; 400 bar to 800 bar; 500 bar to 700 bar; or 600 bar. In some embodiments, the pressure during homogenization is 2,000 psi to 20,000 psi; 2,000 psi to 18,000 psi; 2,000 psi to 16,000 psi; 2,000 psi to 14,000 psi; 2,000 psi to 12,000 psi; 2,000 psi to 10,000 psi; 2,000 psi to 8,000 psi; 2,000 psi to 6,000 psi; 2,000 psi to 4,000 psi; 4,000 psi to 20,000 psi; 4,000 psi to 18,000 psi; 4,000 psi to 16,000 psi; 4,000 psi to 14,000 psi; 4,000 psi to 12,000 psi; 4,000 psi to 10,000 psi; 4,000 psi to 8,000 psi; 4,000 psi to 6,000 psi; 6,000 psi to 20,000 psi; 6,000 psi to 18,000 psi; 6,000 psi to 16,000 psi; 6,000 psi to 14,000 psi; 6,000 psi to 12,000 psi; 6,000 psi to 10,000 psi; 6,000 psi to 8,000 psi; 8,000 psi to 20,000 psi; 8,000 psi to 18,000 psi; 8,000 psi to 16,000 psi; 8,000 psi to 14,000 psi; 8,000 psi to 12,000 psi; 8,000 psi to 10,000 psi; 10,000 psi to 20,000 psi; 10,000 psi to 18,000 psi; 10,000 psi to 16,000 psi; 10,000 psi to 14,000 psi; 10,000 psi to 12,000 psi; 12,000 psi to 20,000 psi; 12,000 psi to 18,000 psi; 12,000 psi to 16,000 psi; 12,000 psi to 14,000 psi; 14,000 psi to 20,000 psi; 14,000 psi to 18,000 psi; 14,000 psi to 16,000 psi; 16,000 psi to 20,000 psi; 16,000 psi to 18,000 psi; or 18,000 psi to 20,000 psi.

[0037] In some embodiments, the microbial cells are mixed in high shear mixer before being homogenized. In some embodiments, the high shear mixer is operated in a range of at least 5,000 rpm; at least 7,500 rpm; at least 10,000 rpm; at least 12,500 rpm; at least 15,000 rpm; 5,000 rpm to 15,000 rpm; 5,000 rpm to 12,500 rpm; 5,000 rpm to 10,000 rpm; 5,000 rpm to 7,500 rpm; 7,500 rpm to 15,000 rpm; 7,500 rpm to 12,500 rpm; 7,500 rpm to 10,000 rpm; 10,000 rpm to 15,000 rpm; 10,000 rpm to 12,500 rpm; or 12,500 rpm to 15,000 rpm.

[0038] Physical treatment includes, but is not limited to, heating, which includes, but is not limited to, resistive, convection, steam, fluid bath, solar, and combinations thereof. In some

embodiments, the cells are heated in a tank with resistive coils in/on its walls. In some embodiments, the cells are heated in a liquid bath with tubes passing there through.

[0039] Chemical treatment includes, but is not limited to, raising the pH of the cells; lowering the pH of the cells; contacting the cells with a chemical; and combinations thereof.

[0040] In some embodiments, the cells are lysed by raising the pH of the cells. In some embodiments, the pH is raised by adding a base. The bases include, but are not limited to, hydroxides (e.g., LiOH, NaOH, KOH, and $Ca(OH)_2$, and combinations thereof); carbonates (e.g., Na_2CO_3, K_2CO_3, $MgCO_3$, and combinations thereof); bicarbonates (e.g., $LiHCO_3$, $NaHCO_3$, $KHCO_3$, and combinations thereof); and combinations thereof. The base can be in the form of a solid (e.g., crystals, granulates, and pellets); a liquid (e.g., an aqueous solution; and combinations thereof.

[0041] In some embodiments, the base has a pK_b of 1 to 12, 1 to 10, 1 to 8, 1 to 6, 1 to 5, 2 to 12, 2 to 10, 2 to 8, 2 to 6, 2 to 5, 3 to 10, 3 to 6, 3 to 5, 4 to 10, 4 to 8, 4 to 6, 5 to 10, or 5 to 8. As used herein, the term "pK_b" refers to the negative logarithm of the base association constant, K_b, of the base. K_b refers to the equilibrium constant for the ionization of the base in water, wherein:

$B + H_2O \rightleftharpoons HB^+ + OH^-$; and the K_b of base, B, is defined as: $K_b = \frac{[HB^+][OH^-]}{[B]}$.

[0042] In some embodiments, the pH is selected from about 8 or above; about 9 or above; about 10 or above; about 11 or above; and about 12 or above. In other embodiments, the pH is selected from 7 to 13; 7 to 12; 7 to 11; 7 to 10; 7 to 9; 8 to 13; 8 to 12; 8 to 11; 8 to 10; 8 to 9; 9 to 12; 9 to 11; 9 to 10; 10 to 12; and 10 to 11.

[0043] In some embodiments, the pH of the cells can be raised by a chloralkali process. In some embodiments, the fermentation broth containing sodium chloride and the cells is subjected to electrolysis that results in the formation of sodium hydroxide, which raises the pH of

the cell. In some embodiments, the fermentation broth includes calcium chloride or potassium chloride instead of, or in addition to, sodium chloride, and electrolysis results in the formation of calcium hydroxide or potassium hydroxide, respectively, thereby raising the pH of the cell.

[0044] In some embodiments, the cells are lysed by lowering the pH of the cells. In some embodiments, the pH is lowered by adding an acid. The acids include, but are not limited to, sulfuric; phosphoric; hydrochloric; hydrobromic; hydroiodic; hypochlorous; chlorous; chloric; perchloric; fluorosulfuric; nitric; fluoroantimonic; fluoroboric; hexafluorophosphoric; chromic; boric; acetic; citric; formic; and combinations thereof. In some embodiments, the pH is selected from about 7 or below; about 6.5 or below; about 6 or below; about 5.5 or below; about 5 or below; about 4.5 or below; about 4 or below; about 3.5 or below; about 3 or below; about 2.5 or below; about 2 or below; about 1.5 or below; about 1 or below; and about 0.5 or below. In other embodiments, the pH is selected from about 0.5 to about 7; about 0.5 to about 6.5; about 0.5 to about 6; about 0.5 to about 5.5; about 0.5 to about 5; about 0.5 to about 4.5; about 0.5 to about 4; about 0.5 to about 3.5; about 0.5 to about 3; about 0.5 to about 2.5; about 0.5 to about 2; about 0.5 to about 1.5; about 0.5 to about 1; about 1 to about 7; about 1 to about 6.5; about 1 to about 6; about 1 to about 5.5; about 1 to about 5; about 1 to about 4.5; about 1 to about 4; about 1 to about 3.5; about 1 to about 3; about 1 to about 2.5; about 1 to about 2; about 1 to about 1.5; about 1.5 to about 7; about 1.5 to about 6.5; about 1.5 to about 6; about 1.5 to about 5.5; about 1.5 to about 5; about 1.5 to about 4.5; about 1.5 to about 4; about 1.5 to about 3.5; about 1.5 to about 3; about 1.5 to about 2.5; about 1.5 to about 2; about 2 to about 7; about 2 to about 6.5; about 2 to about 6; about 2 to about 5.5; about 2 to about 5; about 2 to about 4.5; about 2 to about 4, about 2 to about 3.5; about 2 to about 3; about 2 to about 2.5; about 2.5 to about 7; about 2.5 to about 6.5; about 2.5 to about 6; about 2.5 to about 5.5; about 2.5 to about 5; about 2.5 to about 4.5; about 2.5 to about 4; about 2.5 to about 3.5; about 2.5 to about 3; about 3 to about 7; about 3 to about 6.5; about 3 to about 6; about 3 to about 5.5; about 3 to about 5; about 3 to about 4.5; about 3 to about

4; about 3 to about 3.5; about 3.5 to about 7; about 3.5 to about 6.5; about 3.5 to about 6; about 3.5 to about 5.5; about 3.5 to about 5; about 3.5 to about 4.5; about 3.5 to about 4; about 4 to about 7; about 4 to about 6.5; about 4 to about 6; about 4 to about 5.5; about 4 to about 5; about 4 to about 4.5; about 4.5 to about 7; about 4.5 to about 6.5; about 4.5 to about 6; about 4.5 to about 5.5; about 4.5 to about 5; about 5 to about 7; about 5 to about 6.5; about 5 to about 6; about 5 to about 5.5; about 5.5 to about 7; about 5.5 to about 6.5; about 5.5 to about 6; about 6 to about 7; about 6 to about 6.5; and about 6.5 to about 7.

[0045] In some embodiments, an acid is added in an amount of about 2% to about 10%, about 2% to about 9%, about 2% to about 8%, about 2% to about 7%, about 2% to about 6%, about 3% to about 6%, about 4% to about 6%, about 5% to about 6%, about 2% to about 5%, about 2% to about 4%, about 2% to about 3%, about 3% to about 5%, about 3% to about 4%, or about 4% to about 5% by weight (or volume) of the cell broth to lower the pH.

[0046] Enzymatic treatment refers to contacting the cells with one or more enzymes. Enzymes include, but are not limited to, proteases, cellulases, hemicellulases, chitinases, pectinases, and combinations thereof. Non-limiting examples of proteases include serine proteases, theronine proteases, cysteine proteases, aspartate proteases, metalloproteases, glutamic acid proteases, alacase, and combinations thereof. Non-limiting examples of cellulases include sucrase, maltase, lactase, alpha-glucosidase, beta-glucosidase, amylase, lysozyme, neuraminidase, galactosidase, alpha-mannosidase, glucuronidase, hyaluronidase, pullulanase, glucocerebrosidase, galactosylceramidase, acetylgalactosaminidase, fucosidase, hexosaminidase, iduronidase, maltase-glucoamylase, and combinations thereof. A non-limiting example of a chitinase includes chitotriosidase. Non-limiting examples of pectinases include pectolyase, pectozyme, polygalacturonase, and combinations thereof. In some embodiments, some enzymes are activated by heating. In some embodiments, lysis does not include the use of enzymes.

[0047] As used herein, a "lysed cell composition" refers to a composition comprising one or more lysed cells, including cell debris and other contents of the cell, in combination with microbial oil (from the lysed cells), and optionally, a fermentation broth that contains liquid (e.g., water), nutrients, and microbial cells. In some embodiments, a microbial cell is contained in a fermentation broth or media comprising water. In some embodiments, a lysed cell composition refers to a composition comprising one or more lysed cells, cell debris, microbial oil, the natural contents of the cell, and aqueous components from a fermentation broth. In one embodiment, the lysed cell composition comprises liquid, cell debris, and microbial oil. In some embodiments, a lysed cell composition is in the form of an oil-in-water emulsion comprising a mixture of a continuous aqueous phase and a dispersed oil phase. In some embodiments, a dispersed oil phase is present in a concentration of about 1% to about 60%; about 1% to about 50%; about 1% to about 40%; about 1% to about 30%; about 1% to about 20%; about 5% to about 60%; about 5% to about 50%; about 5% to about 40%; about 5% to about 30%; about 5% to about 20%; about 10% to about 60%; about 10% to about 50%; about 10% to about 40%; about 20% to about 60%; 20% to 50%, 20% to about 40%; about 30% to about 60%; about 30% to about 50%; or about 40% to about 60% by weight (or volume) of an emulsified lysed cell composition.

[0048] In some embodiments, lysing microbial cells results in the formation of an emulsion from endogenous materials in the cell or cell biomass including, but not limited to, proteins, phospholipids, carbohydrates, and combinations thereof. While not being bound to any particular theory, it is believed the processes of the present invention break-up or demulsify an emulsified lysed cell composition, allowing the microbial oil to be separated from the lysed cell composition. The terms "emulsion" and "emulsified" refers to a mixture of two or more immiscible phases or layers wherein one phase or layer is dispersed in another phase or layer. The terms "break," "break-up," "demulsify," "demulsification," "demulsifying," and "breaking" refer to a process of separating immiscible phases or layers of an emulsion. For example,

demulsifying or breaking an emulsified lysed cell composition refers to a process by which an emulsified lysed cell composition changes from an emulsion having one or more phases or layers to a composition having two or more phases or layers. For example, in some embodiments, a process of the present invention breaks an emulsified lysed cell composition from a single-phase to two or more phases. In some embodiments, the two or more phases include an oil phase and an aqueous phase. In some embodiments, a process of the present invention breaks an emulsified lysed cell composition into at least three phases. In some embodiments, the three phases are selected from an oil phase, an aqueous phase, and a solid phase. In some embodiments, the phases are selected from an oil phase, an emulsion phase, an aqueous phase, and a solid phase.

[0049] In some embodiments, the mean particle size of the oil droplets formed during demulsification is selected from 5 microns to 50 microns; 5 microns to 45 microns; 5 microns to 40 microns; 5 microns to 35 microns; 5 microns to 30 microns; 5 microns to 25 microns; 5 microns to 20 microns; 5 microns to 15 microns; 10 microns to 50 microns; 10 microns to 45 microns; 10 microns to 40 microns; 10 microns to 35 microns; 10 microns to 30 microns; 10 microns to 25 microns; 10 microns to 20 microns; 10 microns to 15 microns; 15 microns to 50 microns; 15 microns to 45 microns; 15 microns to 40 microns; 15 microns to 35 microns; 15 microns to 30 microns; 15 microns to 25 microns; 15 microns to 20 microns; 20 microns to 50 microns; 20 microns to 45 microns; 20 microns to 40 microns; 20 microns to 35 microns; 20 microns to 30 microns; 20 microns to 25 microns; 25 microns to 50 microns; 25 microns to 45 microns; 25 microns to 40 microns; 25 microns to 35 microns; 25 microns to 30 microns; 30 microns to 50 microns; 30 microns to 45 microns; 30 microns to 40 microns; 30 microns to 35 microns; 35 microns to 50 microns; 35 microns to 45 microns; 35 microns to 40 microns; 40 microns to 50 microns; 40 microns to 45 microns; and 45 microns to 50 microns. In a further embodiment, the mean particle size of the oil droplets formed during demulsification is selected from at least 10 microns, at least 15 microns, at least 20 microns, at least 25 microns, at least 30

microns, at least 35 microns, and at least 40 microns or above. In further embodiments, the mean particle size of the oil droplets formed during demulsification is selected from at least 10 microns, at least 15 microns, at least 20 microns, and at least 25 microns. In some embodiments, the mean particle size can be measured using, e.g., a Beckman Coulter LS 13 320 particle size analyzer (Beckman Coulter, Brea, CA). In some embodiments, the mean particle size can be measured using, e.g., a Malvern MS2000 particle size analyzer (Malvern Instruments Ltd., Worcestershire, United Kingdom).

[0050] In some embodiments, an emulsifier is added to a lysed cell composition. In some embodiments, the emulsifier replaces the emulsion formed by the endogenous materials because the PUFAs have a stronger affinity for the emulsifier than the endogenous materials (e.g., proteins, phospholipids, and carbohydrates). In some embodiments, the emulsion formed by the emulsifier is more stable than the emulsion formed by the endogenous materials. In some embodiments, an emulsion formed by the emulsifier is more easily demulsified using the processes described herein, than an emulsion formed by the endogenous materials.

[0051] In some embodiments, the emulsifier is a detergent. In some embodiments, the emulsifier is a surfactant. In some embodiments, the emulsifier is added prior to, during, or after lysis. In one embodiment, the emulsifier is added after lysis. In some embodiments, the emulsifier is added to the lysed cell composition. As used herein, the term "emulsifier" refers to a substance that stabilizes an emulsion. Emulsifiers are selected from ionic emulsifiers, nonionic emulsifiers, and combinations thereof. In some embodiments, the emulsifier is an ionic emulsifier.

[0052] In some embodiments, the ionic emulsifier is selected from anionic emulsifiers, cationic emulsifiers, and combinations thereof. In some embodiments, the anionic emulsifiers can be anionic sulfate emulsifiers, such as, for example, alkyl sulfates (e.g., ammonium lauryl sulfate, sodium lauryl sulfate (SLS)/sodium dodecyl sulfate (SDS), and combinations thereof),

alkyl ether sulfates (e.g., sodium laureth sulfate/sodium lauryl ether sulfate, sodium myreth sulfate, and combinations thereof), and combinations thereof; anionic sulfonate emulsifiers, such as, for example, docusates (e.g., dioctyl sodium sulfosuccinate, sulfonate fluorosurfactants (e.g., perfluorooctanesulfonate and perfluorobutanesulfonate), alkyl benzene sulfonates, and combinations thereof); anionic phosphate emulsifiers (e.g., alkyl aryl ether phosphate, alkyl ether phosphate, and combinations thereof); anionic carboxylate emulsifiers (e.g., alkyl carboxylates, (e.g., sodium stearate, sodium lauroyl sarcosinate. carboxylate fluorosurfactants (e.g., pefluorononanoate, perfluorooctanoate, and combinations thereof), and combinations thereof); and combinations thereof. In some embodiments, the emulsifier is an anionic emulsifier. In one embodiment, the anionic emulsifier is selected from an anionic sulfate emulsifier, an anionic sulfonate emulsifier, an anionic phosphate emulsifier, an anionic carboxylate emulsifier, and combinations thereof. In another embodiment, an anionic emulsifier is an anionic sulfate emulsifier. In a still further embodiment, an anionic sulfate emulsifier is selected from ammonium lauryl sulfate, sodium dodecyl sulfate, sodium laureth sulfate, sodium lauryl ether sulfate, sodium myreth sulfate, and combinations thereof. In yet an even further embodiment, an anionic sulfate emulsifier is sodium dodecyl sulfate.

[0053] In some embodiments, the cationic emulsifier can be a pH-dependent primary amine; a pH-dependent secondary amine; a pH-dependent tertiary amine; octenidine dihydrochloride; a permanently charged quaternary ammonium cation (e.g., alkyltrimethylammonium salts (e.g., cetyl trimethylammonium bromide (CTAB)/hexadecyl trimethyl ammonium bromide, cetyl trimethylammonium chloride (CTAC), and combinations thereof), cetylpyridinium chloride (CPC), benzalkonium chloride (BAC), benzethonium chloride (BZT), 5-bromo-5-nitro-1,3-dioxane, dimethyldioctadecylammonium chloride, dioctadecyldimethylammonium bromide (DODAB), and combinations thereof); and combinations thereof.

[0054] In some embodiments, the molecular weight of the emulsifier is selected from 500 g/mole or less, 450 g/mole or less, 400 g/mole or less, 350 g/mole or less, and 300 g/mole or less. In a further embodiment, the molecular weight of the emulsifier is selected from 250 g/mole to 500 g/mole, 250 g/mole to 450 g/mole, 250 g/mole to 400 g/mole, 250 g/mole to 350 g/mole, 250 g/mole to 300 g/mole, 300 g/mole to 500 g/mole, 300 g/mole to 450 g/mole, 300 g/mole to 400 g/mole, 300 g/mole to 350 g/mole, 350 g/mole to 500 g/mole, 350 g/mole to 450 g/mole, 350 g/mole to 400 g/mole, 400 g/mole to 500 g/mole, 400 g/mole to 450 g/mole, and 450 g/mole to 500 g/mole. For example, the molecular weight of SDS is 288 g/mole, and the molecular weight of CTAB is 364 g/mole. In yet a further embodiment, the molecular weight of the emulsifier is selected from 250 g/mole to 450 g/mole, 250 g/mole to 400 g/mole, 250 g/mole to 350 g/mole, and 250 g/mole to 300 g/mole.

[0055] In some embodiments, an emulsifier is added as a powder. In some embodiments, an emulsifier is added in a solution having a concentration of emulsifier in an amount of 5% to 50%, 5% to 45%, 5% to 40%, 5% to 35%, 5% to 30%, 10% to 50%, 10% to 45%, 10% to 40%, 10% to 35%, 10% to 30%, 15% to 50%, 15% to 45%, 15% to 40%, 15% to 35%, 15% to 30%, 20% to 50%, 20% to 45%, 20% to 40%, 20% to 35%, 20% to 30%, 25% to 50%, 25% to 45%, 25% to 40%, 25% to 35%, 25% to 30%, 30% to 50%, 30% to 45%, 30% to 40%, and 30% to 35%.

[0056] In some embodiments, an emulsifier (e.g., in powder form or in solution) is added in an amount selected from 0.2% to 10%, 0.2% to 9.5%, 0.2% to 9%, 0.2% to 8.5%, 0.2% to 8%, 0.2% to 7.5%, 0.2% to 7%, 0.2% to 6.5%, 0.2% to 6%, 0.2% to 5.5%, 0.2% to 5%, 0.2% to 4.5%, 0.2% to 4%, 0.2% to 3.5%, 0.2% to 3%, 0.2% to 2.5%, 0.2% to 2%, 0.2% to 1.5%, 0.2% to 1%, 0.2% to 0.5%, 0.5% to 10%, 0.5% to 9.5%, 0.5% to 9%, 0.5% to 8.5%, 0.5% to 8%, 0.5% to 7.5%, 0.5% to 7%, 0.5% to 6.5%, 0.5% to 6%, 0.5% to 5.5%, 0.5% to 5%, 0.5% to 4.5%, 0.5% to 4%, 0.5% to 3.5%, 0.5% to 3%, 0.5% to 2.5%, 0.5% to 2%, 0.5% to 1.5%, 0.5% to 1%, 1% to

10%, 1% to 9.5%, 1% to 9%, 1% to 8.5%, 1% to 8%, 1% to 7.5%, 1% to 7%, 1% to 6.5%, 1% to 6%, 1% to 5.5%, 1% to 5%, 1% to 4.5%, 1% to 4%, 1% to 3.5%, 1% to 3%, 1% to 2.5%, 1% to 2%, 1% to 1.5%, 1.5% to 10%, 1.5% to 9.5%, 1.5% to 9%, 1.5% to 8.5%, 1.5% to 8%, 1.5% to 7.5%, 1.5% to 7%, 1.5% to 6.5%, 1.5% to 6%, 1.5% to 5.5%, 1.5% to 5%, 1.5% to 4.5%, 1.5% to 4%, 1.5% to 3.5%, 1.5% to 3%, 1.5% to 2.5%, 1.5% to 2%, 2% to 10%, 2% to 9.5%, 2% to 9%, 2% to 8.5%, 2% to 8%, 2% to 7.5%, 2% to 7%, 2% to 6.5%, 2% to 6%, 2% to 5.5%, 2% to 5%, 2% to 4.5%, 2% to 4%, 2% to 3.5%, 2% to 3%, 2% to 2.5%, 2.5% to 10%, 2.5% to 9.5%, 2.5% to 9%, 2.5% to 8.5%, 2.5% to 8%, 2.5% to 7.5%, 2.5% to 7%, 2.5% to 6.5%, 2.5% to 6%, 2.5% to 5.5%, 2.5% to 5%, 2.5% to 4.5%, 2.5% to 4%, 2.5% to 3.5%, 2.5% to 3%, 3% to 10%, 3% to 9.5%, 3% to 9%, 3% to 8.5%, 3% to 8%, 3% to 7.5%, 3% to 7%, 3% to 6.5%, 3% to 6%, 3% to 5.5%, 3% to 5%, 3% to 4.5%, 3% to 4%, and 3% to 3.5% by weight (or volume) of the fermentation broth or lysed cell composition. In another embodiment, an emulsifier (e.g., in powder form or in solution) is added in an amount selected from 0.2% to 5%, 0.5% to 5%, 1% to 5%, 1.5% to 5%, 2% to 5%, 2.5% to 5%, and 3% to 5% by weight (or volume) of the fermentation broth or lysed cell composition. In yet a further embodiment, an emulsifier is added in an amount of from 0.2% to 10% by weight of the lysed cell composition.

[0057] In some embodiments, the emulsifier decreases the interfacial tension (i.e., surface tension) of the fermentation broth or lysed cell composition. As used herein, the term "interfacial tension" or "surface tension" refers to the force which acts on an imaginary line one meter in length at the interface between two phases. In some embodiments, the interfacial tension of the emulsion formed by the emulsifier is lower than an emulsion formed by the endogenous materials. In some embodiments, the interfacial tension can be measured in dynes/cm.

[0058] In some embodiments, the emulsifier increases an absolute value of the zeta potential of the fermentation broth or lysed cell composition (i.e., increases a positive zeta potential or decreases a negative zeta potential). In some embodiments, the addition of an

anionic emulsifier can result in a downward shift in the zeta potential of the fermentation broth or lysed cell composition (e.g., decreases a positive zeta potential or increases a negative zeta potential). In some embodiments, the addition of a cationic emulsifier can result in an upward shift in zeta potential of the fermentation broth or lysed cell composition (e.g., increases a positive zeta potential or increases a negative zeta potential). As used herein, the term "zeta potential" refers to the electrokinetic potential between particles in the emulsion. In some embodiments, the zeta potential can be measured in mV. In some embodiments, the absolute value of the zeta potential of the emulsion formed by the emulsifier is higher than an emulsion formed by the endogenous materials.

[0059] In some embodiments, the addition of an ionic emulsifier creates an oil-in-water emulsion. In some embodiments, the oil-in-water emulsion includes, but is not limited to, oil, water, and an ionic emulsifier.

[0060] In some embodiments, the process further comprises raising the pH of the lysed cell composition. In some embodiments, the pH is raised by adding a base to the lysed cell composition. The bases that can be used to demulsify the lysed cell composition are the same as those set forth hereinabove. In some embodiments, the pH is selected from about 8 or above; about 9 or above; about 10 or above; about 11 or above; and about 12 or above. In other embodiments, the pH is selected from a pH of 7 to 13; 7 to 12; 7 to 11; 7 to 10; 7 to 9; 8 to 13; 8 to 12; 8 to 11; 8 to 10; 8 to 9; 9 to 12; 9 to 11; 9 to 10; 10 to 12; and 10 to 11.

[0061] In some embodiments, the process further comprises adding a salt to the lysed cell composition. The term "salt" refers to an ionic compound formed by replacing a hydrogen ion from an acid with a metal (e.g., an alkali metal, an alkali earth metal, and a transition metal) or a positively charged compound (e.g., NH_4^+). In some embodiments, the salt can be an alkali metal salt, alkali earth metal salts, sulfate salts, or combinations thereof. Negatively charged ionic species present in a salt include, but are not limited to, halides, sulfate, bisulfate, sulfite,

phosphate, hydrogen phosphate, dihydrogen phosphate, carbonate, bicarbonate, and combinations thereof. In some embodiments, a salt is selected from sodium chloride, sodium sulfate, sodium carbonate, calcium chloride, potassium sulfate, magnesium sulfate, monosodium glutamate, ammonium sulfate, potassium chloride, iron chloride, iron sulfate, aluminum sulfate, ammonium acetate, and combinations thereof. In some embodiments, a salt does not include NaOH. A salt can be added as a solid (e.g., in crystalline, amorphous, pelletized, and/or granular form), and/or as a solution (e.g., a dilute solution, a saturated solution, or a super-saturated solution) containing, for example, water.

[0062] In some embodiments, the salt is added in an amount of 5 g/l to 25 g/l, 5 g/l to 10 g/l, 10 g/l to 15 g/l, 15 g/l to 20 g/l, 20 g/l to 25 g/l, or 10 g/l to 20 g/l.

[0063] In other embodiments, a salt is added to the lysed cell composition in an amount of 20% or less, 15% or less, 10% or less, 7.5% or less, 5% or less, or 2% or less by weight (or volume), of the lysed cell composition. In some embodiments, a salt is added to the lysed cell composition in an amount of from about 0.05% to about 20%, about 0.1% to about 20%, about 0.1% to about 15%, about 0.1% to about 10%, about 0.5% to about 20%, about 0.5% to about 15%, about 0.5% to about 10%, about 0.5% to about 5%, about 0.5% to about 4%, about 0.5% to about 3%, about 0.5% to about 2.5%, about 0.5% to about 2%, about 0.5% to about 1.5%, about 0.5% to about 1%, about 1% to about 20%, about 1% to about 15%, about 1% to about 10%, about 1% to about 5%, about 1% to about 4%, 1% to about 3%, about 1% to about 2.5%, about 1% to about 2%, about 1% to about 1.5%, about 1.5% to about 5%, about 1.5% to about 4%, about 1.5% to about 3%, about 1.5% to about 2.5%, about 1.5% to about 2%, about 2% to 20%, about 2% to about 15%, about 2% to about 10%, about 2% to about 5%, about 2% to about 4%, about 2% to about 3%, about 2% to about 2.5%, about 2.5% to about 5%, about 2.5% to about 4%, about 2.5% to about 3%, about 3% to about 5%, about 3% to about 4%, about 4% to about 5%, about 5% to about 20%, about 5% to about 15%, about 5% to about 10%, about 10% to

about 20%, about 10% to about 15%, or about 15% to about 20%, by weight (or volume), lysed cell composition (e.g., a total broth weight (or volume)). For example, when a lysed cell composition weighs 1,000 kg, salt that is added in an amount of 0.5% to 20%, by weight (or volume), requires the addition of 5 kg to 200 kg salt to the lysed cell composition. In some embodiments, a salt is added to the lysed cell composition in an amount of from about 0.05% to about 20%, about 0.1% to about 20% by weight (or volume), about 0.5% to about 15% by weight (or volume), or about 2% to about 10% by weight (or volume) of the lysed cell composition.

[0064] In some embodiments, the process further comprises heating prior to, during, or after lysing the cells. In some embodiments, the heating is performed prior to adding an emulsifier, during adding an emulsifier, after adding an emulsifier, prior to adding a salt, during adding a salt, after adding a salt, or combinations thereof. In some embodiments, the process comprises heating the lysed cell composition and/or cells to at least 10 °C, at least 20 °C, at least 25 °C, at least 30 °C, at least 35° C, at least 40° C, at least 45° C, at least 50° C, at least 55° C, at least 60° C, at least 65° C, at least 70° C, at least 75° C, at least 80° C, at least 85° C, at least 90° C, at least 95° C, or at least 100° C. In other embodiments, the process comprises heating the lysed cell composition and/or cells to from about 10 °C to about 100 °C, about 10 °C to about 90 °C, about 10 °C to about 80 °C, about 10 °C to about 70 °C, about 20 °C to about 100 °C, about 20 °C to about 90 °C, about 20 °C to about 80 °C, about 20 °C to about 70 °C, about 30° C to about 100° C, about 30° C to about 90° C, about 30° C to about 80° C, about 30 °C to about 70 °C, about 40° C to about 100° C, about 40° C to about 90° C, about 40° C to about 80° C, about 50° C to about 100° C, about 50° C to about 90° C, about 50° C to about 80° C, about 50° C to about 70° C, about 60° C to about 100° C, about 60° C to about 90° C, about 60° C to about 80° C, about 70° C to about 100° C, about 70° C to about 90° C, about 80° C to about 100° C, about 80° C to about 90° C, or about 90° C to about 100° C. In further embodiments, the process comprises heating the lysed cell composition from about 70° C to about 100° C, about 70° C to about 90° C, about 80° C

to about 100° C, about 80° C to about 90° C, or about 90° C to about 100° C. In yet further embodiments, the process comprises heating the lysed cell composition to at least 70° C, at least 75° C, at least 80° C, at least 85° C, at least 90° C, at least 95° C, or at least 100° C.

[0065] In some embodiments, the lysed cell composition is heated for a sufficient period of time to demulsify the lysed cell composition.

[0066] In some embodiments, cells and/or a lysed cell composition can be heated in a closed system or in a system with an evaporator. In some embodiments, cells and/or a lysed cell composition can be heated in a system with an evaporator such that a portion of the water present in the cells and/or the lysed cell composition is removed by evaporation. In some embodiments, the process comprises heating cells and/or a lysed cell composition in a system with an evaporator to remove up to 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45% or 50% by weight (or volume) of water present in the cells and/or the lysed cell composition. In some embodiments, the process comprises heating cells and/or a lysed cell composition in a system with an evaporator to remove 1% to 50%, 1% to 45%, 1% to 40%, 1% to 35%, 1% to 30%, 1% to 25%, 1% to 20%, 1% to 15%, 1% to 10%, 1% to 5%, 5% to 50%, 5% to 45%, 5% to 40%, 5% to 35%, 5% to 30%, 5% to 25%, 5% to 20%, 5% to 15%, 5% to 10%, 10% to 50%, 10% to 45%, 10% to 40%, 10% to 35%, 10% to 30%, 10% to 25%, 10% to 20%, 10% to 15%, 15% to 50%, 15% to 45%, 15% to 40%, 15% to 35%, 15% to 30%, 15% to 25%, 15% to 20%, 20% to 50%, 20% to 45%, 20% to 40%, 20% to 35%, 20% to 30%, 20% to 25%, 25% to 50%, 25% to 45%, 25% to 40%, 25% to 35%, 25% to 30%, 30% to 50%, 30% to 45%, 30% to 40%, 30% to 35%, 35% to 50%, 35% to 45%, 35% to 40%, 40% to 50%, 40% to 45%, or 45% to 50% by weight (or volume) of water.

[0067] In some embodiments, the process further comprises agitating (i) prior to, during, or after lysing the cells; (i) prior to, during, or after demulsifying the lysed cell composition; or a combination thereof. The terms "agitating" and "agitation" refer to a process of affecting motion

in the cells and/or the lysed cell composition through an application of force. In some embodiments, the process comprises agitating the cells and/or the lysed cell composition by stirring, mixing, blending, shaking, vibrating, or a combination thereof.

[0068] In some embodiments, the agitator is a dispersion style agitator that disperses a base and/or salt in the cells and/or the lysed cell composition. In some embodiments, the agitator has a heating plate. In some embodiments, the agitator has a mantle for stirring. In some embodiments, an agitator has one or more impellers. As used herein, "impeller" refers to a device arranged to impart motion to the cells or a lysed cell composition when rotated. Impellers suitable for use with the present invention include straight blade impellers, Rushton blade impellers, axial flow impellers, radial flow impellers, concave blade disc impellers, high-efficiency impellers, propellers, paddles, turbines, and combinations thereof.

[0069] In some embodiments, the process of the invention comprises agitating the cells and/or the lysed cell composition at 0.1 hp/1,000 gal to 10 hp/1,000 gal, 0.5 hp/1,000 gal to 8 hp/1,000 gal, 1 hp/1,000 gal to 6 hp/1,000 gal, or 2 hp/1,000 gal to 5 hp/1,000 gal of composition. In some embodiments, the process comprises agitating the cells and/or the lysed cell composition at 0.1 hp/1000 gal to 10 hp/1000 gal of composition.

[0070] In some embodiments the invention comprises agitating at 10 rpm or below, 20 rpm or below, 50 rpm or below, 100 rpm or below, 150 rpm or below, 200 rpm or below, 250 rpm or below, 300 rpm or below, 350 rpm or below, 400 rpm or below, 10 rpm to 400 rpm, 10 rpm to 350 rpm, 10 rpm to 300 rpm, 10 rpm to 250 rpm, 10 rpm to 200 rpm, 10 rpm to 150 rpm, 10 rpm to 100 rpm, 10 rpm to 50 rpm, 10 rpm to 20 rpm, 20 rpm to 400 rpm, 20 rpm to 350 rpm, 20 rpm to 300 rpm, 20 rpm to 250 rpm, 20 rpm to 200 rpm, 20 rpm to 150 rpm, 20 rpm to 100 rpm, 20 rpm to 50 rpm, 50 rpm to 400 rpm, 50 rpm to 350 rpm, 50 rpm to 300 rpm, 50 rpm to 250 rpm, 50 rpm to 200 rpm, 50 rpm to 150 rpm, 50 rpm to 100 rpm, 100 rpm to 400 rpm, 100 rpm to 350 rpm, 100 rpm to 300 rpm, 100 rpm to 250 rpm, 100 rpm to 200 rpm, 100 rpm to 150

rpm, 150 rpm to 400 rpm, 150 rpm to 350 rpm, 150 rpm to 300 rpm, 150 rpm to 250 rpm, 150 rpm to 200 rpm, 200 rpm to 400 rpm, 200 rpm to 350 rpm, 200 rpm to 300 rpm, 200 rpm to 250 rpm, 250 rpm to 400 rpm, 250 rpm to 350 rpm, 250 rpm to 300 rpm, 300 rpm to 400 rpm, 300 rpm to 350 rpm, or 350 rpm to 400 rpm. In some embodiments, the agitating occurs at a rate of 350 rpm or less.

[0071] In some embodiments, the process includes agitating cells and/or a lysed cell composition using an agitator having an impeller tip speed of 90 ft/min to 1,200 ft/min, 200 ft/min to 1,000 ft/min, 300 ft/min to 800 ft/min, 400 ft/min to 700 ft/min, or 500 ft/min to 600 ft/min. In some embodiments, the process comprises agitating with an agitator having an impeller tip speed of 200 ft/min to 1000 ft/min.

[0072] In some embodiments, a process includes agitating cells and/or a lysed cell composition using an agitator having an impeller tip speed of 5 cm/sec to 900 cm/sec, 5 cm/sec to 750 cm/sec, 5 cm/sec to 500 cm/sec, 5 cm/sec to 350 cm/sec, 5 cm/sec to 300 cm/sec, 5 cm/sec to 250 cm/sec, 5 cm/sec to 200 cm/sec, 5 cm/sec to 150 cm/sec, 5 cm/sec to 100 cm/sec, 5 cm/sec to 50 cm/sec, 5 cm/sec to 25 cm/sec, 25 cm/sec to 900 cm/sec, 25 cm/sec to 750 cm/sec, 25 cm/sec to 500 cm/sec, 25 cm/sec to 350 cm/sec, 25 cm/sec to 300 cm/sec, 25 cm/sec to 250 cm/sec, 25 cm/sec to 200 cm/sec, 25 cm/sec to 150 cm/sec, 25 cm/sec to 100 cm/sec, 25 cm/sec to 50 cm/sec, 50 cm/sec to 900 cm/sec, 50 cm/sec to 750 cm/sec, 50 cm/sec to 500 cm/sec, 50 cm/sec to 350 cm/sec, 50 cm/sec to 300 cm/sec, 50 cm/sec to 250 cm/sec, 50 cm/sec to 200 cm/sec, 50 cm/sec to 150 cm/sec, 50 cm/sec to 100 cm/sec, 100 cm/sec to 900 cm/sec, 100 cm/sec to 750 cm/sec, 100 cm/sec to 500 cm/sec, 100 cm/sec to 350 cm/sec, 100 cm/sec to 300 cm/sec, 100 cm/sec to 250 cm/sec, 100 cm/sec to 200 cm/sec, 100 cm/sec to 150 cm/sec, 150 cm/sec to 900 cm/sec, 150 cm/sec to 750 cm/sec, 150 cm/sec to 500 cm/sec, 150 cm/sec to 350 cm/sec, 150 cm/sec to 300 cm/sec, 150 cm/sec to 250 cm/sec, 150 cm/sec to 200 cm/sec, 200 cm/sec to 900 cm/sec, 200 cm/sec to 750 cm/sec, 200 cm/sec to 500 cm/sec, 200 cm/sec to 350

cm/sec, 200 cm/sec to 300 cm/sec, 200 cm/sec to 250 cm/sec, 250 cm/sec to 900 cm/sec, 250 cm/sec to 750 cm/sec, 250 cm/sec to 500 cm/sec, 250 cm/sec to 350 cm/sec, 250 cm/sec to 300 cm/sec, 300 cm/sec to 900 cm/sec, 300 cm/sec to 750 cm/sec, 300 cm/sec to 500 cm/sec, 300 cm/sec to 350 cm/sec, 350 cm/sec to 900 cm/sec, 350 cm/sec to 850 cm/sec, 350 cm/sec to 800 cm/sec, 350 cm/sec to 750 cm/sec, 350 cm/sec to 700 cm/sec, 350 cm/sec to 650 cm/sec, 350 cm/sec to 600 cm/sec, 350 cm/sec to 550 cm/sec, 350 cm/sec to 500 cm/sec, 350 cm/sec to 450 cm/sec, 350 cm/sec to 400 cm/sec, 400 cm/sec to 900 cm/sec, 400 cm/sec to 850 cm/sec, 400 cm/sec to 800 cm/sec, 400 cm/sec to 750 cm/sec, 400 cm/sec to 700 cm/sec, 400 cm/sec to 650 cm/sec, 400 cm/sec to 600 cm/sec, 400 cm/sec to 550 cm/sec, 400 cm/sec to 500 cm/sec, 400 cm/sec to 450 cm/sec, 450 cm/sec to 900 cm/sec, 450 cm/sec to 850 cm/sec, 450 cm/sec to 800 cm/sec, 450 cm/sec to 750 cm/sec, 450 cm/sec to 700 cm/sec, 450 cm/sec to 650 cm/sec, 450 cm/sec to 600 cm/sec, 450 cm/sec to 550 cm/sec, 450 cm/sec to 500 cm/sec, 500 cm/sec to 900 cm/sec, 500 cm/sec to 850 cm/sec, 500 cm/sec to 800 cm/sec, 500 cm/sec to 750 cm/sec, 500 cm/sec to 700 cm/sec, 500 cm/sec to 650 cm/sec, 500 cm/sec to 600 cm/sec, 500 cm/sec to 550 cm/sec, 550 cm/sec to 900 cm/sec, 550 cm/sec to 850 cm/sec, 550 cm/sec to 800 cm/sec, 550 cm/sec to 750 cm/sec, 550 cm/sec to 700 cm/sec, 550 cm/sec to 650 cm/sec, 550 cm/sec to 600 cm/sec, 600 cm/sec to 900 cm/sec, 600 cm/sec to 850 cm/sec, 600 cm/sec to 800 cm/sec, 600 cm/sec to 750 cm/sec, 600 cm/sec to 700 cm/sec, 600 cm/sec to 650 cm/sec, 650 cm/sec to 900 cm/sec, 650 cm/sec to 850 cm/sec, 650 cm/sec to 800 cm/sec, 650 cm/sec to 750 cm/sec, 650 cm/sec to 700 cm/sec, 700 cm/sec to 900 cm/sec, 700 cm/sec to 850 cm/sec, 700 cm/sec to 800 cm/sec, 700 cm/sec to 750 cm/sec, 750 cm/sec to 900 cm/sec, 750 cm/sec to 850 cm/sec, 750 cm/sec to 800 cm/sec, 800 cm/sec to 900 cm/sec, 800 cm/sec to 850 cm/sec, or 850 cm/sec to 900 cm/sec. The term "impeller tip speed" refers to the speed of the outer most portion of the impeller as it rotates around its central axis.

[0073] In some embodiments, the agitating (and optional additional steps as described herein) is performed in a container comprising an impeller, wherein a ratio of the impeller diameter to the container volume is 0.1 to 0.5, 0.1 to 0.4, 0.2 to 0.5, 0.2 to 0.4, 0.3 to 0.5, or 0.3 to 0.4.

[0074] In some embodiments, the agitating (and optional additional steps as described herein) is performed in a container comprising an impeller, wherein a ratio of the impeller diameter to the inner diameter of the container is at least 0.25, at least 0.34, at least 0.65, 0.25 to 0.65, 0.25 to 0.33, 0.3 to 0.6, 0.3 to 0.5, 0.3 to 0.4, 0.34 to 0.65, 0.34 to 0.6, 0.34 to 0.55, 0.37 to 0.55, 0.4 to 0.65, 0.4 to 0.6, 0.4 to 0.5, or 0.42 to 0.55.

[0075] In some embodiments, agitating comprises mixing cells and/or a lysed cell composition such that the cells and/or the lysed cell composition is placed under flow conditions described by a Reynolds number of 10 to 10,000, 1,000 to 10,000, 1,500 to 10,000, or 2,000 to 10,000. In some embodiments, a lysed cell emulsion during the agitating has a Reynolds number of 2,000 or more, 3,000 or more, or 5,000 or more, or 2,000 to 10,000, 3,000 to 10,000, or 5,000 to 10,000.

[0076] In some embodiments, the agitation vessels can have two impellers. In some embodiments, the impellers are Rushton blade impellers. In some embodiments, the impellers are separated from each other by a distance at least equal to a diameter of the smallest impeller. In some embodiments, the impellers are 30 inches to 40 inches, 33 inches to 37 inches, 33 inches, 34 inches, 35 inches, 36 inches or 37 inches from tip to tip. In some embodiments, the agitation vessels have a volume of at least 10,000 liters, at least 20,000 liters, at least 30,000 liters, at least 40,000 liters or at least 50,000 liters. In some embodiments, the agitation vessels have an inner diameter of 90 inches to 110 inches, 95 inches to 105 inches, 98 inches, 99 inches, 100 inches, 101 inches, or 102 inches. In some embodiments, a first impeller is located 15 inches to 20 inches, 16 inches to 19 inches, or 17 inches to 18 inches from a bottom of the agitation vessel and

a second impeller is located 60 inches to 80 inches, 65 inches to 75 inches, 68 inches, 69 inches, 70 inches, 71 inches, 72 inches, 73 inches, 74 inches, or 75 inches above the first impeller. In some embodiments, a lysed cell composition is agitated at least 50 rpm, at least 60 rpm, or at least 70 rpm. In some embodiments, a lysed cell composition is agitated at 50 rpm to 70 rpm, 50 rpm to 60 rpm, or 60 rpm to 70 rpm.

[0077] In some embodiments, the process further comprises agitating the lysed cell composition. In some embodiments, the process of agitating a lysed cell composition demulsifies the lysed cell composition.

[0078] In some embodiments, the lysed cell composition is subjected to one or more of an emulsifier, a base, a salt, heat, and agitation to form a demulsified lysed cell composition.

[0079] In some embodiments, the process comprises demulsifying unwashed lysed cells comprising microbial oil to for an unwashed demulsified lysed cell composition; and then separating the oil from the unwashed demulsified lysed cell composition. An unwashed demulsified lysed cell composition refers to the process whereby the lysed cell composition is not washed, e.g., with water or buffer that is then removed by, e.g., centrifugation. Washing a lysed cell composition can decrease the overall yield of oil obtained from a cell.

[0080] In an alternative embodiment, the number of times the lysed cell composition is washed can be decreased by 1 time, 2 times, 3 times or more. In some embodiments, the washing is no more than 1 time, 2 times, or 3 times.

[0081] In some embodiments, the various combinations of forming a lysed cell composition, adding an emulsifier to the lysed cell composition, contacting a lysed cell composition with a base or raising the pH of a lysed cell composition, contacting a lysed cell composition with a salt, heating the lysed cell composition, and agitating a lysed cell composition can occur in a single vessel. In some embodiments, the various combinations of forming cells, contacting cells with a base or raising the pH of cells, adding an emulsifier to the lysed cell

composition, contacting cells with a salt, heating the cells, and agitating cells can occur in a single vessel. In some embodiments, the single vessel includes a fermentation vessel. In some embodiments, the fermentation vessel can have a volume of at least 20,000 liters, at least 50,000 liters, at least 100,000 liters, at least 120,000 liters, at least 150,000 liters, at least 200,000 liters, or at least 220,000 liters. In some embodiments, the fermentation vessel can have a volume of 20,000 liters to 220,000 liters, 20,000 liters to 100,000 liters, 20,000 liters to 50,000 liters, 50,000 liters to 220,000 liters, 50,000 liters to 150,000 liters, 50,000 liters to 100,000 liters, 100,000 liters to 220,000 liters, 100,000 liters to 150,000 liters, 100,000 liters to 120,000 liters, 150,000 liters to 220,000 liters, 150,000 liters to 200,000 liters, or 200,000 liters to 220,000 liters.

[0082] In some embodiments, a quantity of cells or a lysed cell composition formed in a vessel can be transferred into one or more agitation vessels. In some embodiments, the agitation vessels can have a volume of at least 20,000 liters, at least 30,000 liters, at least 40,000 liters or at least 50,000 liters. In some embodiments, the agitation vessels can have a volume of 20,000 liters to 50,000 liters, 20,000 liters to 40,000 liters, 20,000 liters to 30,000 liters, 30,000 liters to 50,000 liters, 30,000 liters to 40,000 liters or 40,000 liters to 50,000 liters.

[0083] In general, the processes described herein do not utilize an organic solvent to obtain, separate, or otherwise recover a microbial oil from the microbial cells. In some embodiments, no organic solvent is used in obtaining microbial oil from microbial cells. In another embodiment, an organic solvent is not added to cells, is not added to a lysed cell composition, and/or is not added to an oil during the processes disclosed herein in an amount or concentration sufficient to obtain a microbial oil. Organic solvents include polar solvents, non-polar solvents, water-miscible solvents, water-immiscible solvents, and combinations thereof.

[0084] In some embodiments, the process further comprises separating an oil from a demulsified lysed cell composition. In some embodiments, the process comprises separating an oil from a demulsified lysed cell composition by centrifuging the demulsified lysed cell

composition. In some embodiments, an oil is separated from a demulsified lysed cell composition by allowing the demulsified lysed cell composition to stand, wherein the oil separates (e.g., as a separate layer) from the demulsified lysed cell composition using gravity. In some embodiments, separation is achieved by a lipophilic membrane. In some embodiments, the separating comprises centrifuging at a temperature of 10° C to 100° C. In some embodiments, the oil is separated from the demulsified lysed cell composition by first raising the pH (e.g. by adding a base that is described hereinabove) and then centrifuging the demulsified lysed cell composition to obtain the oil.

[0085] In some embodiments, the process comprises separating an oil from the demulsified lysed cell composition by centrifuging the demulsified lysed cell composition at a temperature of at least 10 °C, at least 20 °C, at least 25 °C, at least 30 °C, at least 35° C, at least 40° C, at least 45° C, at least 50° C, at least 55° C, at least 60° C, at least 65° C, at least 70° C, at least 75° C, at least 80° C, at least 85° C, at least 90° C, at least 95° C, or at least 100° C. In some embodiments, the process comprises separating an oil from the demulsified lysed cell composition by centrifuging the demulsified lysed cell composition at a temperature of 10 °C to 100 ° C, 10 °C to 90 °C, 10 °C to 80 °C, 20 °C to 100 °C, 20 °C to 90 °C, 20 °C to 80 °C, 25 °C to 100 °C, 25 °C to 90 °C, 25 °C to 80 °C, 25 °C to 75 °C, 30° C to 100° C, 30° C to 90° C, 30° C to 80° C, 40° C to 100° C, 40° C to 90° C, 40° C to 80° C, 50° C to 100° C, 50° C to 90° C, 50° C to 80° C, 50° C to 70° C, 60° C to 100° C, 60° C to 90° C, 60° C to 80° C, 60° C to 70° C, 70° C to 100° C, 70° C to 90° C, 70° C to 80° C, 80° C to 100° C, 80° C to 90° C, or 90° C to 100° C.

[0086] In some embodiments, centrifuging is conducted at a feed rate (of a demulsified lysed cell composition into a centrifuge) of 1 kilogram per minute (kg/min) to 500 kg/min, 1 kg/min to 400 kg/min, 1 kg/min to 300 kg/min, 1 kg/min to 200 kg/min, 1 kg/min to 100 kg/min, 1 kg/min to 75 kg/min, 1 kg/min to 50 kg/min, 1 kg/min to 40 kg/min, 1 kg/min to 30 kg/min, 1 kg/min to 25 kg/min, 1 kg/min to 10 kg/min, 10 kg/min to 500 kg/min, 10 kg/min to

400 kg/min, 10 kg/min to 300 kg/min, 10 kg/min to 200 kg/min, 10 kg/min to 100 kg/min, 10 kg/min to 75 kg/min, 10 kg/min to 50 kg/min, 10 kg/min to 40 kg/min, 10 kg/min to 30 kg/min, 20 kg/min to 500 kg/min, 20 kg/min to 400 kg/min, 20 kg/min to 300 kg/min, 20 kg/min to 200 kg/min, 20 kg/min to 100 kg/min, 20 kg/min to 75 kg/min, 20 kg/min to 50 kg/min, 20 kg/min to 40 kg/min, 25 kg/min to 500 kg/min, 25 kg/min to 400 kg/min, 25 kg/min to 300 kg/min, 25 kg/min to 200 kg/min, 25 kg/min to 100 kg/min, 25 kg/min to 75 kg/min, 25 kg/min to 50 kg/min, 30 kg/min to 60 kg/min, 30 kg/min to 50 kg/min, 30 kg/min to 40 kg/min, 50 kg/min to 500 kg/min, 100 kg/min to 500 kg/min, or 200 kg/min to 500 kg/min.

[0087] In some embodiments, the process comprises centrifuging a demulsified lysed cell composition at a centrifugal force of 1,000 g to 25,000 g, 1,000 g to 20,000 g, 1,000 g to 10,000 g, 2,000 g to 25,000 g, 2,000 g to 20,000 g, 2,000 g to 15,000 g, 3,000 g to 25,000 g, 3,000 g to 20,000 g, 5,000 g to 25,000 g, 5,000 g to 20,000 g, 5,000 g to 15,000 g, 5,000 g to 10,000 g, 5,000 g to 8,000 g, 10,000 g to 25,000 g, 15,000 g to 25,000 g, or at least 1,000 g, at least 2,000, g, at least 4,000 g, at least 5,000 g, at least 7,000 g, at least 8,000 g, at least 10,000 g, at least 15,000 g, at least 20,000 g, or at least 25,000 g. As used herein, "g" refers to standard gravity or approximately 9.8 m/s^2. In some embodiments, the process comprises centrifuging a demulsified lysed cell composition at 4,000 rpm to 14,000 rpm, 4,000 rpm to 10,000 rpm, 6,000 rpm to 14,000 rpm, 6,000 rpm to 12,000 rpm, 8,000 to 14,000 rpm, 8,000 rpm to 12,000 rpm, or 8,000 rpm to 10,000 rpm.

[0088] In some embodiments, the oil can be recovered, for example, by decanting, skimming, vacuuming, pumping, sucking off, drawing off, siphoning, or otherwise recovering the microbial oil from the surface of the separated composition.

[0089] In some embodiments, the process comprises drying the oil that has been recovered to remove water from the oil. In some embodiments, drying the oil can include, but is not limited to, heating the oil to evaporate water. In some embodiments, after drying, the oil has

a water content by weight (or volume) percentage of oil that is less than 3%, less than 2.5%, less than 2%, less than 1.5%, less than 1%, less than 0.5%, less than 0.1%, or 0%. In some embodiments, after drying, the oil has a water content by weight (or volume) percentage of oil of 0% to 3%, 0% to 2.5%, 0% to 2%, 0% to 1.5%, 0% to 1%, 0% to 0.5%, 0.1% to 3%, 0.1% to 2.5%, 0.1% to 2%, 0.1% to 1.5%, 0.1% to 1%, 0.1% to 0.5%, 0.5% to 3%, 0.5% to 2.5%, 0.5% to 2%, 0.5% to 1.5%, 0.5% to 1%, 1% to 3%, 1% to 2.5%, 1% to 2%, 1% to 1.5%, 1.5% to 3%, 1.5% to 2.5%, 1.5% to 2%, 2% to 3%, 2% to 2.5%, or 2.5% to 3%.

[0090] Disclosed herein is a microbial oil that can be obtained from microbial cells by any of the processes disclosed herein. In some embodiments, the oil comprises at least 30% by weight (or volume) arachidonic acid. In some embodiments, the oil comprises at least 30% by weight (or volume) docosahexaenoic acid.

[0091] The Anisidine value (AV) is determined in accordance with AOCS Official Method Cd 18-90. In one embodiment, the oil described herein has an AV of less than about 50; less than about 40; less than about 30; less than about 20; less than about 15; or less than about 10. In some embodiments, the oil has an AV of less than about 50. The term anisidine value refers to the measure of secondary reaction products, such as aldehydes and ketones that occur during oxidation of the oil.

[0092] The peroxide value (PV) is determined in accordance with the AOCS Official Method Cd 8-53. In one embodiment, the oil described herein has a PV less than about 20 meq/kg; less than about 10 meq/kg; or less than about 5 meq/kg. In some embodiments, the oil has a PV of less than about 5 meq/kg. The term peroxide value refers to the measure of primary reaction products, such as peroxides and hydroperoxides, that occur during oxidation of the oil. As used herein peroxide value is measured in meq/kg.

[0093] In some embodiments, the oil has a phosphorus content of 100 ppm or less, 95 ppm or less, 90 ppm or less, 85 ppm or less, 80 ppm or less, 75 ppm or less, 70 ppm or less, 65

ppm or less, 60 ppm or less, 55 ppm or less, 50 ppm or less, 45 ppm or less, 40 ppm or less, 35 ppm or less, 30 ppm or less, 25 ppm or less, 20 ppm or less, 15 ppm or less, 10 ppm or less, 9 ppm or less, 8 ppm or less, 7 ppm or less, 6 ppm or less, 5 ppm or less, 4 ppm or less, 3 ppm or less, 2 ppm or less, or 1 ppm or less. In some embodiments, the oil has a phosphorus content of about 8 ppm or less.

[0094] In some embodiments, the oil comprises one or more PUFAs. In some embodiments, the oil comprises at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70% or at least 80% PUFA (by PUFA weight). In some embodiments, the oil comprises at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70% or at least 80% DHA (by DHA weight), and/or at least 10%, at least 15%, or at least 20% DPA n-6 (by DPA n-6 weight), and/or at least 10%, at least 15%, or at least 20% EPA (by EPA weight), and/or at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, or at least 80% ARA (by ARA weight). In some embodiments, an oil comprises less than 50%, less than 40%, less than 30%, less than 20%, less than 15%, less than 10%, or less than 5% EPA (by EPA weight). In some embodiments, an oil comprises less than 50%, less than 40%, less than 30%, less than 20%, less than 15%, less than 10%, or less than 5% DHA (by DHA weight). In some embodiments, an oil comprises less than 10%, less than 5%, less than 2%, less than 1%, or less than 0.5% by weight (or volume) sterols.

[0095] In some embodiments, an oil comprises at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 50% to 95%, 50% to 90%, 50% to 85%, 50% to 80%, 50% to 75%, 60% to 95%, 60% to 90%, 60% to 85%, 70% to 95%, 70% to 90%, 70% to 85%, 75% to 95%, 75% to 90%, or 75% to 85%, by weight (or volume) of triglycerides.

[0096] In some embodiments, the triglycerides comprise at least 10%, at least 20%, at least 30%, at least 35%, at least 40%, at least 50%, at least 60%, at least 70% or at least 80% by weight (or volume) DHA. In some embodiments, the triglycerides comprise at least 10%, at least 20%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, or at least 80% by weight (or volume) ARA. In some embodiments, the triglycerides comprise at least 50%, at least 40%, at least 30%, at least 20%, at least 15%, at least 10%, or at least 5% by weight (or volume) EPA.

[0097] In one embodiment, the microbial oil obtained and/or recovered by any of the processes described herein is a crude oil. In another embodiment, the oil described herein is a refined oil. A "crude oil" is an oil obtained from microbial cells without further processing. A "refined oil" is an oil obtained by treating a crude oil with standard processing of refining, bleaching, and/or deodorizing. *See*, e.g., U.S. Patent No. 5,130,242. In some embodiments, refining includes, but is not limited to, caustic refining, degumming, acid treatment, alkali treatment, cooling, heating, bleaching, deodorizing, deacidification, and combinations thereof.

[0098] In some embodiments, the process comprises concentrating a fermentation broth comprising microbial cells. In some embodiments, the process comprises concentrating the lysed cell composition. As used herein, "concentrating" refers to removing water from a composition. Concentrating can include, but is not limited to, evaporating, chemical drying, centrifuging, and the like, and combinations thereof. In some embodiments, a cell composition or a lysed cell composition is concentrated to provide an oil concentration of at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, or at least 30% by weight (or volume) of the composition. In some embodiments, a cell composition or a lysed cell composition is concentrated to provide an oil concentration of 4% to 40%, 4% to 30%, 4% to 20%, 4% to 15%, 5% to 40%, 5% to 30%, 5% to 20%, 10% to 40%, 10% to 30%, 10% to 20%, 15% to 40%, 15%

to 30%, 20% to 40%, 20% to 30%, 25% to 40%, or 30% to 40% by weight (or volume) of the composition.

[0099] Effective culture conditions for a microbial cell for use with the invention include, but are not limited to, effective media, bioreactor, temperature, pH, and oxygen conditions that permit oil production. An effective medium refers to any medium in which a microbial cell, e.g., Thraustochytriales microbial cell, is typically cultured. Such media typically comprises an aqueous medium having assimilable carbon, nitrogen, and phosphate sources, as well as appropriate salts, minerals, metals, and other nutrients, such as vitamins. Microbial cells for use with the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes, and petri plates.

[00100] In some embodiments, a microbial cell comprises at least 30% by weight (or volume) oil, at least 35% by weight (or volume) oil, at least 40% by weight (or volume) oil, at least 50% by weight (or volume) oil, at least 60% by weight (or volume) oil, at least 70% by weight (or volume) oil, or at least 80% by weight (or volume) oil. In some embodiments, a microbial cell for use with the present invention is capable of producing at least 0.1 grams per liter per hour (g/L/h) of DHA, at least 0.2 g/L/h of DHA, at least 0.3 g/L/h of DHA, or at least 0.4 g/L/h of DHA. In some embodiments, a microbial cell for use with the present invention is capable of producing at least 0.01 grams per liter per hour (g/L/h) of ARA, at least 0.05 g/L/h of ARA, at least 0.1 g/L/h of ARA, at least 0.2 g/L/h of ARA, at least 0.3 g/L/h of ARA, or at least 0.4 g/L/h of ARA.

[00101] In some embodiments, an oil obtained according to any of the processes described herein, the spent biomass, or combinations thereof can be used directly as a food or food ingredient, such as an ingredient in baby food, infant formula, beverages, sauces, dairy based foods (such as milk, yogurt, cheese and ice-cream), oils (e.g., cooking oils or salad dressings), and baked goods; nutritional supplements (e.g., in capsule or tablet forms); feed or feed

supplement for any non-human animal (e.g., those whose products (e.g., meat, milk, or eggs) are consumed by humans); food supplements; and pharmaceuticals (in direct or adjunct therapy application). The term "animal" refers to any organism belonging to the kingdom *Animalia* and includes any human animal, and non-human animal from which products (e.g., milk, eggs, poultry meat, beef, pork or lamb) are derived. In some embodiments, the oil and/or biomass can be used in seafood. Seafood is derived from, without limitation, fish, shrimp and shellfish. The term "products" includes any product derived from such animals, including, without limitation, meat, eggs, milk or other products. When the oil and/or biomass is fed to such animals, polyunsaturated oils can be incorporated into the flesh, milk, eggs or other products of such animals to increase their content of these oils.

EXAMPLES

Example 1

[00102] A cell broth (250 mL) containing microbial cells (*Mortierella*) was sent through a shear mixer for 30 seconds and then mechanically homogenized to lyse the cells by passing the broth through a Microfluidics M-110Y microfluidizer (Microfluidics, Newton, MA) two times. The first and second passes through the microfluidizer were at 10,000 psi. Sodium dodecyl sulfate (SDS) powder was added to the lysed cell composition in an amount of 1% by weight of the lysed cell composition. The lysed cell composition was heated to 90 ºC while agitating at a speed of 180 rpm for 3 hours. The pH of the lysed cell composition was adjusted to 8.42 by adding from 2% to 4%, by weight, of the lysed cell composition of a 12.5% w/w solution of NaOH. Solid NaCl in an amount of 2%, by weight, of the lysed cell composition was added and the composition was agitated at 180 rpm at 90 ºC for 30 hours. During agitation, the pH of the lysed cell composition was allowed to drop and the pH adjusted to 8 by adding from 2% to 4% by weight of the lysed cell composition of a 12.5% w/w solution of NaOH. The lysed cell

composition was centrifuged at 8,000 g for 5 minutes to provide a crude oil, which yielded 69.7% ARA (by ARA weight). The Anisidine Value (AV) of the crude oil was 27.9.

Example 2

[00103] A cell broth (250 mL) containing microbial cells (*Mortierella*) was sent through a shear mixer for 30 seconds and then mechanically homogenized to lyse the cells by passing the broth through a Microfluidics M-110Y microfluidizer (Microfluidics, Newton, MA) two times. The first and second passes through the microfluidizer were at 10,000 psi. The pH of the lysed cell composition was adjusted to 10.02 by adding from 4% to 8% by weight of the lysed cell composition of a 12.5% w/w solution of NaOH. SDS powder was added in an amount of 1% by weight of the lysed cell composition, and the composition was heated to 90 ºC while being agitated at a speed of 180 rpm for 3 hours. The pH of the lysed cell composition was allowed to decrease to 8.5, at which point solid NaCl in an amount of 2% by weight of the lysed cell composition was added, and the composition was agitated at 180 rpm at 90 ºC for 40 hours. During agitation, the pH of the lysed cell composition was allowed to drop and the pH adjusted to 8 by adding from 2% to 4% by weight of the lysed cell composition of a 12.5% w/w solution of NaOH. The lysed cell composition was then centrifuged at 8,000 g for 5 minutes to provide a crude oil, which yielded 78.9% ARA (by ARA weight). The AV of the crude oil was 11.2.

Example 3

[00104] A cell broth (716 kg) containing microbial cells (*Mortierella*) was mechanically homogenized to lyse the cells by passing the broth through a GEA Niro homogenizer (GEA Niro Soavi North America, Bedford, NH) twice to form a lysed cell composition. The first and second passes through the microfluidizer were at 10,000 psi. SDS powder was added in amount of 1% by weight of the lysed cell composition, and the composition was heated to 90 ºC while being

agitated at a speed of 180 rpm for 3 hours. The pH of the lysed cell composition was adjusted to about 8.5 by adding 1.3 kg of a 50% w/w solution of NaOH. Solid NaCl in an amount of 2%, by weight, of the lysed cell composition was added, and the composition was agitated at 90 °C for 60 hours. During agitation, the pH of the lysed cell composition was allowed to drop and the pH was subsequently adjusted to 8 by adding from 0.5% to 1% by weight of the lysed cell composition a 50% w/w solution of NaOH. The composition was centrifuged at 6,000 rpm at a feed rate of 40 kg/min (a Westfalia RSE-110 centrifuge was used (Westfalia Separator AG, Germany)) to provide a crude oil, which yielded 85% ARA (by ARA weight). The crude oil had an AV of 48.9 and a phosphorus content of 3.2 ppm.

Example 4

[00105] A cell broth (1,600 kg) containing microbial cells (*Mortierella*) was sent through a shear mixer for 60 minutes and then mechanically homogenized to lyse the cells by passing the broth through a Microfluidics M-110Y microfluidizer (Microfluidics, Newton, MA) twice to form a lysed cell composition. The first and second passes through the microfluidizer were at 10,000 psi. The pH of the lysed cell composition was adjusted to about 10 by adding 1% by weight of the lysed cell composition of a 50% w/w solution of NaOH. A 30% w/w solution of SDS was added in an amount of 3% by weight of the lysed cell composition, and the composition was heated to 90 °C while being agitated at a speed of 180 rpm for 3 hours. The pH of the lysed cell composition was allowed to drop to about 8.5, at which point solid NaCl in an amount of 2% by weight of the lysed cell composition was added and the composition agitated at 180 rpm at 90 °C for 100 hours. During agitation, the pH of the lysed cell composition was allowed to drop and the pH was readjusted to 8 by adding from 0.5% to 1% by weight of the lysed cell composition of a 50% w/w solution of NaOH. The composition was centrifuged at 11,000 rpm at a feed rate of 8L/min (a Seital SR 1010 centrifuge was used (Seital srl, Italy)) to provide a crude oil, which

yielded 66.9% ARA (by weight ARA). The crude oil had an AV of 14.6 and phosphorus content of 1 ppm.

Example 5

[00106] A cell broth (250 g) containing microbial cells (*Mortierella*) was sent through a shear mixer for 30 seconds and then mechanically homogenized to lyse the cells by passing the broth through a Microfluidics M-110Y microfluidizer (Microfluidics, Newton, MA) two times. The first and second passes through the microfluidizer were at 10,000 psi. The pH of the lysed cell composition was adjusted to 10 by adding a 12.5% w/w solution of NaOH. Tween 80 (polysorbate 80) was added to the lysed cell composition in amount of 1% by weight of the lysed cell composition, and the composition was heated to 90 °C while being agitated at a speed of 250 rpm for 3 hours. The pH of the lysed cell composition was allowed to drop to 8.3, at which point solid NaCl in an amount of 2% by weight of the lysed cell composition was added and the composition was agitated at 250 rpm at 90 °C for 20 hours. Samples were taken throughout the 20 hour agitation period and centrifuged at 8,000 g for 5 minutes. There was no sign of separation of an oil. The experiment was repeated by adding Tween 80 (polysorbate 80) in an amount of 2% by weight of the lysed cell composition. There was no sign of separation of an oil. It is believed that SDS facilitated the separation of oil from the lysed cell composition of Examples 1-4 because SDS is an ionic emulsifier (Tween 80 is a nonionic emulsifier) and has a smaller molecular weight (288 g/mol) than Tween 80 (1310 g/mol).

Example 6

[00107] A cell broth (300g) containing microbial cells (S*chizochytrium sp.*) was pasteurized at 60° C for 1 hour. The cell broth was agitated at a speed of 180 RPM and heated to 60° C. The cells were lysed by adding a 50wt% NaOH solution to pH adjust the unwashed broth

to 7-7.5 and Alcalase® 2.4 FG (available from, Novozymes (Franklinton, NC)) in an amount of 0.5% based on broth weight. The broth reacted for 2 hours and then the pH was adjusted to 10-11 by adding a 12.5wt% NaOH solution. Simultaneously, SDS powder was added in an amount of 2% by weight of the lysed cell composition and the composition heated to 80-90° C for 2-5 hours. After the hold time, solid NaCl in an amount of 2% by weight of the lysed cell composition was added and the composition heated to 90° C for 19 hours. The lysed cell composition was centrifuged (Thermo Scientific Sorvell ST40R Centrifuge) at 8000 RPM for 5 minutes to provide a crude oil, which yielded 51.44% DHA (by DHA weight).

Example 7

[00108] A cell broth (300g) containing microbial cells (S*chizochytrium sp.*) was pasteurized at 60° C for 1 hour. The unwashed cell broth was mechanically homogenized to lyse the cells by passing the broth through a Microfluidics M-110Y microfluidizer (Microfluidics, Newton, MA) at 10,000 psi one time. While agitating the lysed cell composition at a speed of 180 RPM, the pH of the composition was adjusted to 10-11 by adding a 12.5wt% NaOH solution. Simultaneously, SDS powder was added in an amount of 2% by weight of the lysed cell composition and the composition heated to 80-90° C for 2-5 hours. After the hold time, solid NaCl in an amount of 2% by weight of the lysed cell composition was added and the composition heated to 90° C and held for a few hours. The lysed cell composition was centrifuged (Thermo Scientific Sorvell ST40R Centrifuge) at 8000 RPM for 5 minutes to provide a crude oil, which yielded 59.32% DHA (by DHA weight).

Example 8

[00109] A cell broth (300g) containing microbial cells (S*chizochytrium sp.*) was pasteurized at 60° C for 1 hour. The cell broth was agitated at a speed of 180 RPM and heated to

60° C. The cells were lysed by adding a 50wt% NaOH solution to pH adjust the unwashed broth to 7-7.5 and Alcalase® 2.4 FG (available from, Novozymes (Franklinton, NC)) in an amount of 0.5% based on broth weight. While maintaining the agitation, the pH of the lysed cell composition was adjusted to 10-11 by adding a 12.5wt% NaOH solution. Simultaneously, SDS powder was added in an amount of 1% by weight of the lysed cell composition and the composition heated to 80-90° C for 1.5 hours. After the hold time, solid NaCl in an amount of 2% by weight of the lysed cell composition was added and the composition heated to 90° C and held for a few hours. The lysed cell composition was centrifuged (Thermo Scientific Sorvell ST40R Centrifuge) at 8000 RPM for 5 minutes to provide a crude oil.

Example 9

[00110] A cell broth (300g) containing microbial cells (S*chizochytrium sp.*) was pasteurized at 60° C for 1 hour. The cell broth was agitated at a speed of 180 RPM and heated to 60° C. The cells were lysed by adding a 50wt% NaOH solution to pH adjust the unwashed broth to 7-7.5 and Alcalase® 2.4 FG (available from, Novozymes (Franklinton, NC)) in an amount of 0.5% based on broth weight. While maintaining the agitation, the pH of the lysed cell composition was adjusted to 10-11 by adding a 12.5wt% NaOH solution. Simultaneously, SDS powder was added in an amount of 0.5% by weight of the lysed cell composition and the composition heated to 80-90° C for 2 hours. After the hold time, solid NaCl in an amount of 2% by weight of the lysed cell composition was added and the composition heated to 90° C and held for 18 hours. The lysed cell composition was centrifuged (Thermo Scientific Sorvell ST40R Centrifuge) at 8000 RPM for 5 minutes to yield 80% crude oil, which yielded 85% DHA (by DHA weight).

Example 10

[00111] A cell broth (300g) containing microbial cells (S*chizochytrium sp.*) was pasteurized at 60° C for 1 hour. The cell broth was agitated at a speed of 180 RPM and heated to 60° C. The cells were lysed by adding a 50wt% NaOH solution to pH adjust the unwashed broth to 7-7.5 and Alcalase® 2.4 FG (available from, Novozymes (Franklinton, NC)) in an amount of 0.5% based on broth weight. While maintaining the agitation, the pH of the lysed cell composition was adjusted to 10-11 by adding a 12.5wt% NaOH solution. Simultaneously, SDS powder was added in an amount of 1% by weight of the lysed cell composition and the composition heated to 90° C. After 2.5 hours at 90° C, the composition was centrifuged (Thermo Scientific Sorvell ST40R Centrifuge) at 8000 RPM for 5 minutes to provide a crude oil, which yielded 92% DHA (by DHA weight).

What is claimed is:

1. A process for obtaining a microbial oil comprising one or more polyunsaturated fatty acids from one or more microbial cells, wherein the process comprises:
(a) lysing the cells comprising the microbial oil to form a lysed cell composition;
(b) demulsifying the lysed cell composition to form a demulsified lysed cell composition;
(c) separating the oil from the demulsified lysed cell composition; and
(d) recovering the oil;
wherein (b) comprises adding an ionic emulsifier after step (a), and wherein (b) further comprises adding a salt selected from the group consisting of alkali metal salts, alkali earth metal salts, sulfate salts, and combinations thereof.

2. The process of claim 1, wherein at least one of (a) or (b) further comprises heating the cells or the composition to at least 70° C.

3. The process of claim 1, wherein at least one of (a) or (b) further comprises heating the cells or the composition to from about 70° C. to about 100° C.

4. The process of claim 1, wherein (b) further comprises adding a base to the lysed cell composition.

5. The process of claim 1, wherein (b) further comprises raising the pH of the lysed cell composition to about 8 or above.

6. The process of claim 1, wherein the salt is added in an amount of from about 0.05% to about 20%, by weight, of the lysed cell composition.

7. The process of claim 1, wherein (b) further comprises agitating the lysed cell composition.

8. The process of claim 1, wherein (a) further comprises agitating the lysed cell composition.

9. The process of claim 1, wherein the cells of (a) are unwashed.

10. The process of claim 1, wherein the cells of (a) are contained in a fermentation broth.

11. The process of claim 1, wherein (c) comprises centrifuging the demulsified lysed cell composition.

12. The process of claim 1, wherein the polyunsaturated fatty acid is selected from an omega-3 fatty acid, an omega-6 fatty acid, and mixtures thereof.

13. The process of claim 1, wherein the polyunsaturated fatty acid is selected from docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), arachidonic acid (ARA), gamma-linolenic acid (GLA), dihomo-gamma-linolenic acid (DGLA), stearidonic acid (SDA), and mixtures thereof.

14. The process of claim 13, wherein the polyunsaturated fatty acid is docosahexaenoic acid (DHA).

15. The process of claim 13, wherein the polyunsaturated fatty acid is arachidonic acid (ARA).

16. The process of claim 1, wherein the microbial cells are algae, yeast, fungi, protest, or bacteria cells.

17. The process of claim 1, wherein the microbial cells are from the genus *Mortierella*, genus *Crypthecodinium*, or order Thraustochytriales.

18. The process of claim 16, wherein the microbial cells are from the order Thraustochytriales.

19. The process of claim 17, wherein the microbial cells are from the genus *Thraustochytrium*, *Schizochytrium*, or mixtures thereof.

20. The process of claim 16, wherein the microbial cells are from *Mortierella Alpina*.

21. The process of claim 1, wherein the lysed cell composition comprises liquid, cell debris, and microbial oil.

22. The process of claim 1 wherein an organic solvent is not used to obtain the oil from the cells.

23. The process of claim 1, wherein the mean particle size of the demulsified lysed cell composition is at least 10 microns.

24. The process of claim 1, wherein the ionic emulsifier is an anionic emulsifier selected from an anionic sulfate emulsifier, an anionic sulfonate emulsifier, an anionic phosphate emulsifier, an anionic carboxylate emulsifier, and combination thereof.

25. The process of claim 24, wherein the anionic emulsifier is an anionic sulfate emulsifier.

26. The process of claim 25, wherein the anionic sulfate emulsifier is selected from ammonium lauryl sulfate, sodium dodecyl sulfate, sodium laureth sulfate, sodium lauryl ether sulfate, sodium myreth sulfate, and combinations thereof.

27. The process of claim 1, wherein the ionic emulsifier is added in an amount of 0.2% to 10% by weight of the lysed cell composition.

28. The process of claim 1, wherein the oil of (d) is a crude oil.

29. The process of claim 28, wherein (d) further comprises refining the crude oil to obtain a refined oil.

30. The process of claim 1, wherein the oil comprises at least 30% by weight arachidonic acid.

31. The process of claim 1, wherein the oil comprises at least 30% by weight docosahexaenoic acid.

32. The process of claim 1, wherein the oil has an anisidine value of less than about 50.

33. The process of claim 1, wherein the oil has a phosphorus content of about 8 ppm or less.

34. The process of claim 1, wherein the oil has a peroxide value of less than about 5 meq/kg.