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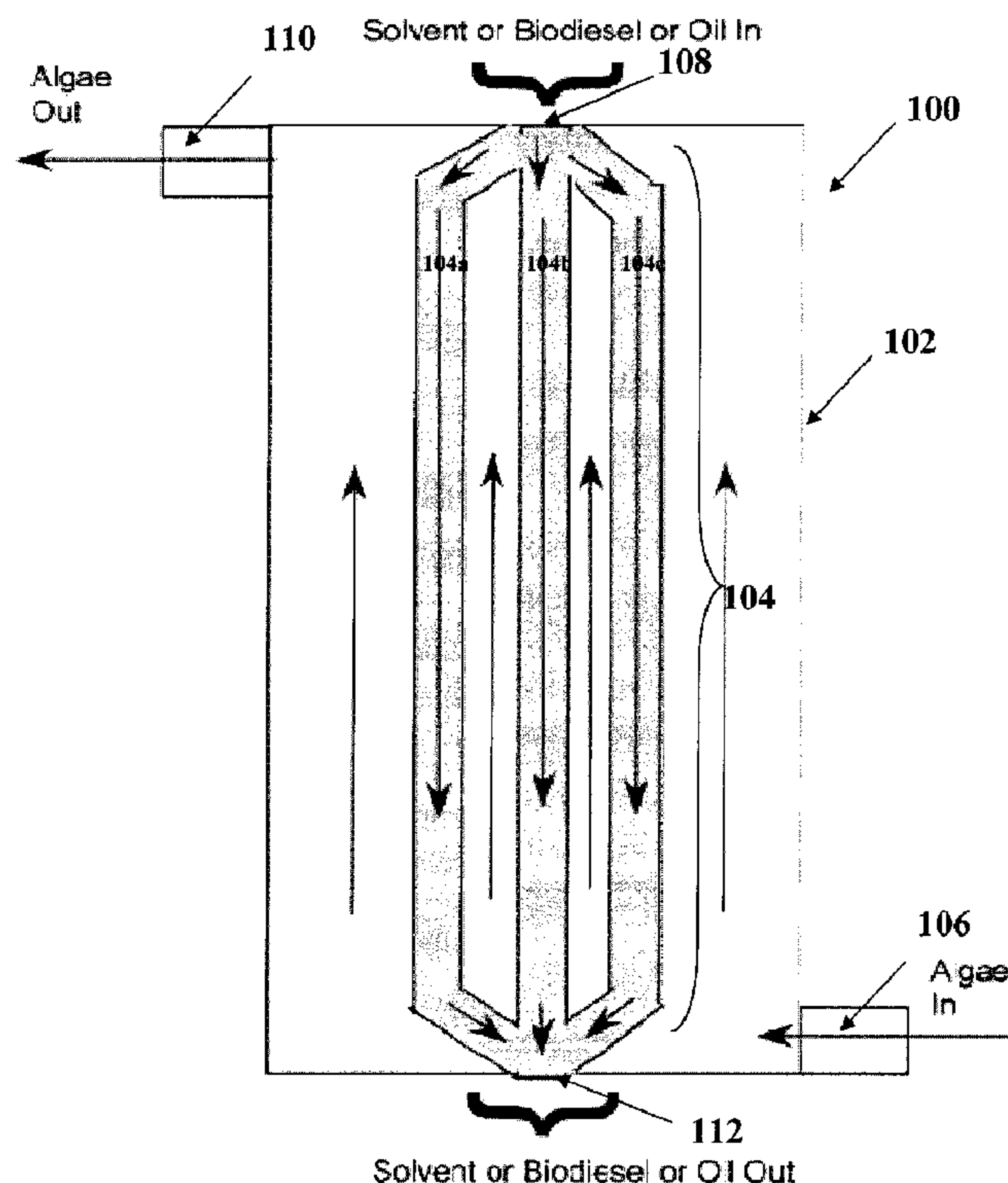
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(54) **Titre : PROCÉDE NON DISPERSIF POUR LA RECUPERATION D'HUILE INSOLUBLE A PARTIR DE BOUILLIES AQUEUSES**

(54) **Title: NON-DISPERSIVE PROCESS FOR INSOLUBLE OIL RECOVERY FROM AQUEOUS SLURRIES**



(57) **Abrégé/Abstract:**

The development and application of a novel non-polar oil recovery process utilizing a non-dispersive solvent extraction method to coalesce and recover oil from a bio-cellular aqueous slurry is described herein. The process could apply to recovery of algal oil from

(57) Abrégé(suite)/Abstract(continued):

a lysed algae slurry, recovery of Omega fatty acids from a bio-cellular aqueous feed, recovery of Beta-carotene from a bio-cellular aqueous feed and for the removal from produced water in oil production and similar type applications. The technique of the present invention utilizes a microporous hollow fiber (MHF) membrane contactor. The novel non-polar oil recovery process described herein can be coupled to a collecting fluid (a non-polar solvent such as heptane, a biodiesel mixture or the previously extracted oil) that is circulated through the hollow fiber membrane. In cases where the biodiesel mixture or the previously extracted oil is used the solvent recovery step (e.g. distillation) can be eliminated.

Abstract

The development and application of a novel non-polar oil recovery process utilizing a non-dispersive solvent extraction method to coalesce and recover oil from a bio-cellular aqueous slurry is described herein. The process could apply to recovery of algal oil from a lysed algae slurry, recovery of Omega fatty acids from a bio-cellular aqueous feed, recovery of Beta-carotene from a bio-cellular aqueous feed and for the removal from produced water in oil production and similar type applications. The technique of the present invention utilizes a microporous hollow fiber (MHF) membrane contactor. The novel non-polar oil recovery process described herein can be coupled to a collecting fluid (a non-polar solvent such as heptane, a biodiesel mixture or the previously extracted oil) that is circulated through the hollow fiber membrane. In cases where the biodiesel mixture or the previously extracted oil is used the solvent recovery step (e.g. distillation) can be eliminated.

NON-DISPERSIVE PROCESS FOR INSOLUBLE OIL RECOVERY FROM AQUEOUS SLURRIES

This application is a divisional application of Canadian application serial number 2,786,709 filed January 13, 2011.

5 **Technical Field of the Invention**

The present invention relates in general to the field of insoluble oil recovery from aqueous slurries, and more particularly, to a microporous membrane based method for recovering oil from a lysed algal concentrate and other aqueous slurries.

Background Art

10 Without limiting the scope of the invention, its background is described in connection with recovery methods for insoluble and low solubility compounds having economic value from aqueous mixtures that may include one or more types of biological cells or cellular debris.

United States Patent No. 4,439,629 issued to Ruegg (1984) describes a process for extracting either or both beta-carotene or glycerine from algae containing these substances, especially from algae of the
15 genera *Dunaliella*. According to the Ruegg patent either or both of beta-carotene or glycerine can be extracted from algae. If it is desired to extract beta-carotene, the algae are first treated with calcium hydroxide and then filtered. The residue from this filtration is treated with a beta-carotene solvent, which removes the beta-carotene from the residue and into the solvent. The beta-carotene can be recovered from the solvent by conventional means. If it is desired to extract glycerine, the filtrate from
20 the treatment of the algae with calcium hydroxide is neutralized, concentrated and the residue from the solid is treated with a lower alkanol to remove glycerine from the residue.

United States Patent No. 5,378,639 issued to Rose et al. (1995) discloses a method for the solvent-extraction of β -carotene from an aqueous algal biomass suspension, whereby a vegetable oil which is immiscible with water is mixed with an aqueous biomass suspension, the biomass containing the β -
25 carotene, to form a mixture of the organic phase and the aqueous suspension, whereby the β -carotene is caused to dissolve in the organic phase. This is followed by separation of the organic phase from the aqueous phase by passing the organic phase containing the dissolved β -carotene through a semi-permeable membrane to effect microfiltration or ultrafiltration of the organic phase. The membrane is of a material which is hydrophobic and the organic phase is passed through the membrane with a
30 pressure drop across the membrane which is lower than that which causes the aqueous phase to pass through the membrane.

Disclosure of the Invention

The present invention describes a method for recovering insoluble oil from aqueous slurries using a hydrophobic microporous hollow fiber membrane followed by circulation of a collection fluid

through the membrane. The collection fluid as described herein comprises an appropriate solvent for the insoluble or low solubility compound to be recovered, for e.g. heptane or a biodiesel mixture or the extracted oil or combinations thereof. The extracted algal oil can be used as the collection fluid for the recovery of additional algal oil, allowing the process to be conducted without a chemical solvent such as heptane. The novel process could be used in a wide variety of commercially significant applications such as: (i) recovery of released or secreted algae oil from an aqueous mixture, (ii) recovery of insoluble hydrocarbon and hydrocarbon-rich molecules from aqueous mixtures, (iii) recovery of Omega fatty acids from an aqueous mixture, (iv) recovery of Beta-carotene from an aqueous mixture, and (v) removal of oil from produced water in petroleum exploration and production.

In one aspect of the invention there is provided a method of separating one or more insoluble oils from a liquid source using one or more membrane contactors comprising the steps of: contacting the liquid source with a first surface of the one or more membrane contactors; coalescing the one or more insoluble oils in the liquid source in the one or more membrane contactors at a second surface of the one or more membrane contactors; and removing the one or more coalesced insoluble oils from the membrane contactor at the second surface.

In conventional liquid-liquid extraction and coalescing processes involving large drops of oil (greater than 1,000 microns), the mixing and separation of the oil and water phases by a dispersive process is routinely practiced with relative ease. However, when the oil drops are significantly smaller in diameter (less than 10 microns) and solids are present, the complete separation of the immiscible liquids is extremely difficult, if not impossible using dispersive methods routinely practiced for larger oil droplets. When routine methods are applied to try to recover small oil droplets from water in the presence of solids (such as cells or cell debris), a solid-liquid-liquid emulsion layer is created resulting in an incomplete and inefficient separation of the two liquids. Therefore a new process is required that will allow for a more efficient separation and elimination of the solid-liquid-liquid-emulsion problem. The process of the present invention enables the recovery of micron and submicron sized insoluble oil drops from an aqueous slurry utilizing a novel non-dispersive process.

A non-dispersive process promotes a one-way flow of specific compounds into and through a membrane to remove the compounds from the shell side feed to the tube side. A non-dispersive separation process is currently used to remove dissolved gases from liquids such as the removal of dissolved oxygen from water to produce ultra pure water for the microelectronics industry. The present invention is a first successful demonstration of the application of non-dispersive processes to recover insoluble oil from water or aqueous slurries. The non-dispersive process disclosed herein uses a microporous hollow fiber membrane composed of hydrophobic fibers. The aqueous slurry containing the insoluble oil is fed on the shell-side of the hollow fiber module and a hydrocarbon-

2a

appropriate solvent, for example, a biodiesel, or similar oil recovered in previous application of the described process is fed on the tube side of the hollow fiber module as a collection fluid. The aqueous phase passes around the outside of the large surface area of hydrophobic fibers containing the hydrophobic collection fluid as it passes through and eventually out of the module. As the aqueous liquid with the insoluble oil drops passes through the module, the insoluble oil droplets coalesce on to the walls of hydrophobic fibers and dissolve into the hydrocarbon-appropriate collection fluid on the tube side of the module and are carried out of the module with the collection fluid. In this process, the tube side collection fluid does not make prolonged contact with the aqueous phase or disperse into the

aqueous phase. The absence of this mixing as hypothesized by the inventors prevents the formation of a solid-liquid-liquid emulsion, when solids were present, allowing insoluble oil to be recovered efficiently from an aqueous slurry containing solids. The above hypothesis was successfully demonstrated herein to efficiently recover insoluble oil from an aqueous mixture including cells
5 without the formation of a solid-liquid-liquid emulsion.

In typical membrane filtration processes, small amounts of solids quickly build up on the surface of the membrane (commonly called membrane fouling) reducing the efficiency and cost effectiveness of the filtration process. In the process discovered and disclosed herein using the microporous hollow fiber membrane module, the inventors hypothesized that membrane fouling is not a concern within
10 specific operating parameters. The inventors show that if the module was operated using hydrophilic cells that were small enough to pass through the dimensions of the module, and an appropriate pressure differential was maintained between the aqueous fluid and collection fluid, that the hydrophilic cells would flow through the module and be repelled from the surface of the membrane because the membrane is coated with a hydrophobic collection fluid. The results presented herein at
15 the prescribed operating conditions do not indicate any evidence of membrane fouling.

The novel extraction process of the present invention utilizes a non-dispersive solvent extraction method to coalesce and recover an insoluble oil from an aqueous slurry. As an example, the recovery of non-polar algal oil from an algal concentrate is described. The technique utilizes a microporous hollow fiber membrane contactor. The inventors have tested the Liqui-Cel Extra Flow Contactor,
20 commercially used for gas/liquid contacting, to obtain >80% extraction efficiency and process concentrates up to 10% bio-cellular solids without membrane fouling. The novel technique of the present invention utilizes the large coalescing area provided by the surface of the microporous hollow fibers when filled with a hydrophobic collection fluid and minimizes the actual contact of the solvent with the (e.g. algae) biomass and aqueous phase.

25 The novel extraction process described herein can be coupled with a variety of appropriate collection fluids for recovery of insoluble compounds, depending upon the types of compound or compounds to be recovered. The choice of collection fluid will impact both the sub-set of compounds recovered from the aqueous slurry as well as the downstream steps needed to economically and efficiently use compounds from the collection fluid. Differential extraction of desired molecules, for example,
30 recovery of non-polar oils, but not polar oils, can be achieved by choice of collection fluid. Segregation of non-polar oils from polar oils, specifically polar oils containing phosphorous (e.g., phospholipids), is highly advantageous as phosphorus containing compounds complicate both the refining and transesterification processes used to create transportation fuels. Polar oils could be recovered using the process described herein using a different collection fluid, for example as a
35 secondary recovery step once non-polar oils are already removed.

Downstream steps needed to recover desired molecules from the collection fluid are also application specific. If heptane is used as the collection fluid, compounds of interest may be recovered by distillation without the need of a steam stripper. If biodiesel (Fatty Acid Methyl Ester [FAME]) is used as the collection fluid, e.g., recovered oils may not require processing prior to transesterification to FAME. Importantly, the present invention can also use a “self” oil that has been previously extracted from an aqueous slurry as the collection fluid thereby completely eliminating the need and expense of having to separate the recovered compounds from the collection fluid. In this application, the collection fluid is a quantity of oil derived from a previously processed aqueous slurry or extracted by a different method. The microporous hollow fiber membrane contactor as described in the present invention is small, portable, economical, and is capable of handling large aqueous slurry feed rates.

In one embodiment the present invention discloses a method of extracting one or more insoluble oils comprising algal lipid components, algal oils or both from an aqueous (lysed algal slurry) preparation using one or more hydrophobic membranes or membrane modules. The method of the present invention comprises the following steps: (i) feeding an aqueous slurry comprising the insoluble oil by pumping in a contactor or a vessel, (ii) pumping one or more collection fluids through the one or more membranes or membrane modules. The one or more collection fluids counterflows with the aqueous slurry in the contactor or the vessel and comprise one or more solvents, a biodiesel, a non-polar oil extracted from process (e.g. algal oil), or mixtures and combinations thereof, (iii) contacting the preparation in the contactor or the vessel with one or more collection fluids pumped through the one or more membranes or membrane modules, (iv) removing a first stream from the contactor or the vessel, wherein the first stream comprises the algal biomass, and (v) removing a second stream from the contactor or the vessel, wherein the second stream comprises the one or more collection fluids, one or more extracted (algal lipids), one or more algal oils or both.

In another embodiment, the present invention describes a method of extracting one or more hydrocarbons or hydrocarbon-rich molecules (e.g., farnesene, squalane, aldehydes, triglycerides, diglycerides, etc.) or combinations thereof, from an aqueous preparation using one or more hydrophobic membranes or membrane modules. Without limiting the scope of the invention, an example includes recovery of hydrocarbon and hydrocarbon-rich molecules produced by microbial fermentation. Microbial fermentation processes are described in which organisms including algae, yeast, *E. coli*, fungi, etc. are used to metabolize carbon sources (e.g., sugars, sugarcane bagasse, glycerol, etc.) into hydrocarbons and hydrocarbon-rich molecules that are secreted from (or accumulate within) the cells. Such organisms are expected, by design, to produce physically small oil droplets; the inventors hypothesized that these droplets will not readily resolve from water by gravity alone and that the process described herein will be immediately applicable to recover insoluble oils produced by microbial platforms. The companies commercializing microbial fermentation to oil technologies have implied that the recovery of the oil product is trivial, but emerging company disclosures and scientific data suggest recovering the oil from the aqueous growth media is a mission-

critical problem. Technologies currently in use, for e.g. centrifugal force sufficient to pellet *E. coli* cells are not sufficient to break the oil/water emulsion that is created in the aqueous growth media by the hydrocarbon-producing *E. coli*.

In addition to the steps listed herein above the method of the present invention further involves the steps of collecting the one or more extracted algal lipid components, algal oils or both in a collection vessel, recycling the separated solvent by pumping through the one or more membranes or membrane modules to process a subsequent batch of lysed algae, converting the one or more extracted algal lipid components, algal oils or both in the collection vessel to Fatty Acid Methyl Esters (FAMES) or a biodiesel by transesterification or alternatively, refinery-based processing such as hydrocracking or pyrolysis, and processing the first stream comprising the algal biomass by drying the algal biomass to be optionally used as animal feed, feedstock for chemical production, or for energy generation. In the event one or more solvents are used as the collection fluids, the method includes an optional step for separating the one or more extracted algal lipid components, algal oils or both from the one or more solvents. The lysed algal preparation used in the method of the present invention comprises a concentrate, a slurry, a suspension, a dispersion, an emulsion, a solution or any combinations thereof. In one aspect the hydrophobic membrane or membrane module comprises microporous hollow fiber membranes, selected from polyethylene, polypropylene, polyolefins, polyvinyl chloride (PVC), amorphous Polyethylene terephthalate (PET), polyolefin copolymers, poly(etheretherketone) type polymers, surface modified polymers, mixtures or combinations thereof. The surface modified polymers comprise polymers modified chemically at one or more halogen groups or by corona discharge or by ion embedding techniques. In another aspect of the method of the present invention the algae are selected from the group consisting of the diatoms (bacillariophytes), green algae (chlorophytes), blue-green algae (cyanophytes), golden-brown algae (chrysophytes), haptophytes, Amphipleura, Amphora, Chaetoceros, Cyclotella, Cymbella, Fragilaria, Hantzschia, Navicula, Nitzschia, Phaeodactylum, Thalassiosira Ankistrodesmus, Botryococcus, Chlorella, Chlorococcum, Dunaliella, Monoraphidium, Oocystis, Scenedesmus, Nanochloropsis, Tetraselmis, Chlorella, Dunaliella, Oscillatoria, Synechococcus, Boekelovia, Isochysis and Pleurochysis. In yet another aspect of the method of the present invention the one or more counterflowing solvents comprise non-polar solvents, alkanes such as hexane, aromatic solvents such as benzene, toluene, ethers such as diethyl ether, halogenated solvents such as chloroform, dichloromethane, and esters such as ethyl acetate. In one aspect the counterflowing non-polar oil comprises algal oils, components of biodiesels selected from monoglycerides, diglycerides, triglycerides, and fatty acid methyl esters.

The present invention also provides for a method of extracting one or more algal lipid components, algal oils or both from a lysed algal preparation using one or more hydrophobic membranes or membrane modules. In the first step the lysed algal preparation is fed to a contactor or a vessel by pumping while at the same time, pumping a solvent, biodiesel, an algal oil, a non-polar oil or mixtures thereof through the one or more membranes or membrane modules. The solvent, biodiesel, the algal

oil, the non-polar oil or the mixture is pumped through the membrane such that it counterflows with the lysed algal preparation. Non-limiting examples of the non-polar oil used in the present invention includes non-polar algal oils, palm, canola, corn, etc. The one or more algal lipid components, algal oils or both coalesce on the surface of the membrane or the membrane module. The coalesced algal lipid components and the algal oils are removed from the surface of the membrane or the membrane module by contacting with the counterflowing solvent, biodiesel, the algal oil, the non-polar oil or the mixture. A first stream comprising an algal biomass is removed from the contactor or the vessel, followed by removal of a second stream comprising the counterflowing solvent, biodiesel, the algal oil, the non-polar oil or the mixture, one or more extracted algal lipid components, one or more algal oils or both. The method of extracting the algal oils or lipids without using a solvent further comprises the steps of: (i) collecting the one or more extracted algal lipid components, algal oils or both in a collection vessel, (ii) recycling the counterflowing oil by pumping a part or a whole of the contents of the collection vessel through the one or more membranes or membrane modules to process a subsequent batch of lysed algae, (iii) converting the one or more extracted algal lipid components, algal oils or both in the collection vessel to Fatty Acid Methyl Esters (FAMES) or a biodiesel by transesterification, or delivery of oil to a refinery for processing by hydrocracking or pyrolysis, and (iv) processing the first stream comprising the algal biomass by drying the algal biomass to be optionally used as animal feed, biochemical feedstock, or for energy generation. The method further comprises the optional step of adding one or more natural fatty acids or salts thereof to the lysed algal preparation to aid in lipid transfer to the collection stream.

In one aspect of the method of the present invention the one or more natural fatty acids are designated as [X]:[Y], wherein X represents the number of carbon atoms in the one or more fatty acids ranging from 8-22 and Y represents one or more double bonds in the fatty acids ranging from 0-6. In another aspect the one or more natural fatty acids or salts thereof comprise Myristoleic acid, Palmitoleic acid, Sapienic acid, Oleic acid, Linoleic acid, α -Linolenic acid, Arachidonic acid, Eicosapentaenoic acid, Erucic acid, Docosahexaenoic acid, Lauric acid, Myristic acid, Palmitic acid, Stearic acid, Arachidic acid, and combinations thereof. In yet another aspect the lysed algal preparation comprises a concentrate, a slurry, a suspension, a dispersion, an emulsion, a solution or any combinations thereof.

The counterflowing non-polar oil used in the present invention comprises algal oils, various components of biodiesels selected from monoglycerides, diglycerides, triglycerides, and fatty acid methyl esters. In a related aspect the hydrophobic membrane or membrane module comprises microporous hollow fiber membranes, selected from polyethylene, polypropylene, polyolefins, polyvinyl chloride (PVC), amorphous Polyethylene terephthalate (PET), polyolefin copolymers, poly(etheretherketone) type polymers, surface modified polymers, mixtures or combinations thereof. The surface modified polymers comprise polymers modified chemically at one or more halogen groups or by corona discharge or ion embedding techniques. In one aspect the algae are selected from the group consisting of the diatoms (bacillariophytes), green algae (chlorophytes), blue-green algae

(cyanophytes), golden-brown algae (chrysophytes), haptophytes, Amphipleura, Amphora, Chaetoceros, Cyclotella, Cymbella, Fragilaria, Hantzschia, Navicula, Nitzschia, Phaeodactylum, Thalassiosira, Ankistrodesmus, Botryococcus, Chlorella, Chlorococcum, Dunaliella, Monoraphidium, Oocystis, Scenedesmus, Nanochloropsis, Tetraselmis, Chlorella, Dunaliella, Oscillatoria,
 5 Synechococcus, Boekelovia, Isochysis, and Pleurochysis.

In another embodiment the instant invention describes a contactor or vessel for extracting one or more insoluble oil components from the bio-cellular aqueous slurry such as but not limited to algal oils or both from a lysed algal concentrate. The contactor or vessel as described herein comprises, an external metallic, polypropylene or other polymeric casing, one or more microporous hollow fiber membrane
 10 cartridges comprising a plurality of microporous hollow fiber membranes enclosed by the metal casing, wherein the one or more membrane cartridges divide the casing into a shell-side and a fiber side, one or more baffles on the shell-side of the metal casing, one or more distribution tubes on the fiber-side of the metal casing, two inlet ports connected to the external metal casing, wherein the lysed algal concentrate is pumped to the shell-side through the first inlet port and a strip gas or a
 15 solvent is fed to the fiber side through the second inlet port, and two outlet ports connected to the metal casing, wherein the an algal raffinate comprising the algal biomass is removed from the first outlet port and a solvent/extracted lipid or oil mixture or the strip gas is removed from the second outlet port.

In one aspect the microporous hollow fiber membrane comprises polyethylene, polypropylene,
 20 polyolefins, polyvinyl chloride (PVC), amorphous Polyethylene terephthalate (PET), polyolefin copolymers, poly(etheretherketone) type polymers, surface modified polymers, mixtures or combinations thereof. The surface modified polymers comprise polymers modified chemically at one or more halogen groups or by corona discharge or ion embedding techniques. In another aspect the algae used for the extraction of the algal oil or lipids are selected from the group consisting of the
 25 diatoms (bacillariophytes), green algae (chlorophytes), blue-green algae (cyanophytes), golden-brown algae (chrysophytes), haptophytes, Amphipleura, Amphora, Chaetoceros, Cyclotella, Cymbella, Fragilaria, Hantzschia, Navicula, Nitzschia, Phaeodactylum, Thalassiosira, Ankistrodesmus, Botryococcus, Chlorella, Chlorococcum, Dunaliella, Monoraphidium, Oocystis, Scenedesmus, Nanochloropsis, Tetraselmis, Chlorella, Dunaliella, Oscillatoria, Synechococcus, Boekelovia,
 30 Isochysis, and Pleurochysis.

In yet another embodiment the present invention discloses a method of extracting one or more algal oils from a lysed algal concentrate in a contactor using one or more hydrophobic microporous hollow fiber membrane modules comprising a plurality of microporous hollow fiber membranes comprising the steps of: (i) pumping the lysed algal concentrate through a first inlet port of the contactor to a
 35 shell-side of the contactor, (ii) pumping one or more collection fluids through a second inlet port of the contactor to the one or more hollow fiber membranes on a fiber side of the contactor; wherein the

one or more collection fluids counterflows with the lysed algal preparation on the shell-side of the contactor. The one or more collection fluids comprise one or more solvents, a biodiesel, an algal oil, a non-polar oil or mixtures thereof, (iii) contacting the lysed algal concentrate on the shell-side with the one or more non-polar solvents on the fiber side, (iv) removing a first stream from a first outlet port in the contactor, wherein the first stream comprises an algal biomass, and (v) removing a second stream from a second outlet port in the contactor, wherein the second stream comprises the collection fluid and the one or more extracted algal oils. The extraction method described in the embodiment of the present invention further comprises the steps of: (i) collecting the one or more extracted algal oils in a collection vessel, (ii) recycling the separated solvent by pumping through the one or more microporous hollow fiber membranes to process a subsequent batch of lysed algae, (iii) converting the one or more extracted algal oils in the collection vessel to Fatty Acid Methyl Esters (FAMES) or a biodiesel by transesterification or conversion to fuels by refinery-based methods such as hydrocracking and pyrolysis, and (iv) processing the first stream comprising the algal biomass by drying the algal biomass to be optionally used as animal feed or for energy generation. In one aspect the extraction method as described herein comprises the optional step of separating the one or more extracted algal oils from the one or more solvents.

In another aspect the counterflowing solvents comprise non-polar solvents, alkanes such as hexane, and aromatic solvents such as benzene, toluene, and ethers such as diethyl ether, halogenated solvents such as chloroform, dichloromethane, and esters such as ethyl acetate. In yet another aspect 45-80% of the one or more algal oils in the lysed algal concentrate are extracted by the method of the present invention. As per the method described in the present invention 45%, 55%, 60%, 65%, 70%, 75%, and 80% of the one or more algal oils in the lysed algal concentrate are extracted.

The present invention further describes a method of extracting one or more algal oils from a lysed algal concentrate in a contactor using one or more hydrophobic microporous hollow fiber membrane modules comprising a plurality of microporous hollow fiber membranes. The first step of the method involves pumping a lysed algal concentrate through a first inlet port of the contactor to a shell-side of the contactor followed by pumping a solvent, biodiesel, an algal oil, a non-polar oil or mixtures thereof through a second inlet port of the contactor through the one or more membranes or membrane modules on a fiber side of the contactor. The biodiesel, the algal oil, the non-polar oil or the mixture is pumped through the membrane such that it counterflows with the lysed algal preparation on the shell-side of the contactor. The algal oils coalesce on the microporous hollow fiber membrane and are removed from the surface of the membrane by contacting with the counterflowing solvent, biodiesel, the algal oil, the non-polar oil or the mixture. A first stream comprising an algal biomass is removed from a first outlet port in the contactor followed by the removal of a second stream from a second outlet port in the contactor. The second stream comprises the counterflowing biodiesel, the algal oil, the non-polar oil or the mixture and the one or more extracted algal oils.

The algal oil extraction method as described in an embodiment of the present invention further comprises the steps of: collecting the one or more extracted algal oils in a collection vessel, recycling the counterflowing oil by pumping a part or a whole of the contents of the collection vessel through the one or more microporous hollow fiber membranes to process a subsequent batch of lysed algae, 5 converting the one or more extracted algal oils in the collection vessel to Fatty Acid Methyl Esters (FAMES) or a biodiesel by transesterification or conversion to fuels by refinery-based methods such as hydrocracking and pyrolysis, and processing the first stream comprising the algal biomass by drying the algal biomass to be optionally used as animal feed, biochemical feedstock, or for energy generation. In one aspect the method comprises the optional step of adding one or more natural fatty 10 acids or salts thereof, hydrocarbon and hydrocarbon rich molecules, including aldehydes (flavors and fragrances), terpenes (chemical feedstocks), etc. to the lysed algal preparation. In another aspect the one or more natural fatty acids are designated as [X]:[Y], wherein X represents the number of carbon atoms in the one or more fatty acids ranging from 8-22 and Y represents one or more double bonds in the fatty acids ranging from 0-6. The one or more natural fatty acids (saturated or unsaturated) or salts 15 thereof comprise Myristoleic acid, Palmitoleic acid, Sapienic acid, Oleic acid, Linoleic acid, α -Linolenic acid, Arachidonic acid, Eicosapentaenoic acid, Erucic acid, Docosahexaenoic acid, Lauric acid, Myristic acid, Palmitic acid, Stearic acid, Arachidic acid, and combinations thereof. In another aspect the counterflowing oil comprises non-polar oils, components of biodiesels selected from monoglycerides, diglycerides, triglycerides, and fatty acid methyl esters. In yet another aspect the 20 hydrophobic hollow fiber membrane comprises polyethylene, polypropylene, polyolefins, polyvinyl chloride (PVC), amorphous Polyethylene terephthalate (PET), polyolefin copolymers, poly(etheretherketone) type polymers, surface modified polymers, mixtures or combinations thereof, wherein the polymers are modified chemically at one or more halogen groups or by corona discharge or ion embedding techniques.

25 Another embodiment of the present invention discloses a method of extracting one or more insoluble oils from a liquid source using one or more hydrophobic membranes or membrane modules comprising the steps of: (i) feeding the liquid source comprising the one or more insoluble oils by pumping in a contactor or a vessel, (ii) pumping one or more collection fluids through the one or more membranes or membrane modules, wherein the one or more collection fluids counterflows with the 30 liquid source in the contactor or the vessel, wherein the one or more collection fluids comprise one or more solvents, a biodiesel, an algal oil, a non-polar oil or mixtures and combinations thereof, (iii) contacting the one or more insoluble oils in the liquid source in the contactor or the vessel with one or more collection fluids pumped through the one or more membranes or membrane modules, (iv) removing a first stream from the contactor or the vessel, wherein the first stream comprises the liquid 35 source without the one or more insoluble oils, and (v) removing a second stream from the contactor or the vessel, wherein the second stream comprises the one or more collection fluids and the one or more extracted insoluble oils.

The extraction method as described above further comprises the steps of: collecting the one or more extracted insoluble oils in a collection vessel, recycling the separated solvent by pumping through the one or more membranes or membrane modules to process a subsequent batch of the liquid slurry, and converting the one or more extracted insoluble oils comprising algal lipid components, algal oils or both in the collection vessel to Fatty Acid Methyl Esters (FAMES) or a biodiesel by transesterification or a refinery-based process such as hydrocracking or pyrolysis. The liquid source used in the method of the present invention is selected from the group consisting of industrial water, brine, wastewater, industrial or natural effluents, water-oil mixtures, aqueous slurries, aqueous slurries comprising broken cells, live cells or combinations thereof, bio-cellular mixtures, lysed cellular preparations, and combinations thereof. In one aspect of the method discloses hereinabove the biocellular mixture comprises algae, protists, fungi, yeast, *E. coli*, mixed cultures of cells, and combinations thereof. In another aspect the method extracts 45-100% of the one or more insoluble oils in the liquid source. In yet another aspect 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% and 100% of the one or more insoluble oils in the liquid source are extracted.

15 Description of the Drawings

For a more complete understanding of the features and advantages of the present invention, reference is now made to the detailed description of the invention along with the accompanying figures and in which:

FIG. 1 is a schematic showing the method and the algal oil recovery principle as described in the
20 embodiments of the present invention;

FIG. 2 is a schematic of a general algal oil production process;

FIGS. 3A and 3B shows photographs of an alga cell prior to (3A) and after lysing (3B);

FIGS. 3C and 3D shows photographs of algal cells prior to (3C) and after lysing (3D);

FIG. 4 is a flow diagram of a general algae oil extraction process;

25 FIG. 5 is a flow diagram of the novel algal oil extraction process (with solvent) of the present invention;

FIG. 6 is a flow diagram of the novel algal oil extraction process (without solvent) of the present invention;

FIG. 7 is a schematic of the Liqui-Cel extra flow microporous hollow fiber membrane contactor;

30 FIG. 8 is a HPLC trace (chromatogram) of oil obtained using hollow fiber membrane extraction of a lysed suspension of *Nanochloropsis*. Two main peaks are seen in this sample, the first is a mixture of various long chain hydrocarbons and the second is a triglyceride; and

FIG. 9 shows an alternative process where a solid-liquid-liquid emulsion potentially derived from a dispersive extraction is fed to the shell-side of the microporous hollow fiber membrane for the purpose of separating the two liquids.

Description of the Invention

- 5 While the making and using of various embodiments of the present invention are discussed in detail below, it should be appreciated that the present invention provides many applicable inventive concepts that can be embodied in a wide variety of specific contexts. The specific embodiments discussed herein are merely illustrative of specific ways to make and use the invention and do not delimit the scope of the invention.
- 10 To facilitate the understanding of this invention, a number of terms are defined below. Terms defined herein have meanings as commonly understood by a person of ordinary skill in the areas relevant to the present invention. Terms such as “a”, “an” and “the” are not intended to refer to only a singular entity, but include the general class of which a specific example may be used for illustration. The terminology herein is used to describe specific embodiments of the invention, but their usage does not
- 15 delimit the invention, except as outlined in the claims.

As used herein the term “aqueous slurry” encompasses water based liquids containing any of the following in any combination; insoluble oils (hydrocarbons and hydrocarbon-rich molecules of commercial value), living, dead, damaged and/or broken cells (or not), proteins and other cellular debris, including sugars, DNA, RNA, etc. The slurry may also contain a solvent that was used to pre-

20 treat cells to liberate compounds of interest.

The term “oil” as used herein refers to a single hydrocarbon or hydrocarbon-rich molecule including a complex mixture of lipids, hydrocarbons, free fatty acids, triglycerides, aldehydes, etc. The compounds included herein may be C₈ (jet fuel compatible) and others may be C₆₀ (motor oil compatible). Some compounds are pure hydrocarbons, some have oxygen, and some will have

25 phosphorus.

The present invention describes a method for recovering algae oil from lysed algae concentrate using hydrophobic microporous hollow fiber membrane followed by recovery of the algal oil using a collection fluid which can be a solvent, a biodiesel, an algal oil or mixtures thereof. The technique of the present invention does not require intimate contacting of the lysed algae concentrate and solvent.

30 The use of a hydrophobic microporous hollow fiber membrane provides a non-dispersive method of coalescing and recovering the algal oil. The lysed algae concentrate is fed on the shell side while algal oil or the biodiesel mixture is fed on the fiber side. The algal oil acts to sweep and the remove the coalesced oil within the tube surface of the hollow fibers. A natural fatty acid maybe added to the algae concentrate to minimize fouling on the fiber outer surface and increase oil coalescence. A

35 simple schematic representation of the method of the present invention is depicted in **FIG. 1**.

FIG. 1 shows an algal oil recovery unit **100**. The unit **100** comprises a housing **102**, within which is contained a membrane module **104** comprising a plurality of microporous hollow fiber membrane units depicted as **104a**, **104b**, and **104c**. The unit has two inlet ports **106** and **108**. The lysed algal preparation is fed (pumped) through port **106**. A collection fluid is pumped through inlet port **108**.

- 5 The collection fluid can be a solvent, a biodiesel, an algal oil or mixtures thereof. The algal preparation counterflows with the collection fluid flowing inside the microporous hollow fiber membranes **104a**, **104b**, and **104c**. The algal oils or lipid coalesce on the surface of the hollow fiber membranes and are swept by and recovered by the collection fluid and exit the unit **100** through the outlet port **110**. The exit stream is taken for further processing (e.g. solvent recovery) if necessary.
- 10 The collection fluid flows out of the unit **100** through port **112**.

The method of the present invention using a biodiesel mixture as the collection fluid eliminates the need of a distillation system or a stripper to recover the solvent thereby reducing the capital and operating cost of the overall oil recovery process.

- A wide variety of organisms can be used to generate oils and lipids that can be extracted with the present invention. Non-limiting examples of algae and microalgae may be grown and used with the present invention including one or more members of the following divisions: *Chlorophyta*, *Cyanophyta* (Cyanobacteria), and *Heterokontophyt*. Non-limiting examples of classes of microalgae that may be used with the present invention include: *Bacillariophyceae*, *Eustigmatophyceae*, and *Chrysophyceae*. Non-limiting examples of genera of microalgae used with the methods of the invention include: *Nannochloropsis*, *Chlorella*, *Dunaliella*, *Scenedesmus*, *Selenastrum*, *Oscillatoria*, *Phormidium*, *Spirulina*, *Amphora*, and *Ochromonas*. Non-limiting examples of microalgae species that can be used with the present invention include; *Achnanthes orientalis*, *Agmenellum spp.*, *Amphiprora hyaline*, *Amphora coffeiformis*, *Amphora coffeiformis var. linea*, *Amphora coffeiformis var. punctata*, *Amphora coffeiformis var. taylori*, *Amphora coffeiformis var. tenuis*, *Amphora delicatissima*, *Amphora delicatissima var. capitata*, *Amphora sp.*, *Anabaena*, *Ankistrodesmus*, *Ankistrodesmus falcatus*, *Boekelovia hooglandii*, *Borodinella sp.*, *Botryococcus braunii*, *Botryococcus sudeticus*, *Bracteococcus minor*, *Bracteococcus medionucleatus*, *Carteria*, *Chaetoceros gracilis*, *Chaetoceros muelleri*, *Chaetoceros muelleri var. subsalsum*, *Chaetoceros sp.*, *Chlamydomas perigranulata*, *Chlorella anitrata*, *Chlorella antarctica*, *Chlorella aureoviridis*, *Chlorella candida*, *Chlorella capsulate*, *Chlorella desiccata*, *Chlorella ellipsoidea*, *Chlorella emersonii*, *Chlorella fusca*, *Chlorella fusca var. vacuolata*, *Chlorella glucotropha*, *Chlorella infusionum*, *Chlorella infusionum var. actophila*, *Chlorella infusionum var. auxenophila*, *Chlorella kessleri*, *Chlorella lobophora*, *Chlorella luteoviridis*, *Chlorella luteoviridis var. aureoviridis*, *Chlorella luteoviridis var. lutescens*, *Chlorella miniata*, *Chlorella minutissima*, *Chlorella mutabilis*, *Chlorella nocturna*, *Chlorella ovalis*, *Chlorella parva*, *Chlorella photophila*, *Chlorella pringsheimii*, *Chlorella protothecoides*, *Chlorella protothecoides var. acidicola*, *Chlorella regularis*, *Chlorella regularis var. minima*, *Chlorella regularis var. umbricata*, *Chlorella reisigii*, *Chlorella saccharophila*, *Chlorella saccharophila var.*

ellipsoidea, *Chlorella salina*, *Chlorella simplex*, *Chlorella sorokiniana*, *Chlorella* sp., *Chlorella sphaerica*, *Chlorella stigmatophora*, *Chlorella vanniellii*, *Chlorella vulgaris*, *Chlorella vulgaris* fo. *tertia*, *Chlorella vulgaris* var. *autotrophica*, *Chlorella vulgaris* var. *viridis*, *Chlorella vulgaris* var. *vulgaris*, *Chlorella vulgaris* var. *vulgaris* fo. *tertia*, *Chlorella vulgaris* var. *vulgaris* fo. *viridis*,
5 *Chlorella xanthella*, *Chlorella zofingiensis*, *Chlorella trebouxioides*, *Chlorella vulgaris*, *Chlorococcum infusionum*, *Chlorococcum* sp., *Chlorogonium*, *Chroomonas* sp., *Chrysosphaera* sp., *Cricosphaera* sp., *Crypthecodinium cohnii*, *Cryptomonas* sp., *Cyclotella cryptica*, *Cyclotella meneghiniana*, *Cyclotella* sp., *Dunaliella* sp., *Dunaliella bardawil*, *Dunaliella bioculata*, *Dunaliella granulate*, *Dunaliella maritime*, *Dunaliella minuta*, *Dunaliella parva*, *Dunaliella peircei*, *Dunaliella*
10 *primolecta*, *Dunaliella salina*, *Dunaliella terricola*, *Dunaliella tertiolecta*, *Dunaliella viridis*, *Dunaliella tertiolecta*, *Eremosphaera viridis*, *Eremosphaera* sp., *Effipsoidon* sp., *Euglena* spp., *Franceia* sp., *Fragilaria crotonensis*, *Fragilaria* sp., *Gleocapsa* sp., *Gloeothamnion* sp., *Haematococcus pluvialis*, *Hymenomonas* sp., *Isochrysis* aff. *galbana*, *Isochrysis galbana*, *Lepocinclis*, *Micractinium*, *Micractinium*, *Monoraphidium minutum*, *Monoraphidium* sp., *Nannochloris* sp.,
15 *Nannochloropsis salina*, *Nannochloropsis* sp., *Navicula acceptata*, *Navicula biskanterae*, *Navicula pseudotenelloides*, *Navicula pelliculosa*, *Navicula saprophila*, *Navicula* sp., *Nephrochloris* sp., *Nephroselmis* sp., *Nitzschia communis*, *Nitzschia alexandrina*, *Nitzschia closterium*, *Nitzschia communis*, *Nitzschia dissipata*, *Nitzschia frustulum*, *Nitzschia hantzschiana*, *Nitzschia inconspicua*, *Nitzschia intermedia*, *Nitzschia microcephala*, *Nitzschia pusilla*, *Nitzschia pusilla elliptica*, *Nitzschia*
20 *pusilla monoensis*, *Nitzschia quadrangular*, *Nitzschia* sp., *Ochromonas* sp., *Oocystis parva*, *Oocystis pusilla*, *Oocystis* sp., *Oscillatoria limnetica*, *Oscillatoria* sp., *Oscillatoria subbrevis*, *Parachlorella kessleri*, *Pascheria acidophila*, *Pavlova* sp., *Phaeodactylum tricomutum*, *Phagus*, *Phormidium*, *Platymonas* sp., *Pleurochrysis carterae*, *Pleurochrysis dentate*, *Pleurochrysis* sp., *Prototheca wickerhamii*, *Prototheca stagnora*, *Prototheca portoricensis*, *Prototheca moriformis*, *Prototheca*
25 *zopfii*, *Pseudochlorella aquatica*, *Pyramimonas* sp., *Pyrobotrys*, *Rhodococcus opacus*, *Sarcinoid chrysophyte*, *Scenedesmus armatus*, *Schizochytrium*, *Spirogyra*, *Spirulina platensis*, *Stichococcus* sp., *Synechococcus* sp., *Synechocystis*, *Tagetes erecta*, *Tagetes patula*, *Tetraedron*, *Tetraselmis* sp., *Tetraselmis suecica*, *Thalassiosira weissflogii*, and *Viridiella fridericana*.

Other sources for biomass can be a wild type or genetically modified fungus. Non-limiting examples
30 of fungi that may be used with the present invention include: *Mortierella*, *Mortierella vinacea*, *Mortierella alpine*, *Pythium debaryanum*, *Mucor circinelloides*, *Aspergillus ochraceus*, *Aspergillus terreus*, *Penicillium lilacinum*, *Hansenula*, *Chaetomium*, *Cladosporium*, *Malbranchea*, *Rhizopus*, and *Pythium*. As the source of biomass is not limited using the devices and methods of the present invention can be wild type or genetically modified yeast. Non-limiting examples of yeast that can be
35 used with the present invention include *Cryptococcus curvatus*, *Cryptococcus terricolus*, *Lipomyces starkeyi*, *Lipomyces lipofer*, *Endomycopsis vernalis*, *Rhodotorula glutinis*, *Rhodotorula gracilis*, *Candida* 107, *Saccharomyces paradoxus*, *Saccharomyces mikatae*, *Saccharomyces bayanus*,

Saccharomyces cerevisiae, any *Cryptococcus*, *C. neoformans*, *C. bogoriensis*, *Yarrowia lipolytica*, *Apiotrichum curvatum*, *T. bombicola*, *T. apicola*, *T. petrophilum*, *C. tropicalis*, *C. lipolytica*, and *Candida sp.*, e.g., *Candida albicans*.

The biomass can even be any bacteria that generate lipids, oils, proteins, and carbohydrates, whether naturally or by genetic engineering. Non-limiting examples of bacteria that can be used with the present invention include *Escherichia coli*, *Acinetobacter sp.* any actinomycete, *Mycobacterium tuberculosis*, any streptomycete, *Acinetobacter calcoaceticus*, *P. aeruginosa*, *Pseudomonas sp.*, *R. erythropolis*, *N. erythropolis*, *Mycobacterium sp.*, *B.*, *U. zeae*, *U. maydis*, *B. lichenformis*, *S. marcescens*, *P. fluorescens*, *B. subtilis*, *B. brevis*, *B. polymyxa*, *C. lepus*, *N. erythropolis*, *T. thiooxidans*, *D. polymorphis*, *P. aeruginosa* and *Rhodococcus opacus*.

While algae make oil there is no simple and economical method for extracting the oil directly from an aqueous slurry. Drying algae is usually needed for solvent extraction and the biomass is exposed to toxic solvents. Other methods such as supercritical extraction are uneconomical for commodity products such as fuel. Solvent extraction is somewhat promising but requires distillation of an extract to separate the solvent from the oil. Also, a steam stripper is usually required to recover the residual solvent dissolved or entrained within the exiting algal concentrate. The solvent extraction technique requires contactor equipment or phase separation equipment, a distillation system and a steam stripper along with varying heat exchangers, surge tanks and pumps. Also steam and cooling water are required. The process described herein only requires a membrane system with pumps and tanks. No steam or cooling water is required.

Processing Alternatives: After selection of the appropriate solvent, the next step is to determine whether to extract algae oil from “wet” or “dry” algae. The “dry” process requires dewatering and evaporating the water from the algae biomass and then lysing the algae. Lysing is a process of breaking the cell wall and opening the cell. Solvent may be contacted with the dry algae in special counter current leaching equipment. The solvent and extracted algae oil is separated in a vacuum distillation tower or evaporator. The remaining algae biomass with residual solvent is fed to a special evaporator to remove and recover the solvent and to dry the algae biomass again. The “dry” process suffers from having to dry the algae a second time when the solvent must be evaporated away, handling a high solids stream in multiple steps, and potentially leaving solvent in the residual algae solids.

The “wet” process requires lysing and extraction of the algae concentrate. The wet process requires an excellent lysing technique followed by a solvent extraction process, which provides adequate mass transfer area for dissolving/coalescing the non-polar lipids. The “wet” process offers the advantages of drying the algae only once and leaving less residual solvent in the algae biomass. To minimize the processing cost, the “wet” process appears to offer significant advantages.

The present invention focuses on the “wet” process and the novel non-dispersive extraction contactor used to coalesce and dissolve the desirable non-polar lipids.

As shown in **FIG. 2**, the oil extraction step **212** follows the algae concentration **208** and lysing **210** steps. After growing and initial harvesting, from the pond **202** the dilute algae feed is concentrated significantly. The typical algae concentration obtained from the pond **202** generally ranges from 100 to 300 mg dried algae/liter of solution. The goal of the concentration step **208** is to remove and recycle the water **214** back to the pond. Concentration methods **208** vary from centrifugation to flocculation/settling of the algae. To maximize lysing and extraction efficiency, it is important that concentrate being fed for lysing is not flocculated. After the concentration step **208**, the algae concentrate is sent to the lysing **210** processing step where the algae cell is mechanically or electromechanically broken, thus exposing and freeing the non-polar oil. Various techniques may be used to mechanically or electrically compress and decompress to break the cell. In general after lysing, **212** the algae cell can be disintegrated or opened-up as shown in **FIG. 3**. **FIGS. 3A** and **3C** shows photographs of an alga cell prior to lysing and **FIGS. 3C** and **3D** show photographs of algal cells prior after lysing.

Once the oil has been freed from inside the algae cell, the oil will not simply separate from the cellular biomass due to density differences. Also since the equivalent diameters of most microalgae are extremely small and on the order of 1-5 microns, the oil drop diameter is often much less than 1 micron. Such oil drops do not rise or coalesce with other drops very well and can form a stable emulsion. When solid algae biomass **216** is added to the mixture, the recovery of the oil is even more difficult. Therefore simple gravitational phase settling is not a viable oil separation option after lysing. After lysing, the algae concentrate is fed to the separations step **212** where algae oil **220** is separated from the wet algal biomass **216**. The biomass **216** may be sent for further drying and will be used for animal feed or processed further for energy generation applications.

As shown in **FIG. 4**, the typical solvent extraction process involves 1) an extraction step to recover algae oil from the lysed biomass, 2) a vacuum distillation or evaporation step to separate the oil and solvent where the solvent is returned to step 1, and 3) if necessary, steam stripping step to recover the dissolved and entrained solvent leaving the extraction step with the algal biomass.

FIG. 4 a flow diagram **400** of a general algae oil extraction process using a conventional dispersive extraction column **406**. Lysed algal concentrate **402** and solvent **404** is fed to a column extractor **406** to extract the algal oils and lipids **408**. Stream **406a** comprises the solvent **404** containing the algal oils and lipids. Stream **406a** is then fed to a vacuum distillation unit **408** to recover the solvent **404** and the algal oil **410**. The separated solvent without any oil or other constituents **404** is fed back to the extractor **406**. In the event it needs further purification (separation), the solvent **404** is fed back to the vacuum distillation unit **408** (via stream **408a**). A second stream **406b** from the extractor **406** comprises the algal biomass, solids, and residual solvent. Stream **406b** is passed through a stream

stripper **412**, to separate the wet biomass **418** and other solids from the solvent **404**. The wet biomass **418** is subjected to further drying. The recovered solvent **414** is collected in a decanting vessel **416** before being recycled back to the extractor **406** via stream **414a**. A second stream **414b** from the vessel **416** recycles any dissolved solvent in condensed steam **414** back to the stream stripper **412**.

- 5 Extraction Processing and Equipment: The desired extraction process for algae oil recovery must satisfy certain requirements and avoid potential deficiencies for economic recovery. There are several “wet” extraction processes for oil recovery that are technically feasible but are not necessarily economical. Minimal oil recovery costs are critical if the ultimate use of the recovered algae oil is fuel.
- 10 The optimum oil extraction process should include: (i) processing a bio-cellular aqueous slurry containing oil, (ii) using a non-polar solvent or extracted oil with extremely low miscibility in water, (iii) using a solvent (if necessary), that easily separates from the oil, (iv) using an extraction equipment that can handle high processing feed rates and easily scaled-up, (v) using an extraction equipment that minimizes the entrainment of solvent into the biomass, (vi) using an extraction
15 equipment that provides a high contact area for mass transfer and non-polar lipid coalescence, (vii) using an extraction equipment capable of handling concentrated algae feeds and not be irreversibly fouled by algae solids, (viii) using an extraction equipment that is relatively compact and potentially portable to allow transport to different algae production sites, and (ix) using an extraction equipment that is readily available, inexpensive and safe.
- 20 Membrane based processes for separations have been in existence for a long time. There are many types of membranes. Most membrane processes however use porous membranes wherein the membrane material performs a separation as a result of differences in diffusion and equilibrium between chemical components and on the molecular level. The present inventors however utilize a microporous membrane, which is rarely used commercially except for applications involving the
25 transfer of gases to or from a liquid such as water. The microporous membranes function very differently from the porous membrane because of their relatively large pores. The microporous membranes do not truly separate chemical components on the molecular level like porous membranes do. The present invention relies on the coalescence of non-polar lipids present within the algae slurry to coalesce onto the hydrophobic surfaces provided by the hollow fibers. The vast surface area of the
30 membrane, combined with the hydrophobic collection fluid’s ability to wet the membrane, creates a surface capable of coalescing small lipid droplets. Once coalesced into the collection fluid, the lipids are transported out of the membrane through the inner tubes of the hollow fibers.

Membrane based Oil Recovery Process: For example, the application of a microporous hollow fiber (MHF) membrane contactor as the optimal extraction equipment appears ideally suited for the
35 recovery of algae oil. The MHF contactor provides all of the optimum characteristics listed previously. The application MHF contactor to algae oil recovery is novel, minimizes solvent loss,

eliminates need for the steam stripper, minimizes solids contamination, and is easy to operate. The process does not involve dispersing a solvent into the algae biomass. The non-dispersive nature of the contactor is attractive in minimizing solvent loss and thus potentially eliminating the need for a steam stripper. A collection fluid typically comprising of either a solvent (such as hexane) or a biodiesel mixture, or algal oil is circulated through the hollow fibers for the recovery of the algal oils. The application of the MHF contactor in conjunction with a biodiesel mixture circulated through the microporous hollow fibers eliminates the need for a solvent and distillation column. The two oil extraction processing schemes with solvent and the biodiesel mixture are shown in **FIGS. 5** and **6**, respectively.

FIG. 5 is a schematic **500** depicting the novel algal oil extraction process (with solvent) of the present invention. The process comprises a MHF contactor **502** comprising a plurality of microporous hollow fiber membranes **504** and a central baffle **506**. Solvent **508** is fed (pumped) through the membrane fibers **504** and is contacted with the lysed algal concentrate **512** contained in the shell portion of the MHF contactor **502**. There are two exit streams from the contactor **502**, an algal biomass stream **510** which is processed further (dried) and a solvent stream **508a** which contains the extracted algal oils and lipids **516**. The stream **508a** is passed through a vacuum distillation unit **514** to separate the oil **516** from the solvent **508** and to recover the solvent **508** for recycle and reuse. Exit stream **508b** from the distillation unit **514** comprises pure solvent **508** which is recycled and fed to the contactor **502** to repeat the process and solvent requiring further separation and is recycled back to the distillation unit **514**. Exit stream **508c** from the distillation unit **514** comprises the algal oils **516**. A portion of this stream is vaporized (**518b**) and returned to the distillation unit **514**.

FIG. 6 is a schematic **600** depicting the novel algal oil extraction process (without solvent, using a biodiesel mixture) of the present invention. The process comprises a MHF contactor **602** comprising a plurality of microporous hollow fiber membranes **604** and a central baffle **606**. Non-polar algae oil **608** is fed (pumped) through the membrane fibers **604** and is contacted with the lysed algal concentrate **612** contained in the shell portion of the MHF contactor **602**. The non-polar algae oil functions to dissolve and sweep the coalesced oil from the algae concentrate. The non-polar oil **616** coalesces onto the hydrophobic fiber surface **604** and dissolves into oil contained in the walls and the counterflowing oil phase **608** and can be removed. There are two exit streams from the contactor **602**, an algal biomass stream **610** which is processed further (dried) a stream **608a** which contains the algal oils and lipids **616** that is collected in a tank **614**. Part of the oil **616** can be removed from the tank **614** and fed to the contactor **602** to repeat the process.

Microporous hollow fiber contactors were initially developed in the 1980s. These early studies focused on lab-scale prototype modules containing just a few fibers. These early studies promoted the possibility of liquid-liquid extraction applications. The contacting of two immiscible liquids such as water and a non-polar solvent is unique with MHF contactors in that there is no dispersion of one

liquid into another. This technology is sometimes referred to as non-dispersive extraction. The hollow fibers are generally composed of a hydrophobic material such as polyethylene or polypropylene. These hollow fibers could be made of a different material but it should be hydrophobic to avoid fouling of the fiber surface with the algae solids which are usually hydrophilic. The solvent should be a hydrocarbon with a very low solubility in water and is pumped through the hollow fibers. As a result of the hydrophobicity of the fiber material, the solvent will wet the microporous fibers and fill the micropores. The aqueous-based fluid is pumped through the shell-side of the membrane contactor. To prevent breakthrough of the solvent into the shell-side, the shell or aqueous side is controlled at a higher pressure than the fiber or hydrocarbon side. This results in immobilizing a liquid-liquid interface in the porous walls of the hollow fibers. Unfortunately when these modules were scaled-up for liquid-liquid extraction, the performance was usually disappointingly poor. Further studies identified the poor efficiency was a result of shell-side bypassing. An improved version (referred to as the Liqui-Cel* Extra Flow contactor) was developed which eliminated the possibility of shell-side bypassing by incorporating a shell-side distributor. While the design eliminated the shell-side bypassing, the new design eliminated true counter-current contacting. The overall performance was improved somewhat relative to the original design. Nevertheless, the new design did not correct the fundamental limitations of pore-side mass transfer resistance that would control most commercially significant extraction applications. As a result, only a few commercial liquid extraction applications using MHF contacting technology exist today.

Also, the MHF contactors often required expensive filter systems to avoid plugging with solids associated with most commercial liquid-liquid extraction processes. The Liqui-Cel* contactor used in the present invention has been applied almost exclusively to commercial processes that transfer a gas to or from a liquid such as oxygen stripping from water for the microelectronics industry.

No applications of the MHF contactors are known for enhancing coalescence and removing of submicron oil drops from water. Certainly no applications of MHF technology are known for submicron oil recovery from water involving a significant solids concentration.

FIG. 7 is a schematic **700** of the Liqui-Cel extra flow microporous hollow fiber membrane contactor **702**. The contactor **702** comprises a metallic or polypropylene housing **706**, wherein is contained a cartridge **708** comprising a plurality of hydrophobic microporous hollow fibers **712**, along with a distribution tube **710**, a collection tube **716**, and a central baffle **714**. The housing **706** has 2 inlet ports (**704a** and **704b**) and two outlet ports **704c** and **704d**.

As shown in **FIG. 7**, the aqueous phase **718** is fed through the port **704a** on the shell-side while the solvent (or oil) phase **722** is fed on the fiber side through port **704b**. The non-polar lipids coalesce onto the hydrophobic surface and wet and dissolve into walls and into the counterflowing solvent (or oil) phase. A higher pressure is maintained on the aqueous side to prevent bleed through of the solvent (or oil) phase. However the shell-side pressure is kept below the breakthrough pressure which

*Trade-mark

forces aqueous phase 718 into the solvent (or oil) phase 722. The algae concentrate 718 and solvent feeds 722 could be operated at room temperature or preheated up to 60°C. The solvent (or oil) phase along with the recovered lipids or oils is removed through outlet port 704c, and the aqueous algal raffinate containing the algal biomass and other solids is removed through the port 704d.

- 5 While not intuitive because of the presence of algae solids, the MHF contactor appears ideal for recovering oil from lysed algae. The MHF contactor provides: (i) high contact area for coalescence and mass transfer, (ii) processing of un-flocculated or deflocculated algae solids, (iii) large flow capacities on the shell side, (iv) negligible mass transfer resistance in the pore because of the high equilibrium distribution coefficient of non-polar oils into non-polar solvent, and (v) low cost per unit
10 of algae flow per unit as the contact area is 100X that for the conventional liquid extraction contactor. (e.g. perforated plate column).

The MHF extractor provides four significant advantages: (i) no entrainment of solvent which eliminates the need for a stripping column when the proper solvent is selected, (ii) easy control of the liquid-liquid interface by controlling the pressures, (iii) extremely large area for coalescence of small
15 algae oil drops. The MHF contactor functions primarily as an oil coalescer. The solvent acts to simply remove the coalesced oils from the surface of the fibers, and (iv) while not optimized, commercial MHF contactor modules used for gas transfer are available and reasonably priced. The Liqui-Cel Extra Flow contactor is a good example.

MHF Contactor Performance Data: The present inventors characterize the performance of the MHF
20 contactor for algal oil extraction. The objectives of the studies were to determine the fraction of non-polar algae extracted from the feed and determine if membrane plugging was observed. The 4-inch diameter Liqui-Cel Extra Flow Contactor, purchased from Membrana [Part#G503], was used to extract algae oil from an actual lysed algal concentrate (FIG. 7). Typical oil recoveries from experimentally lysed algae ranged from 45-80% for a single module. The results of the studies are
25 shown in Table 1. Differences in oil recoveries may be attributed to the lysing efficiency, polarity of the algae oil, differences in oil wettability and coalescence onto the membrane fibers. Membrane plugging is not observed when processing lysed algae concentrates where the algae is not flocculated or has been deflocculated. Typical range of conditions associated with the recovery of non-polar algae oil are shown in Table 1. These data are based on the processing of actual lysed algae. Since
30 the non-polar oil recovery efficiency is also affected by the lysing efficiency, controlled experiments were carried out where known quantities of canola oil were injected into a re-circulating algae concentrate stream. In the first set of studies, heptane was re-circulated on the tube side as a non-polar oil specific collection fluid. The results of these studies are shown in Table 2. In the initial small scale studies, 44-64% of the injected oil volume was recovered by the microporous hollow fiber
35 membrane when only 25 mL's of canola oil was injected. When a larger quantity of canola oil was injected (250 mL), more than 90% of the injected oil volume was recovered as shown in Table 2.

These data provide evidence that a fixed volume of oil is likely held up in the walls of the hollow fibers. In a second set of studies using canola oil injected into lysed algae concentrate, canola oil was re-circulated through the hollow fiber tubes as a collection fluid instead of heptane. As shown in Table 3, 93% of the 9 liters of injected canola oil was recovered, conclusively demonstrating that a “like” oil can be used as a collection fluid. The second set of studies validates the mechanism that the process is based on coalescing and recovery of the oil drops from the aqueous slurry can be done using a “like” oil. The canola oil runs also provide supporting data for the application of the non-dispersive microporous hollow fiber technology in removing residual oil from produced water, as canola oil/water emulsions are an accepted experimental proxy to mimic produced water in a laboratory setting. The results from Tables 2 and 3 indicate that oil recoveries approaching 100% are possible. The walls of the hollow fibers will always contain oil during processing.

Table 1: Typical algal oil recoveries from lysed algae with the MHF Contactor.

Parameter	Overall Range	Typical Range
Algae concentration, wt%	0.01-15	1-5
Non-polar Oil in Algae, wt%	0.5-10	2-6
Algae Flow rate, gpm	0.5-2	0.5-1
Heptane Flow rate, gpm	0.04-0.07	0.07
Non-polar Oil Recovery, %	40-90	70-80

Table 2: Results of controlled study using Heptane flowing through the tubes. Basis: Algae feed rate = 1,000 lbs/hr, Heptane feed rate = 50 lbs/hr, Total mass of re-circulating algae = 50 lbs containing approximately 1.5 wt% bio-cellular solids, Oil injection rate = 0.17 lbs/hr.

Test	#1	#2	#3	#4
Oil Injected, ml	25	25	210	210
Oil Recovered, ml	11	16	198	188
Missing Oil	114	99	12	22
% Oil recovery	44	66	94	90

Table 3: Results of the solventless test with Canola oil flowing through the tubes. Shell-side and tube-side flows are re-circulated.

Tube-Side	Canola Oil
Shell-Side	50 lbs of Algae Concentrate
wt% bio-cellular solids in algae	Approximately 1.5 wt%
Tube Side Flow rate	10-15 lbs/hr
Shell Side Flow rate	500, lbs/hr
Canola Oil Injection Rate into Algae	3 ml/min
Run Time	72 hours
% Recovery of Injected Canola Oil	93%

It should be noted that the algae concentrate feed or bio-cellular feed must not contain flocculated algae or solids to prevent plugging within the membrane module. For the case of the MHF contactor described in the present invention, the minimum dimension for shell-side flow is 39 microns which

greater than the size of most single alga. It is likely that flocculated algae will eventually plug the shell-side of the MHF contactor.

In a related and alternative process, the microporous membrane could be used to separate two liquids from a solid-liquid-liquid emulsion. The solid-liquid-liquid emulsion may have been derived from a process for recovering oil from a bio-cellular aqueous feed using a dispersive process. The microporous membrane hollow fiber contactor would allow the hydrocarbon liquid to “wet” and coalesce into the walls of the hollow fibers while preventing the hydrophilic solids or aqueous phase from entering. Thus the hydrocarbon liquid will exit the membrane on the tube side when an appropriate collection fluid is employed, while the aqueous liquid and solids will exit on the shell-side. An alternative process is shown in **FIG. 9**.

The flow diagram **900** shown in **FIG. 9** of the alternative algae oil extraction process comprises a dispersive extraction column **902**, lysed algal concentrate **904** and solvent **908** is fed to a dispersive extractor such as a column extractor, centrifugal type extractor or mixer-settler **902**. The solid-liquid-liquid emulsion (S-L-L) **912** from the column **902** comprising algae-water-solvent is then fed to a shell-side of a microporous membrane extractor (contactor) **910**. Any solids (algal biomass) from the column extractor **902** may be directly subjected to further processing (e.g. drying) as shown by step **914**. The microporous membrane hollow fiber contactor **910** allows the hydrocarbon liquid to “wet” and coalesce into the walls of the hollow fibers while preventing the hydrophilic solids or aqueous phase from entering. The hydrocarbon liquid exits the membrane contactor **910** on the tube side when an appropriate collection fluid (for e.g. solvent **908**) is employed on the tube side, while the aqueous liquid and solids (algal biomass) will exit on the shell-side for further processing (e.g. drying) as shown by step **914**. The hydrocarbon liquid is then fed to a distillation unit **916** (heat exchangers associated with the distillation unit are shown as **918** and **920**) for removal of any residual solvent **906** and to recover the algal oil **924**. The recovered solvent **906** may be circulated back into the process, for e.g. as the collection fluid on the tube-side of the membrane contactor **910** or back to the dispersive extraction column **902**.

The collection fluid on the tube side can be tailored to enhance recovery or selectively recover subsets of desired compounds, and leave others. Study data demonstrates that hydrocarbons and non-polar lipids are removed using heptane or like oil and phospholipids are not. Phospholipid recovery can likely be achieved by employing a more polar collection fluid.

To determine the composition of the extracted oil, the inventors performed a normal phase HPLC using a Sedex 75 evaporative light scattering detector. As shown in **FIG. 8**, two main components were detected in this particular sample of oil, the first peak corresponding to long chain hydrocarbons and the second corresponding to triglycerides. In some samples, 1,3 and 1,2 diglyceride have also been detected.

It will be understood by the skilled artisan that the process described hereinabove is applicable broadly for insoluble oil recovery beyond algae to include protists, fungi, yeast, *E. coli*, etc., mixed cultures of cells, grown by any method (not limited to photosynthetic organisms), aqueous slurries
5 containing broken and/or live cells or no cells (in case pre-treated to remove cells/cell debris or other suspended materials). The process can also be used to recover oil from any liquid source comprising insoluble oils for e.g. industrial water, brine, wastewater, industrial or natural effluents, water-oil mixtures, aqueous slurries, aqueous slurries comprising broken cells, live cells or combinations thereof, bio-cellular mixtures, lysed cellular preparations, and combinations thereof. The process of
10 the present invention is capable of extracting almost up to a 100% of the one or more insoluble oils in the liquid source. The process provides insoluble oil recoveries of 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% and 100% from the liquid source.

The method and the process of the present invention can be expanded for recovery of a variety of molecules depending upon choice of collection fluid and to include single or multi-step, differential
15 recovery processes for e.g., specifically recover non-polar oil with one membrane module, then treat the effluent with a second membrane module employing a different collection fluid. The collection fluids may be selective, partially selective or non-selective for specific compounds. In other specific examples, the present invention may be used to specifically recover non-polar oil with one membrane module, then followed by treatment of the effluent from the first module with a second membrane
20 module employing a different collection fluid.

It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method, kit, reagent, or composition of the invention, and vice versa. Furthermore, compositions of the invention can be used to achieve methods of the invention.

It will be understood that particular embodiments described herein are shown by way of illustration
25 and not as limitations of the invention. The principal features of this invention can be employed in various embodiments without departing from the scope of the invention. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the claims.

30 All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this invention pertains.

The use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,”

“at least one,” and “one or more than one.” The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or.”

5 Throughout this application, the term “about” is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

As used in this specification and claim(s), the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”),
10 “including” (and any form of including, such as “includes” and “include”) or “containing” (and any form of containing, such as “contains” and “contain”) are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

The term “or combinations thereof” as used herein refers to all permutations and combinations of the listed items preceding the term. For example, “A, B, C, or combinations thereof” is intended to
15 include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in a particular context, also BA, CA, CB, CBA, BCA, ACB, BAC, or CAB. Continuing with this example, expressly included are combinations that contain repeats of one or more item or term, such as BB, AAA, MB, BBC, AAABCCCC, CBBAAA, CABABB, and so forth. The skilled artisan will understand that typically there is no limit on the number of items or terms in any combination, unless otherwise
20 apparent from the context.

All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the
25 steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims. The scope of the claims should not be limited by the preferred embodiments set forth in the examples, but should be given the broadest interpretation consistent with the description
30 as a whole.

References

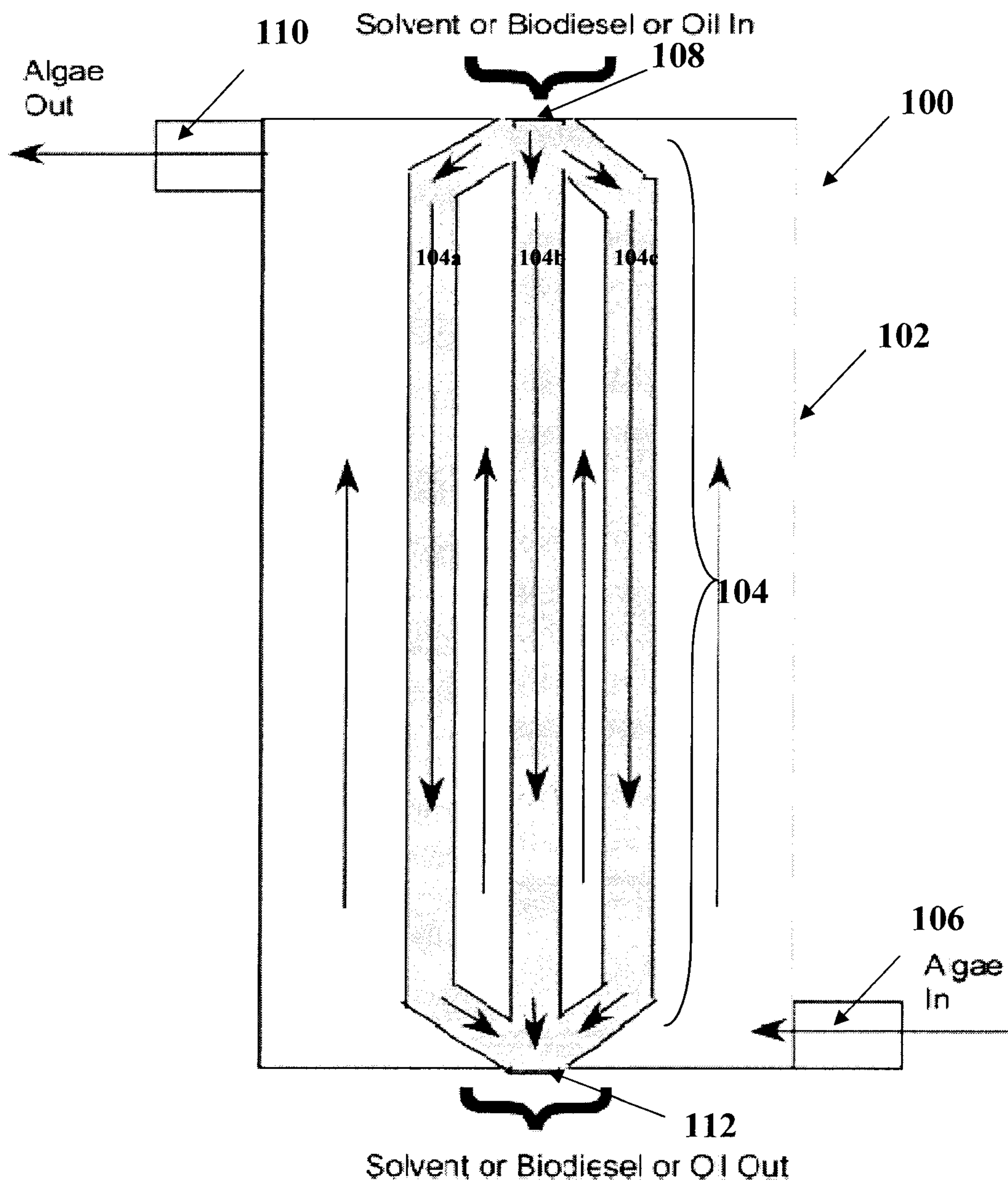
United States Patent No. 4,439,629: *Extraction Process for Beta-Carotene*.

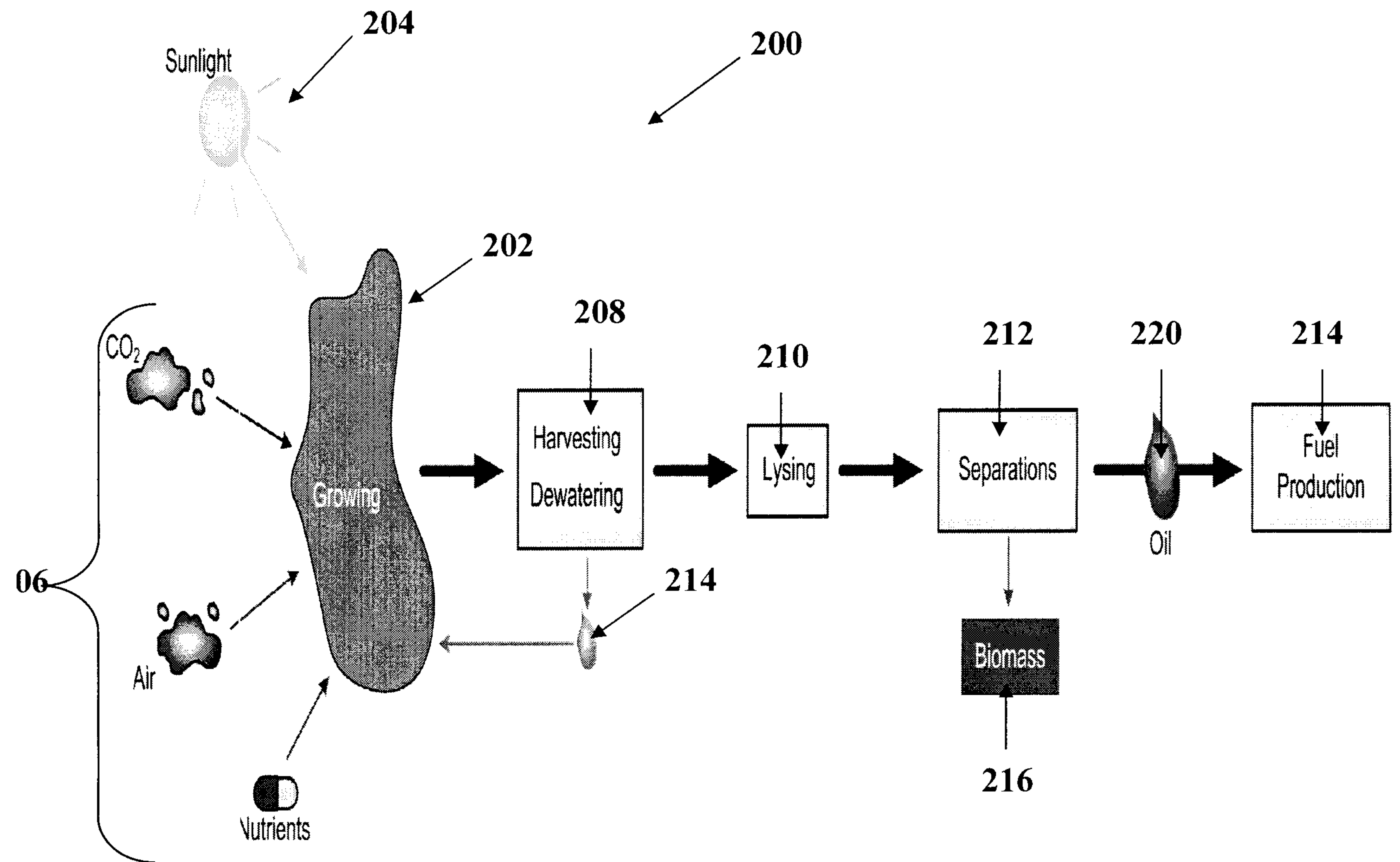
United States Patent No. 5,378,639: *Solvent Extraction*.

CLAIMS

1. A method of separating one or more insoluble oils from a liquid source using one or more membrane contactors comprising the steps of:
 - contacting the liquid source with a first surface of the one or more membrane contactors;
 - coalescing the one or more insoluble oils in the liquid source in the one or more membrane contactors at a second surface of the one or more membrane contactors; and
 - removing the one or more coalesced insoluble oils from the membrane contactor at the second surface.
2. The method of claim 1, further comprising the step of collecting the one or more coalesced insoluble oils in a collection vessel.
3. The method of any of claims 1 or 2, wherein the liquid source is selected from the group consisting of industrial water, brine, wastewater, industrial effluents, natural effluents, water-oil mixtures and combinations thereof.
4. The method of claims 1 to 3, wherein 45-100% of the one or more insoluble oils in the liquid source are coalesced.
5. The method of claims 1 to 4, wherein 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% of the one or more insoluble oils in the liquid source are coalesced.
6. The method of claims 1 to 5, wherein the second surface is contacted with a counterflowing non-polar liquid at the second surface of the one or more membrane contactors to remove the coalesced insoluble oils.
7. The method of claims 1 to 6, wherein the one or more membrane contactors are defined further as a hydrophobic hollow fiber membrane that comprises polyethylene, polypropylene, polyolefins, polyvinyl chloride (PVC), amorphous Polyethylene terephthalate (PET), polyolefin copolymers, poly(etheretherketone) type polymers, surface modified polymers, mixtures or combinations thereof.

8. The method of claims 1 to 7, wherein the one or more membrane contactors are defined further as surface modified polymers that comprise polymers modified chemically at one or more halogen groups, by corona discharge, or by ion embedding.
9. The method of claims 1 to 8, wherein the hydrophobic membrane or membrane module comprises hollow fiber microporous membranes.
10. The method of claims 1 to 9, wherein the method further comprises the step of contacting the second surface of the one or more membrane contactors with a counterflowing fluid selected from at least one of the insoluble oil, monoglyceride, diglyceride, triglyceride, or fatty acid methyl esters.
11. The method of claims 1 to 9, wherein the method further comprises the step of contacting the second surface of the one or more membrane contactors with a counterflowing fluid, wherein the counterflowing fluid is a solvent., an alkane, an aromatic solvent, benzene, toluene, ethers, diethyl ether, a halogenated solvent, chloroform, dichloromethane, an ester or ethyl acetate.
12. The method of claim 11, wherein the solvent is a non-polar solvent, an alkane, an aromatic solvent, an ether, a halogenated solvent or an ester.
13. The method of claim 12, wherein the alkane is hexane; the aromatic solvent is benzene or toluene; the ether is diethyl ether; the halogenated solvent is chloroform or dichloromethane, and the ester is ethyl acetate.

*FIG. 1*



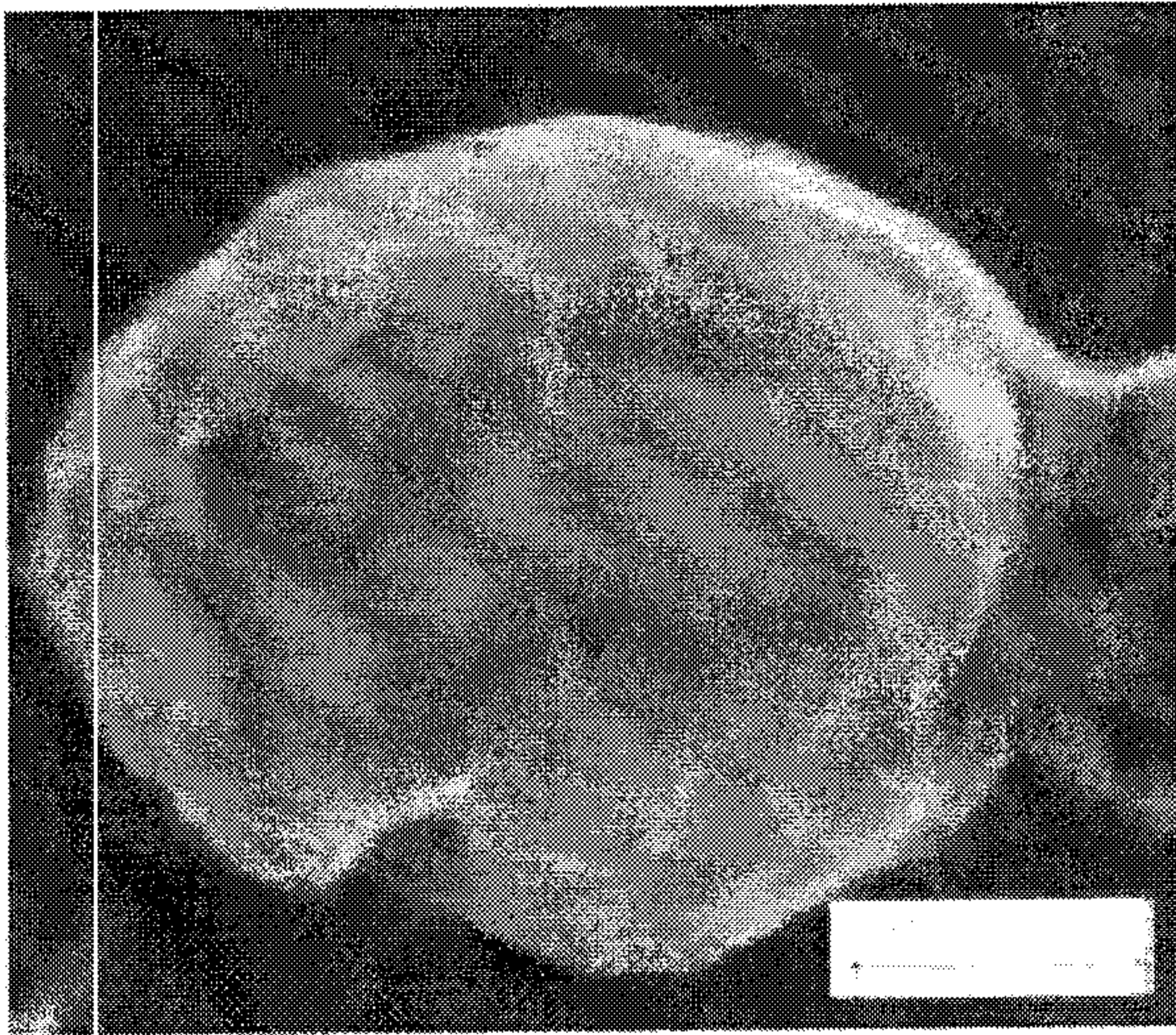


FIG. 3A

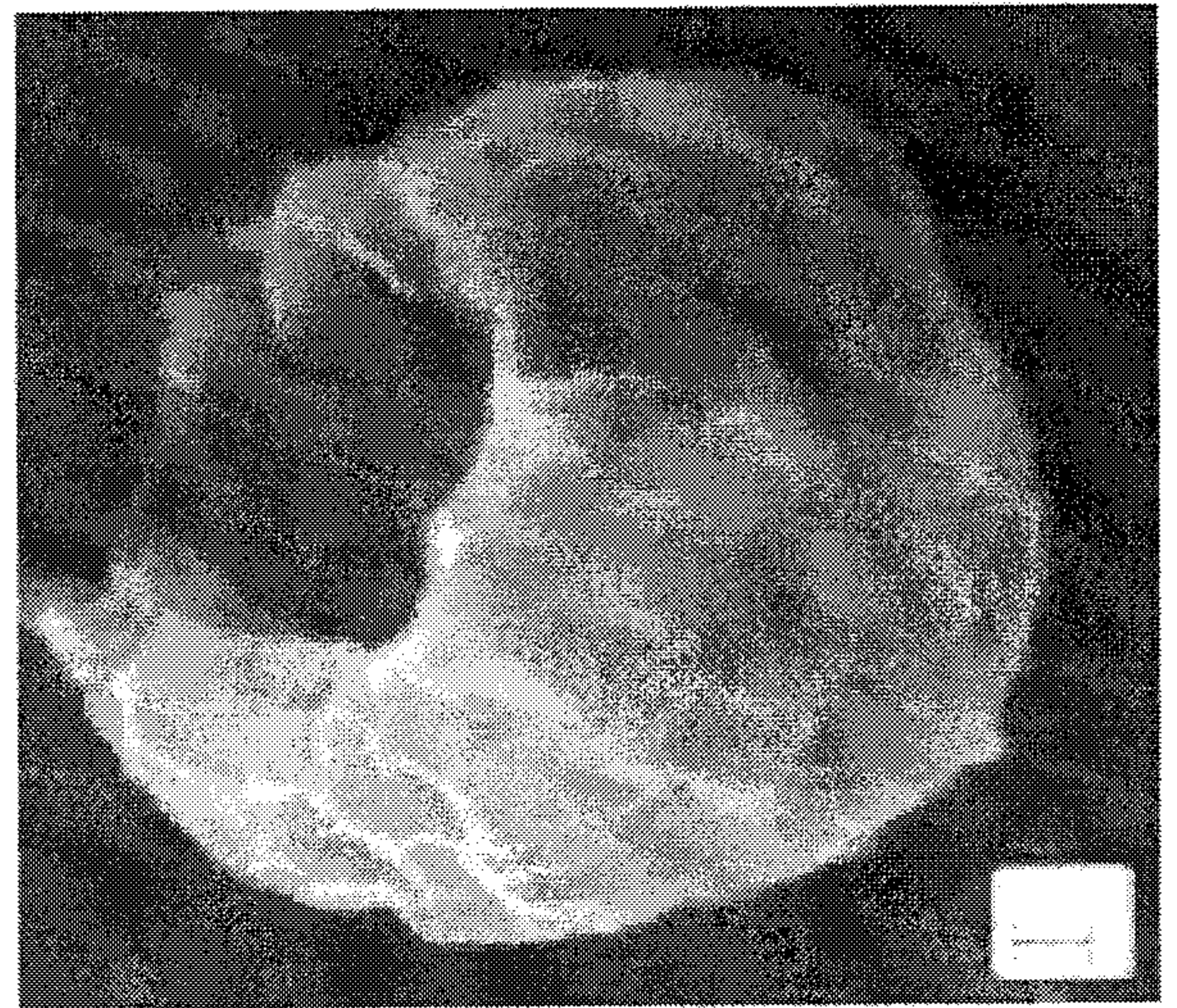


FIG. 3B

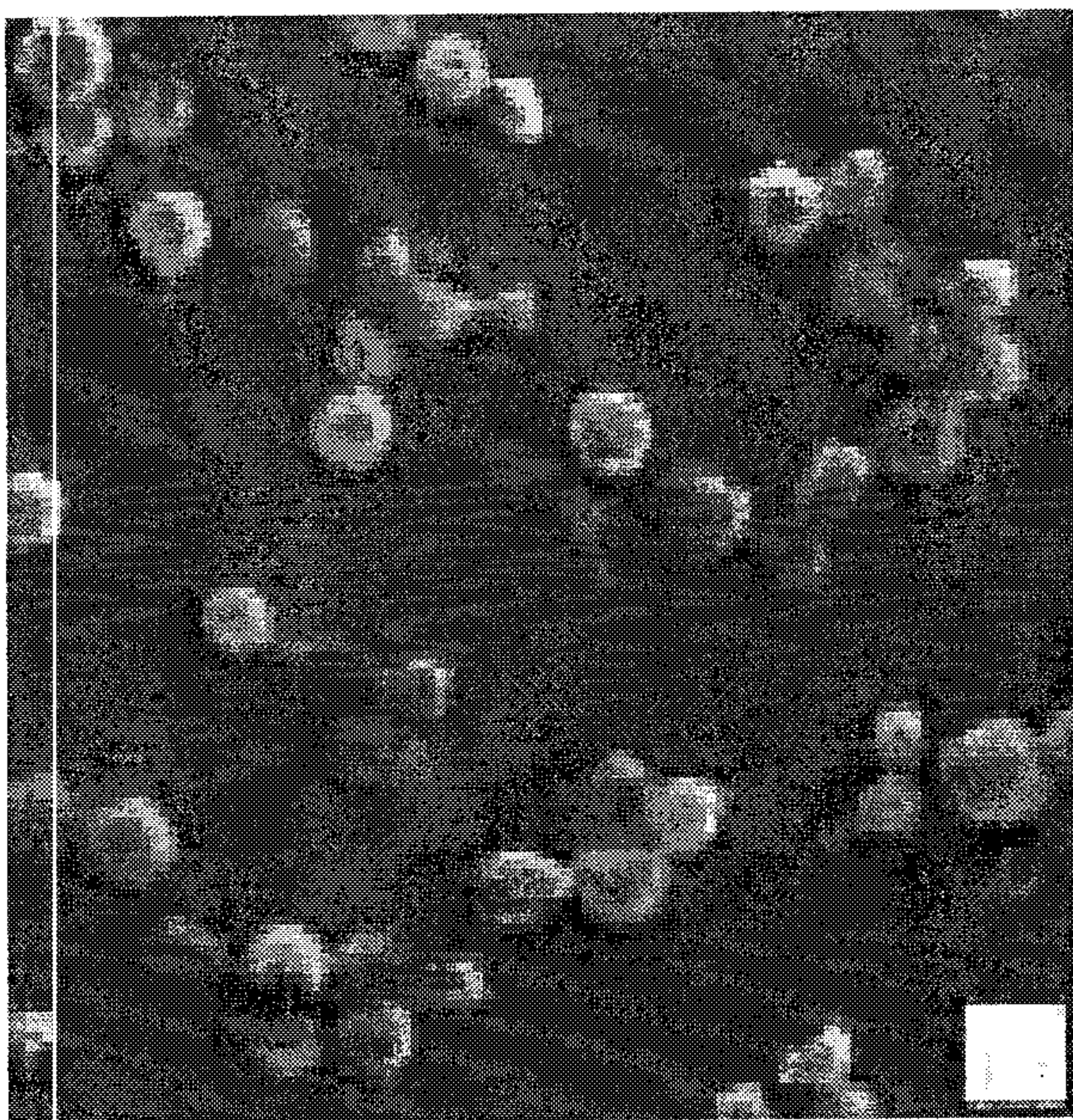


FIG. 3C

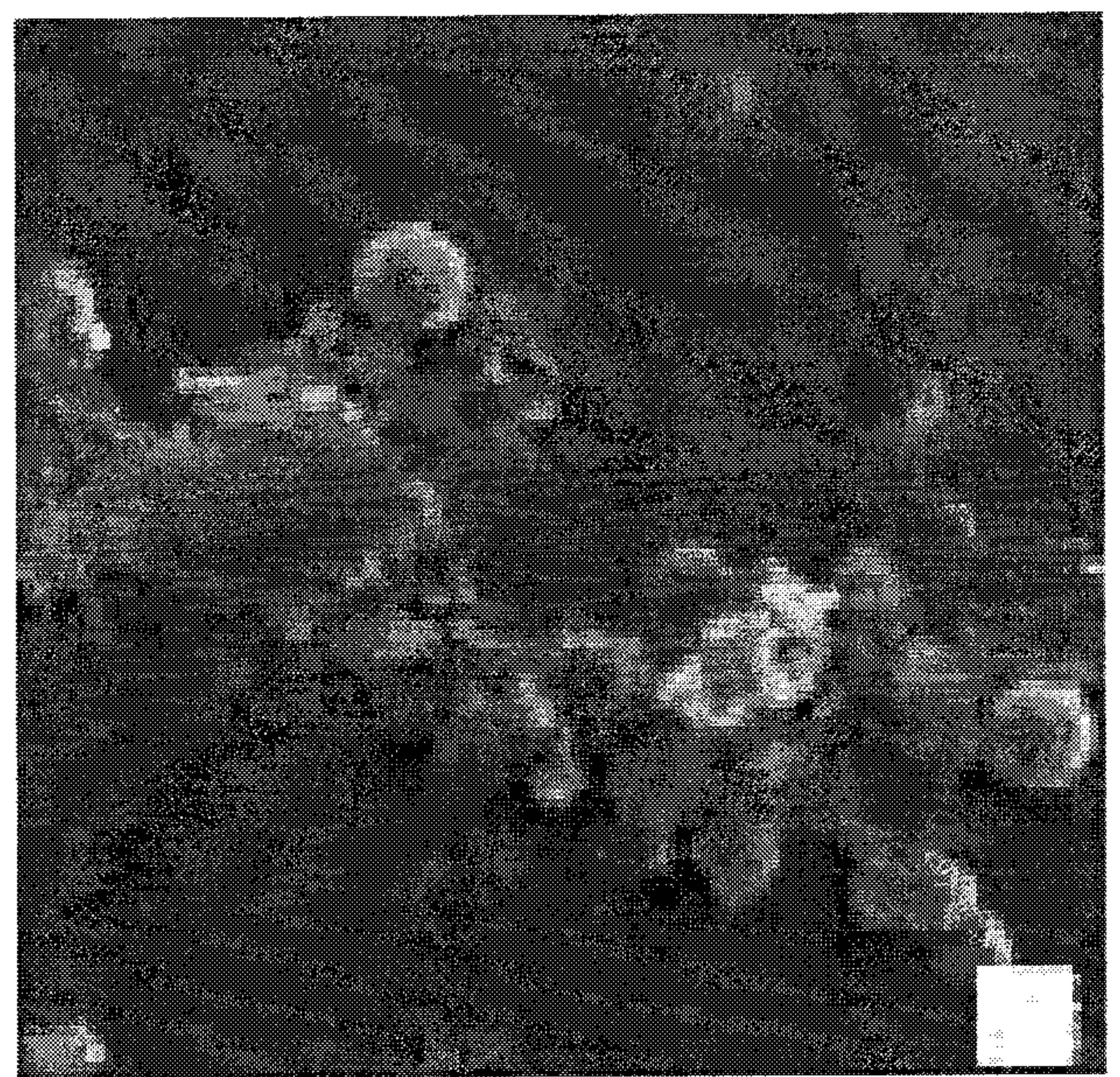
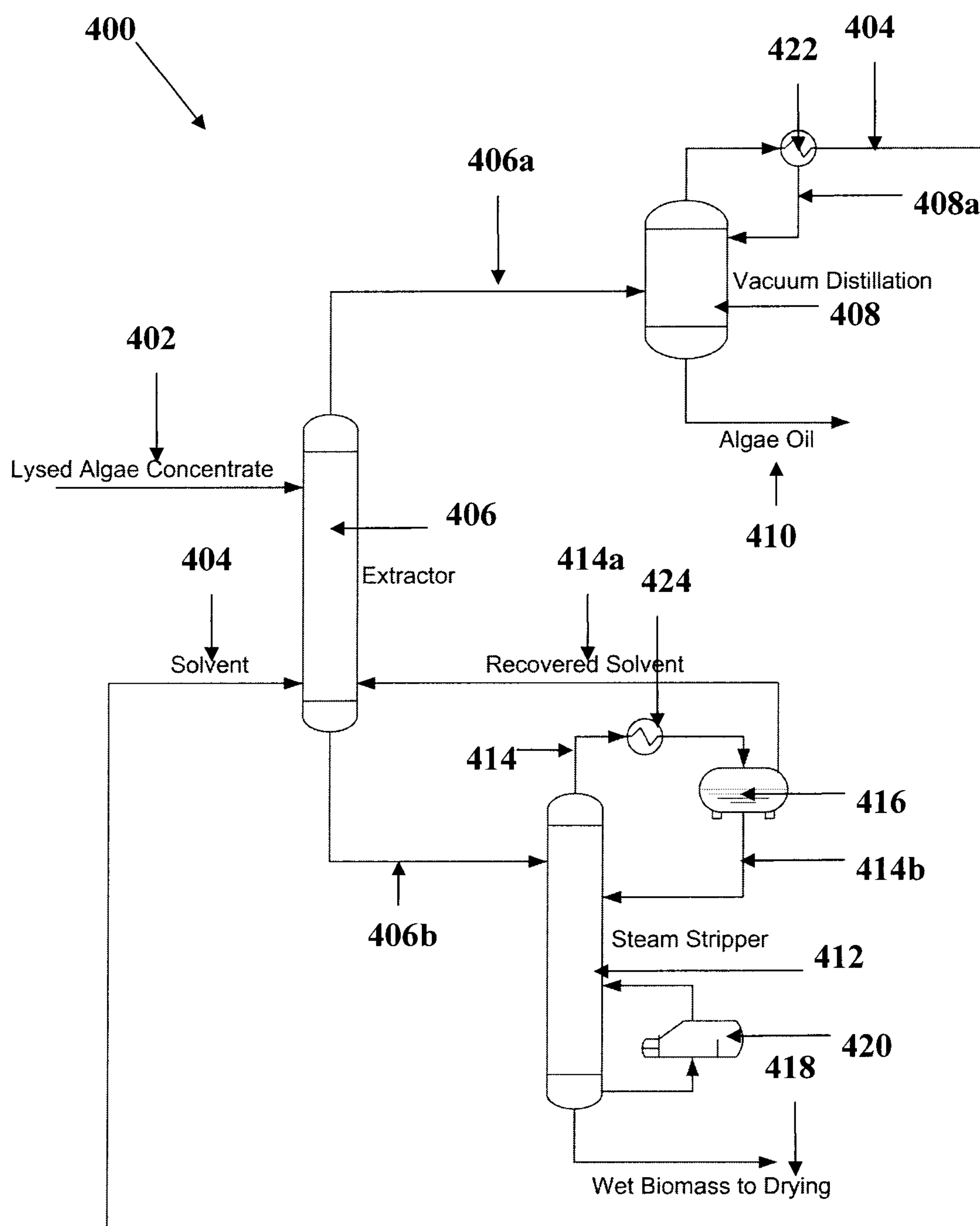


FIG. 3D

*FIG. 4*

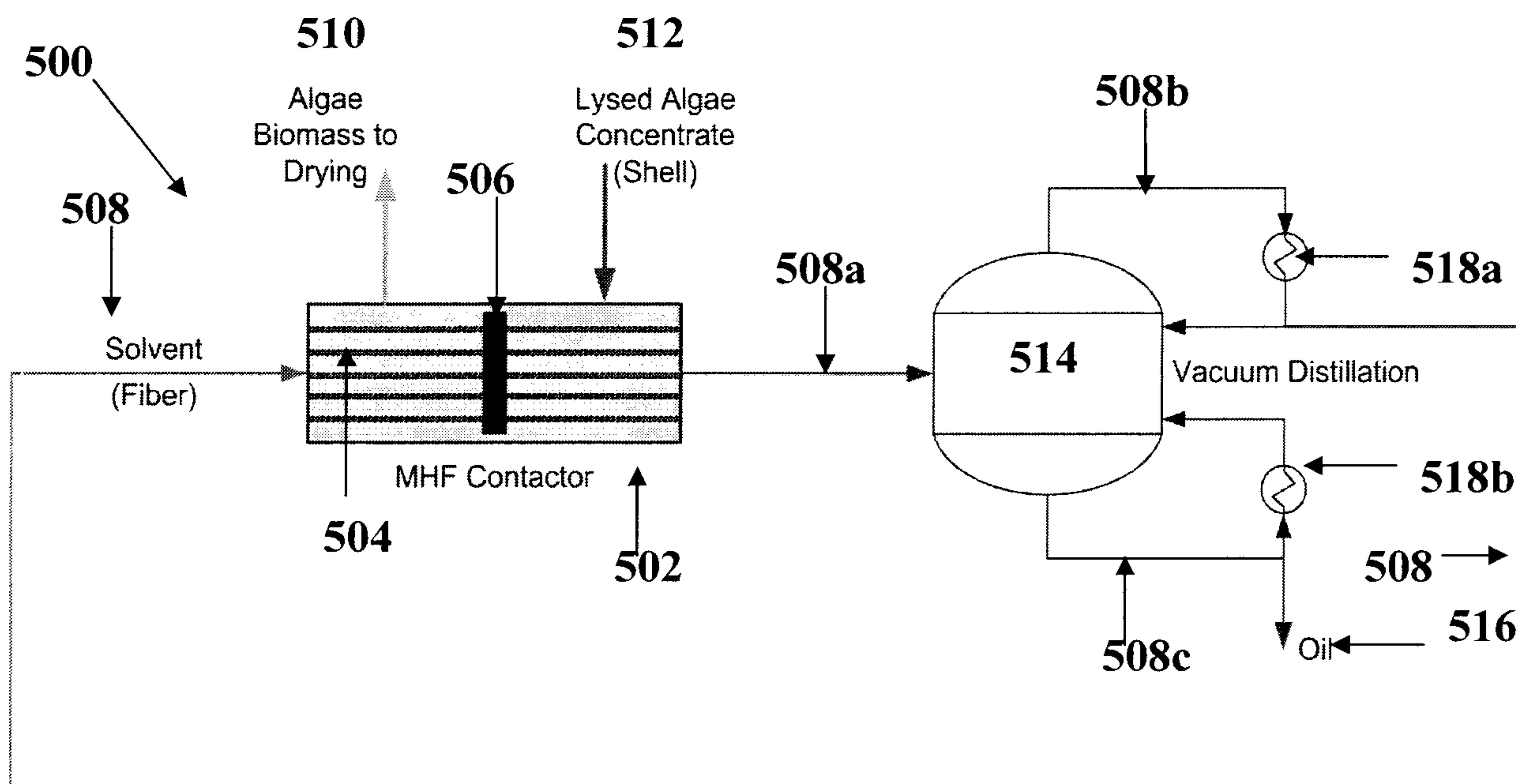


FIG. 5

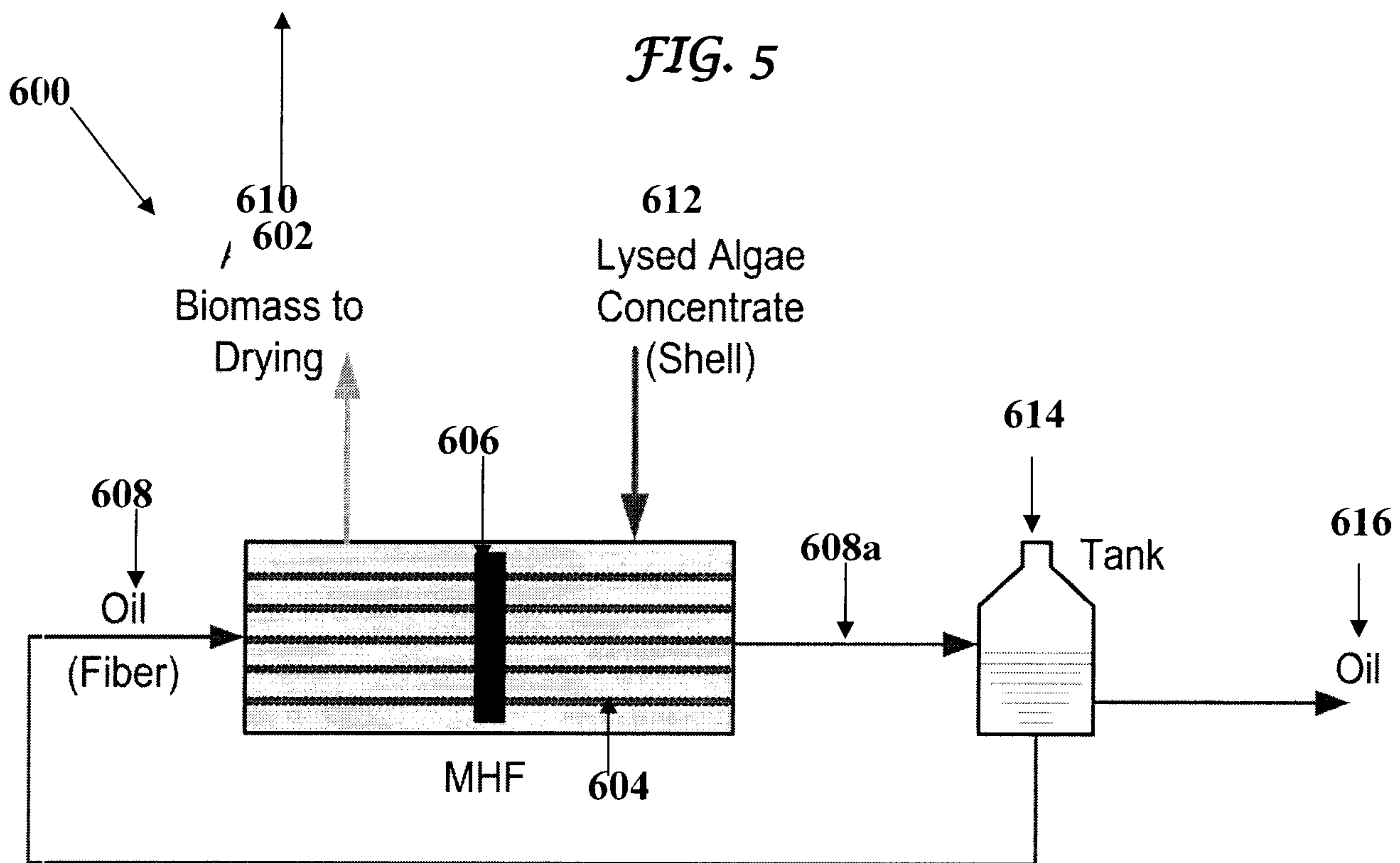


FIG. 6

UTAU:2288

6/8

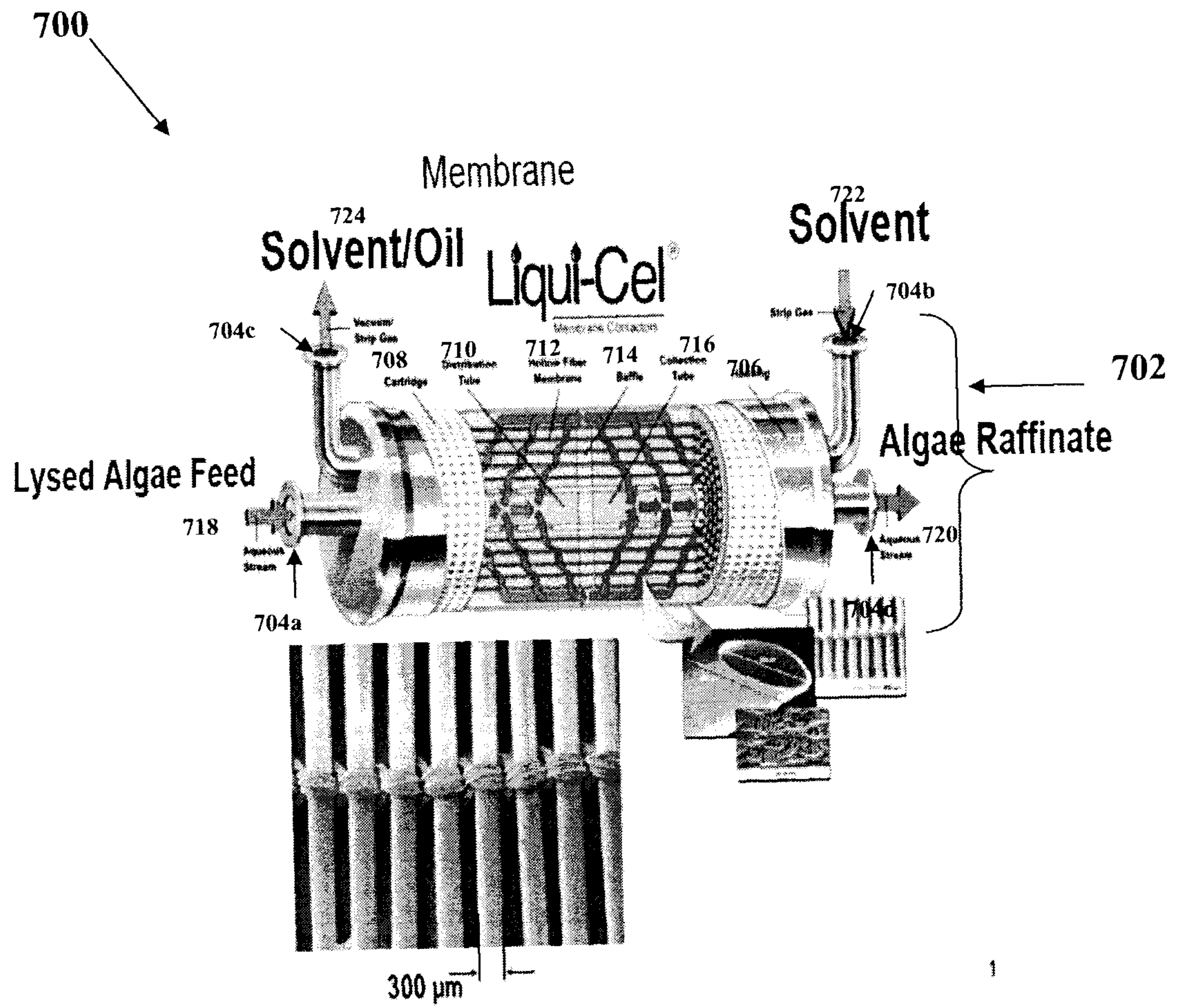
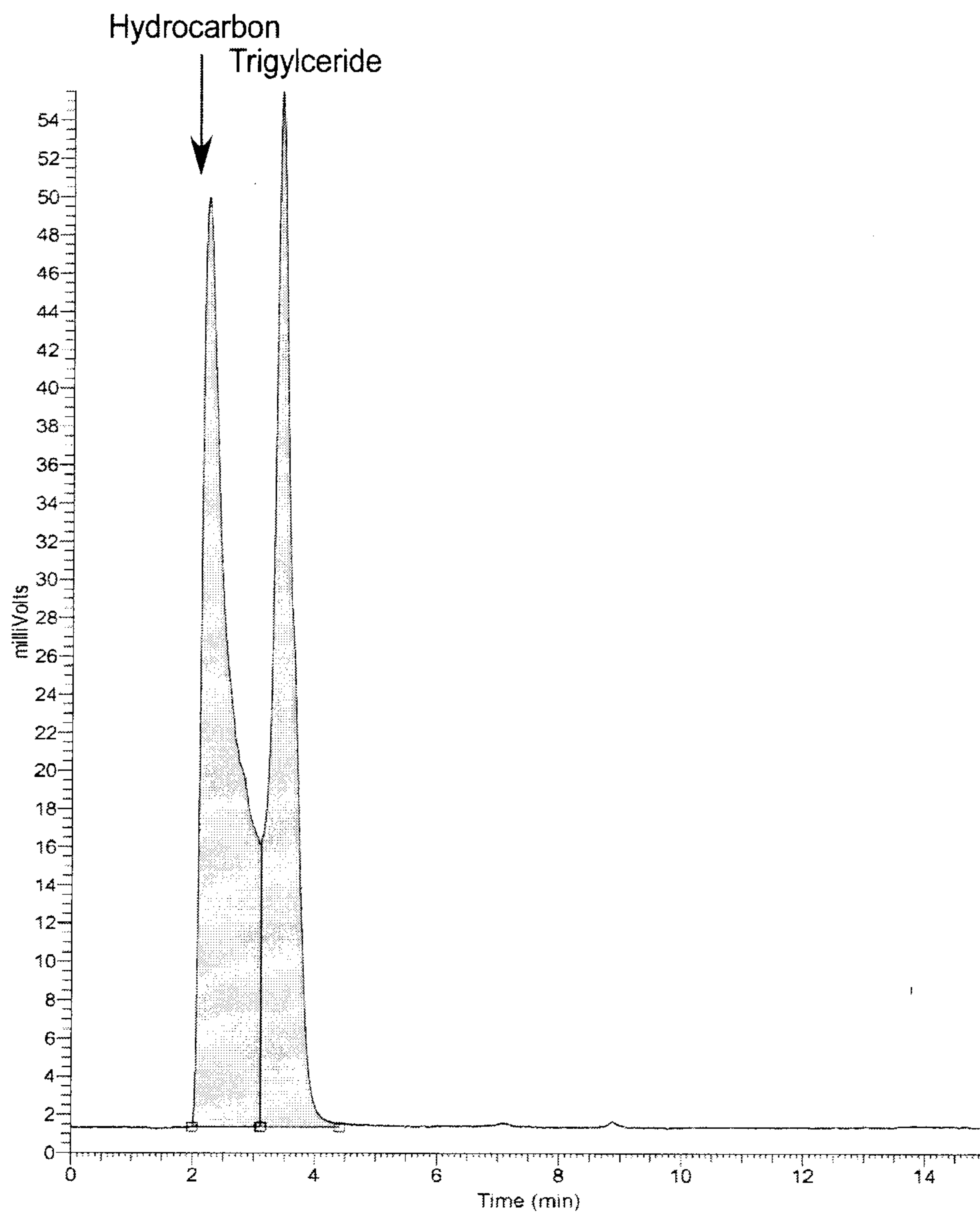
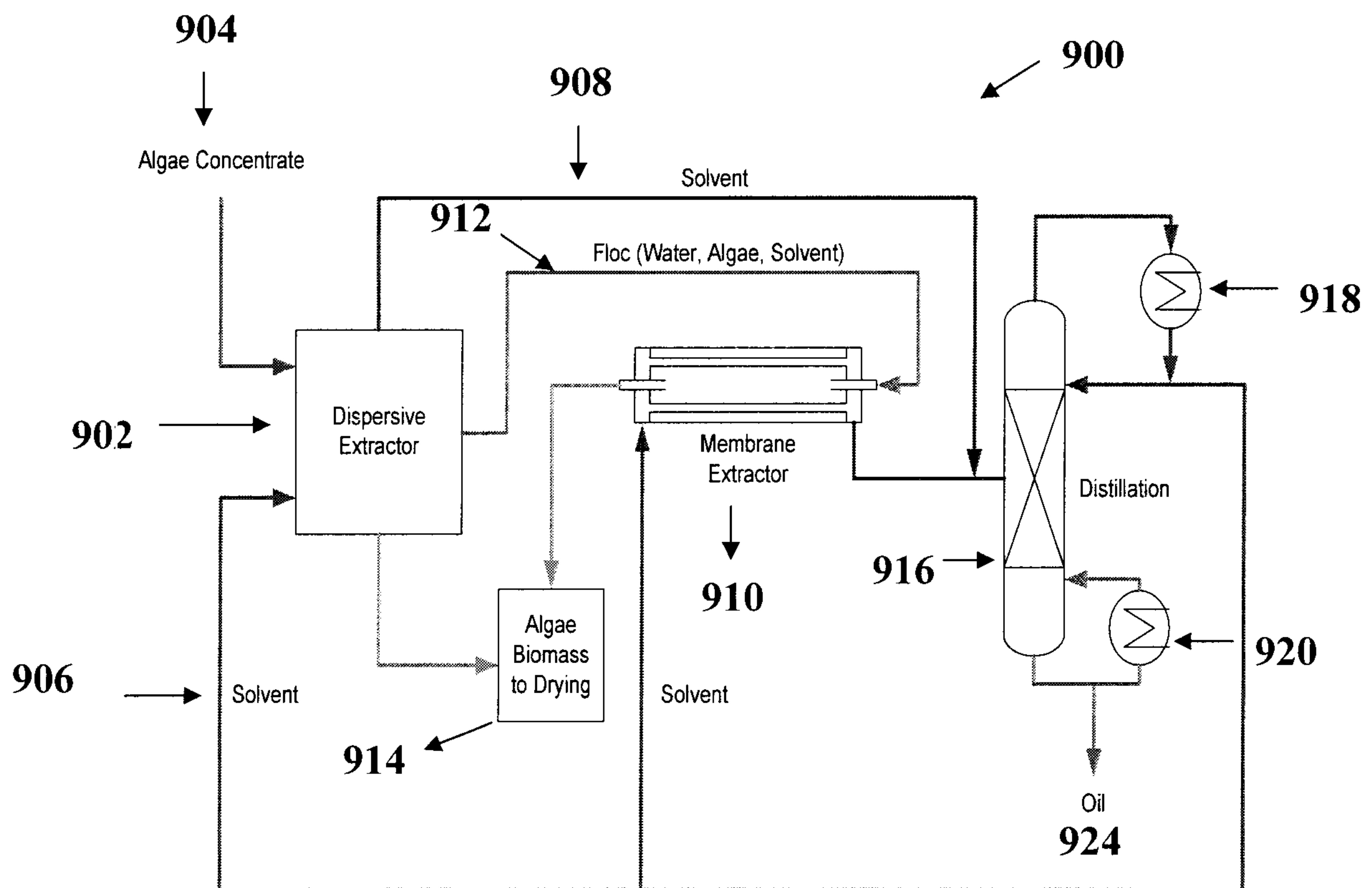


FIG. 7

7/8

**FIG. 8**

*FIG. 9*

