(51) International Patent Classification:
C12N 1/12 (2006.01) C12M 1/00 (2006.01)
C12N 1/34 (2006.01) C12M 1/06 (2006.01)
A01G 33/00 (2006.01)

(21) International Application Number:
PCT/EP2013/073065

(22) International Filing Date:
5 November 2013 (05.11.2013)

(25) Filing Language: English
(26) Publication Language: English

(30) Priority Data:
1219957.6 6 November 2012 (06.11.2012) GB

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Title: GROWING MICROALGAE OR CYANOBACTERIA IN LIQUID-BASED FOAM

Abstract: A flat panel photobioreactor is used to culture microalgae or cyanobacteria. The base of the photobioreactor has a fluid input for growth medium and a pressurised air input via needle spargers. The needle spargers are located in the base of the photobioreactor so that they are situated within the fluid growth medium. Microalgae (or cyanobacteria) are introduced into the growth medium. Sparging of carbon dioxide-rich air through the growth medium produces a foam and the liquid phase of the foam containing the microalgae moves into the entirety of the growth chamber. The microalgae are illuminated and grown in the foam. The foam is then harvested and dewatered to extract the microalgae.
GROWING MICROALGAE OR CYANOBACTERIA IN LIQUID-BASED FOAM

The present invention relates to the culturing of algae or blue-green algae (cyanobacteria) for the purposes of producing biomass to yield useful products such as lipids, oil, biochemical compounds or useful substances of algal origin. The invention also concerns the fields of bioenergy, chemicals, materials, food and feed production from algae.

Microalgae are used in the production of vitamins, pharmaceuticals, natural dyes, a source of fatty acids, proteins and other biochemicals for the health food industry. Microalgae can also be used for biocontrol of agricultural pests, as well as soil conditioners and biofertilizers for agriculture. Other uses include producing oxygen, and removing nitrogen, phosphorus and toxic substances from wastewater or sewage.

There is also considerable interest and progress in the area of production of bulk components for chemicals, materials, food, feed and biofuels. For example, microalgae are used to produce biodiesel or are used as biomass to generate electricity. The traditional photosynthetic fuel process is where triglyceride producing algae are grown and then harvested, dewatered, lysed and then processed to yield a product that is chemically refined to an acyl ester biodiesel product. Lipids can also be converted into biofuels via dehydrogenation and decarboxylation. Over the last few decades, to make improvements, researchers have tried to find strains of algae with higher and/or more desirable lipid content.

Like microalgae, certain cyanobacteria are known to be cultured with the intention of producing useful products such as sugars or biofuels.

Amongst the main methods of algal culture, there are open ponds which can be natural or artificial. One type of artificial pond is a raceway in which algae, water and nutrients are circulated around a track by paddlewheels. Whilst open pond systems are generally easy to construct and use, they have major limitations in terms of poor light utilization by algal cells, evaporative losses and a need for a large land area, for example. Also, if flue gases rich in CO₂ are supplied to open ponds, then there is a
problem of loss of CO₂ to the atmosphere. Biomass productivity in open ponds is mostly recognised as being poor and not cost effective. A primitive closed system may be a pond or raceway inside a greenhouse.

US 4253271 Raymond and US 4320594 Raymond describe covered raceways and a process for culturing algae in a liquid medium. The liquid growth medium circulates in an open trough exposed to the atmosphere and is temperature controlled. The nutrient composition of the liquid medium in the trough is regulated to control growth and reproductive characteristics of cultured algae. Water insoluble gases such as oxygen, hydrogen or ozone are fed into the liquid medium via a porous element so that charged bubbles are formed. These have the effect of attracting small lightweight particles such as organic waste substances. These rise to the surface and help to clean the culture medium, particularly when as a froth forms on the surface of the medium. A foam separation device is used to take away the undesirable organic substances from the liquid medium.

Another method of cultivation is a photobioreactor system. These are closed systems and so risk of infection and contamination can be controlled and minimised. At its most unsophisticated, a photobioreactor system can be a pond covered by a greenhouse. At a more sophisticated level, the photobioreactor can be made of acrylic tubes through which the water, algae and nutrients flow. The tubes can be subjected to artificial light of desired intensity and photoperiod or solar light and the temperature controlled. Photobioreactors may have internal cleaning systems whereby algal growth and production is not stopped. There is also monitoring of oxygen levels and cell density levels to determine the best time for harvesting. Filters are used to extract algal cells from the photobioreactor. Whilst photobioreactors are more productive than ponds, their capital and running cost is much higher, though less area is needed for their installation and operation.

Another form of photobioreactor is a flat panel reactor, which is basically a flat, transparent vessel in which mixing is carried out directly in the reactor with air sparging. Such reactors allow good photosynthetic efficiency and provide a system for cultivating lipid accumulating strains of algae. One design of a flat panel reactor includes baffles to increase liquid turbulence and therefore generate a mixing effect.
Other forms of photobioreactor design which have appeared and been investigated include an annular reactor and a dome reactor.

Apt, K.E. and P.W. Behrens (1999) “Commercial developments in microalgal biotechnology” Journal of Phycology, 35: 215-226 is a review of the progress made in commercial microalgal biotechnology. The article summarises the algal production of nutritional products, including algal oils containing long-chain polyunsaturated fatty acids (LCPUFAs). The production techniques are reviewed and there is mention of how heterotrophic species, e.g. *Chlorella* can be used in a heterotrophic system to achieve greater yield in comparison to an auxotrophic system.

Posten C (2009) “Design principles of photo-bioreactors for cultivation of microalgae” 9: 165 – 177 is a detailed review. Reactor designs are surveyed and whilst the physical parameters and input side of the systems is discussed in detail, all of the disclosure relates to microalgae being grown in a liquid medium.

Clarens *et al.* (2010) “Environmental life cycle comparison of algae to other bioenergy feedstocks” Environ. Sci. Technol. 44: 1813 – 1819 explores how the environmental footprint of algal production in ponds can be reduced by using flue gas and waste water, e.g. municipal waste water providing a source of nitrogen and phosphorus. In looking towards sustainability and economics of pond growth of algae for bioenergy to place it on a par with terrestrial crops, the energy input upstream can be minimised by resorting to waste streams of CO₂ and nitrogen containing waste water.

Norsker *et al.* (2010) “Microalgal production – a close look at economics” Biotechnology Advances doi.10.1016/ibiotechady.2010.08.005 makes a detailed breakdown of variables and makes calculations to compare the costs and efficiency of running a pond system, a tubular photobioreactor and a flat panel photobioreactor in two locations. In the optimal tropical location a flat panel bioreactor is the most cost efficient system.
Robertson, D. et al. (2011) “A new dawn for industrial photosynthesis” Photosynthesis Research 107(3): 269-277 is a scientific article calculating the efficiencies of solar conversion by algae in industrial scale biomass production systems. From a systematic analysis, a most efficient pond process is proposed and involves a direct synthesis of a diesel-like alkane mixture by genetically engineered cyanobacteria which excretes its product during a continuous production process.

US 6524486 Borodyanski et al. describes a process of separating microalgae from water without rupturing cells. The method comprises the steps of flocculation, flotation and dehyration. Microalgae in suspension are mixed with modified starch and other flocculating agents. The suspension is then passed to a flotation column. Carbon dioxide or air is fed into the flotation column to produce bubbles of uniform size in the flocculated suspension. This generates a layer of foam on the top of the fluid column and this contains microalgae which are skimmed off through an overflow outlet. The flotation column is telescopic which advantageously allows the overflow outlet for skimming off the foam to be positioned with the foam for efficient removal. The foam containing the microalgae is then passed to a filtration unit for filtration through a cloth, followed by drying of the filtered algae.


WO2009/040383 (Proviron Holding) describes a bioreactor structure constructed so that it forms a closed volume that can be pressurised compared to atmospheric pressure, thereby being a self-supporting structure.

EP 0207475 A2 KANEGAFUCHI KAGAKU KOGYO KABUSHIKI KAISHA discloses a method of culturing micro-organisms, including microalgae, which are immobilised to a surface. A surfactant is included in the culture medium and this serves to increase the yield and recoverability of the lipids produced by the micro-organism
culture. However, in case of unwanted foaming caused by the surfactant, an antifoaming agent is added to the culture.

WO2007/025145 describes a closed culture system for algae and the production system for biodiesel. To the extent that there is any foaming of culture medium, this is seen to be associated with contamination with unwanted bacteria and so any froth or foam is removed.

Foam bed reactors for chemical-physical treatment of gases are known. See for example, Shah P S, Mahalingam R (1984) "Mass Transfer with Chemical Reaction in Liquid Foam Reactors" AIChE Journal, 30(6):924-934, and Asolekar S R et al (1988) "A Model for a Foam-Bed Slurry Reactor" AIChE Journal, 34(1):150-1540. In a foam bed reactor, a gas is passed through a liquid, usually containing surfactants, so that foam is generated. As the gas and foam rises in a column for example, soluble impurities are transferred from the gas phase to the liquid phase and then as the foam breaks the resulting liquid drains down and can be removed.

In the field of microalgae cultivation, where foam occurs it is used as a way of lifting small organic debris from the liquid growth medium and the foam is then skimmed off. Where foam is a hindrance to cultivation it is suppressed with antifoaming agent or removed.

Where unicellular algae are grown in a liquid medium and harvested, the process includes a dewatering step. This can be carried out using a settler, centrifuge, filter, adsorbent and/or other separation means. Additionally, particularly where the algae are in a dilute suspension, chemical additives such as flocculants and coagulants can be used as an initial step. WO98/28083 (Eastman Chemical Company) refers to disadvantages of chemical additives, especially in relation to nutritional products and describes a process of adsorptive bubble separation in which the cells of harvested microalgae are first ruptured and then subjected to a process of froth flotation. The process involves generating fine bubbles of gas in the liquid medium which make intimate contact with the ruptured algal cells. The ruptured algal bodies agglomerate on the surface of the bubbles. Bubbles are less dense than surrounding liquid and so float forming a froth which can be more readily separated from the liquid medium.
Various forms of froth flotation are described, including mechanical froth flotation, e.g. using a Denver D12 flotation machine, pneumatic froth flotation, e.g. using a Jamieson Cell; or by using spargers in columns or by using an air sparged hydrocyclone (ASH).

CN102127509 (Xinao Technology Dev Co Ltd) describes a harvesting and separation process for cultured microalgae involving the generation of a foam which lifts and concentrates the microalgae from liquid solution. A surfactant is added to the algal suspension and then a gas is bubbled into it to produce foam. The foam floats to the surface to form a layer which is recovered.

GB984403 A (Boeing) describes a process of separating algal cells from a liquid medium comprising the step of acidifying the liquid medium and then bubbling a gas through the medium to cause a foam containing algae to form. The foam is then separated from the liquid medium. The method is also adapted for separating green algae from bleached algae whereby after acidification air is bubbled through the algal suspension to form foam which is taken off; then carbon dioxide is bubbled through the remaining acidified algal suspension to produce foam in which the bleached algae are concentrated and taken off.

US2002/079270 A (Borodyanski) describes a method of separating microalgae from water without rupturing the cells. The process comprises steps of flocculation, flotation and dehydration. The flocculating agent is a modified starch and a flotation column is used to generate a froth of liquid, algae and flocculating agent. The flotation column has a telescopic construction which permits it to be adapted to accommodate a varying depth of the froth layer.

Some inventors have tried to improve algal cell production in cultures by attempts to increase the potential for carbon dioxide uptake and depletion of oxygen produced by the photosynthesising algae. US2009/151241 A (Dressler) describes using perfluorodecalin in a 4% to 20% solution in water for this purpose of increasing the concentration of carbon dioxide in the solution containing the algae. The perfluorodecalin is also believed to further enhance algal growth by carrying away oxygen from the algae.
Some attempts have also been made to enhance the production of certain lipids from moulds or algae. JP62006694 A (Kanegafuchi Chemical KK) describes how a mould or alga having lipid biosynthesis ability, is transplanted to a culture medium consisting of a carbohydrate, hydrocarbon and required nutrient source in the presence of 0.005W5wt% surfactant. The surfactant may be an anionic surfactant, such as polyoxyethylene alkyl(allyl) ether sulfate salt or nonionic surfactant, such as polyoxyethylene sorbitan ester of fatty acid. Following growth of algae in the culture medium the resultant lipids are recoverable.

The inventors have discovered that microalgae species can grow within the liquid film of liquid foams and can withstand the physical processes of foam formation and foam break-up. This has opened the possibility of growing microalgae and cyanobacteria as a component part of liquid foams in a variety of cultivation systems. The terms “cyanobacteria” and “blue-green algae” are used synonymously herein and are interchangeable. References to algae herein include references to both microalgae and blue-green algae.

Accordingly, the present invention provides a method of culturing microalgae or cyanobacteria comprising culturing the microalgae or cyanobacteria when carried by or in the liquid phase of a foam, the foam comprising a gas and a liquid growth medium. The culturing of microalgae or cyanobacteria involves the growing of these organisms in the sense that the numbers of individual cells are increased. The growth medium is one capable of allowing microalgal or cyanobacterial culture.

A foam may be characterised as a mass of bubbles formed of gas and a liquid, particularly a liquid algal growth medium.

Advantageously, the present invention allows for enhanced mass transfer because of the high gas/liquid specific surface area. This is advantageous for O₂, CO₂ but also NOx, also CO and other gases that might be used. The present invention advantageously greatly enhances mass transfer of CO₂ and O₂ in photobioreactors, reduces the energy requirement for mixing and gas-transfer in small and large-scale algal production systems (e.g. photobioreactors) and reduces the liquid volume
resulting in an increase in biomass concentration. The cost for photobioreactor construction is reduced.

When the growth medium is a liquid algal growth medium it may be any suitable medium comprising nitrogen, phosphorus and potassium. Optionally the medium may include one or more of silica, iron, sulphur, magnesium, calcium and/or trace minerals. Being a foam, the liquid growth medium forming the liquid phase may also include a foaming agent, e.g. a protein which may be an algal protein, a plant protein or an animal protein. Albumins are examples of animal proteins which serve as suitable foaming agents, e.g. bovine serum albumin (BSA).

The microalgae or cyanobacteria and/or components of the same may be used as foaming agents in the culture of algae or blue green algae in accordance with the invention.

After harvesting of the algal cells and extracting the useful products, such as lipids and pigments, there are proteins left over. These proteins, or certain fractions, can advantageously be used as foam stabilising agents. (See for example, Schwenzfeier A, Wierenga PA, Gruppen H (2011) “Isolation and characterization of soluble protein from the green microalgae *Tetraselmis sp.*” Bioreour. Technol. 102(19): 9121-9127. Some algal strains can be grown in foams without addition of additional foam agents, such as *Neochloris oleoabundans*.

Methods of the invention may further comprise exposing the microalgae or cyanobacteria suspended in foam to light. The light may be sunlight or artificial light.

The foam is preferably introduced into the growth chamber of a photobioreactor, more preferably at the base of the growth chamber so that the foam may flow upwards through the growth chamber when exposed to light.

In preferred methods, the foam is generated by passing gas through a liquid growth medium supplemented with the foaming agent.
The microalgae or cyanobacteria may be introduced into the liquid prior to foam generation using a foaming agent which is added to the microalgae or cyanobacteria in liquid. Alternatively, the microalgae or cyanobacteria may be introduced into the foam once it is formed. In this respect a foaming agent is needed with the liquid growth medium.

In accordance with the methods of the invention, after a period of time of exposure to light and growth of the microalgae or cyanobacteria, at least a portion of foam is taken from the growth chamber. The methods of the present invention may therefore be run in a continuous or batch mode; preferably continuous. In continuous mode, there is the advantageous extraction of oxygen from the growth chamber so that a steady state of optimal production may be achieved.

Also in accordance with the invention, gas which is, or which comprises, CO₂ is supplied to the growth chamber, whether to produce the foam or not, e.g. by optional separate input to the chamber. The gas containing the CO₂ may be air enriched with CO₂, e.g. combustion gas or flue gas.

Gas is preferably then extracted from the foam taken from the growth chamber and microalgae or cyanobacteria are thereby recovered in a liquid medium. The process of recovering microalgae from foam may involve the use of a foam breaker.

The resulting microalgae or cyanobacteria in liquid medium may then be dewatered and optionally dried. The biomass concentration in the liquid phase is preferably as high as possible, which may be of the order of more than about 10g/l so that advantageously less energy is required for the process of dewatering. In preferred aspects, the biomass concentration in the liquid phase is in the range 1 – 100 g/l; preferably greater than about 10 g/l and/or less than about 50 g/l. The range 10 – 50 g/l is a preferred range, and in some aspects the biomass concentration may be in the range 10 – 30 g/l, e.g. about 20g/l.

The invention also provides a photobioreactor comprising a chamber for culturing microalgae or cyanobacteria under illumination, characterised in that at least a
portion of the illuminated culture chamber contains a foam comprising microalgae or cyanobacteria.

The invention also includes a photobioreactor comprising a foam generator.

The invention further includes a photobioreactor comprising a chamber for culturing microalgae or cyanobacteria under illumination, characterised in that the photobioreactor comprises a foam generator, and at least a portion of the illuminated culture chamber contains a foam comprising microalgae or cyanobacteria.

In embodiments of the photobioreactor which comprise a foam generator, it may be simply at least one hole in a gas supply line, e.g. holes arranged in a gas supply tube. For example a plastics or silicone tube may run the length of the base of the photobioreactor chamber and have a series of pin holes allowing for small volumes of gas under pressure to escape into liquid at the bottom of the photobioreactor chamber. In other embodiments there may be a membrane perforated with holes and gas is supplied to one side of the membrane and escapes into liquid and/or foam in the photobioreactor on the other side. In other embodiments, the gas supply line may carry gas outlets at intervals along its length, or a series of gas supply lines may feed into the photobioreactor, each one terminating in a gas outlet. However the gas supply line(s) may be arranged, the gas may be supplied via one or more spargers which may be, or may include, needle spargers.

A photobioreactor of the invention may further comprise a foam breaker.

Ideally, a photobioreactor of the invention is a flat panel photobioreactor.

Without wishing to be bound by any particular theory, the inventors identify an advantage in a foam reactor of the invention, whereby a high interfacial area between gas and liquid can be created at a low pressure drop. As such, the energy required for transferring gaseous carbon dioxide and oxygen to and from the liquid phase is greatly reduced.
Furthermore, a foam reactor of the invention greatly reduces the energy requirement of separating microalgae from the water phase. This is related to the fact that the microalgae density within liquid films can be an order of magnitude higher than in typical microalgae production systems. At the same time less process water needs to be treated before discharging or recycling the water.

Additionally, a foam reactor of the invention may contain a limited water volume resulting in a low pressure drop relative to the height of cultivation systems (e.g. photobioreactors). The carrier capacity of the structure supporting the photobioreactors therefore can be considerably reduced, thereby saving on construction costs of (large-scale) photobioreactors.

Described herein is a foam comprising a gas phase and a liquid phase which is an algal growth medium. In such a foam, the gas phase may be air, a combustion gas, CO₂-enriched air, or CO₂. The relative composition of individual gases in the foam at locations in the photobioreactor may differ depending on a range of variables, not least the composition of the gas input used to make the foam, the rate of passage of foam in the photosynthetic chamber of the bioreactor, and the amount of photosynthetic activity taking place. A flue gas or combustion gas is a preferred input gas which supports microalgae or cyanobacterial growth.

Foam which includes microalgae may include one or more of the following: Nannochloropsis oculata, Nannochloropsis gaditana, Nannochloropsis salina, Tetraselmis suecica, Tetraselmis chuii, Nannochloropsis sp., Chlorella salina, Chlorella protothecoides, Chlorella ellipsoidea, Dunaliella tertiolecta, Dunaliella salina, Phaeodactulum tricornutum, Botryococcus braunii, Chlorella emersonii, Chlorella minutissima, Chlorella pyrenoidosa, Chlorella sorokiniana, Chlorella vulgaris, Chroomonas salina, Cyclotella cryptica, Cyclotella sp., Ettlia texensis, Euglena gracilis, Gymnodinium nelsoni, Haematococcus pluvialis, Isochrysis galbana, Monoraphidium minutum, Monoraphidium sp., Neochloris oleoabundans, Nitzschia laevis, Onoraphidium sp., Pavlova lutheri, Phaeodactylum tricornutum, Porphyridium cruentum, Scenedesmus obliquus, Scenedesmus quadricaula Scenedesmus sp., Stichococcus bacillaris, Spirulina platensis, Thalassiosira sp. The species of algae may be grown as a monoculture, or as a combination or combinations of species.
Alternatively, the foam may include cyanobacteria (blue-green algae). Species of cyanobacteria suitable for use in the invention may include by way of example, but not limited to the following: Acaryochloris sp., e.g. Acaryochloris marina; Anabaena sp., e.g. Anabaena variabilis; Arthospira sp., e.g. Arthospira platensis; Chlorobium tepidum; Chlorobaculum sp.; Cyanotoce sp.; Gloeobacter sp., e.g. Gloeobacter violaceus; Microcystis sp., e.g. Microcystis aeruginosa; Nostoc sp., e.g. Nostoc punctiforme; Prochlorococcus sp., e.g. Prochlorococcus marinus; Synechococcus sp., e.g. Synechococcus elongates; Synechocystis sp.; Thermosynechococcus elongates; Trichodesmium erythraeum; Rhodopseudomonas sp., e.g. Rhodopseudomonas palustris.

Also described herein are compositions for making a foam comprising a liquid algal growth medium and a foaming agent, as hereinbefore defined.

The invention will now be described in detail by way of examples and with reference to the drawings in which:

Figure 1 shows an overview of a laboratory set-up of a foam bed reactor supporting microalgae growth.

Figure 2 shows a close up of the base of the laboratory scale photobioreactor of figure 1.

Figure 3 is a drawing of the panel photobioreactor and separator shown in figures 1 and 2.

Figure 4 is a graph of data from example 1 showing an increase in biomass concentration in the foam bed reactor shown in Figure 1 and 2 (and diagrammatically in Figure 3) during a growth experiment.

Microalgae are been grown efficiently and successfully within the liquid films of liquid foams. In a process of the invention, a liquid foam is formed and passed into a photobioreactor structure. The algae present in the liquid film layers of the foam will
grow based on the light energy supplied via the transparent photobioreactor surfaces. Within the liquid phase of the foam, inorganic nutrients are dissolved in order to sustain nutrient-replete growth. Carbon dioxide is supplied via the gaseous phase of the foam. Also, photosynthetically produced oxygen is removed via the gaseous phase. The liquid foam is formed by dispersing fine bubbles of air, or air enriched with carbon dioxide, or any combustion gas, inside the liquid via a gas distributor.

The liquid foams are based on water, including the necessary algal nutrients, to which foam forming agents may be added, such as proteins (e.g. bovine serum albumin (BSA)). The following may be used in the formation of foams in accordance with the invention:

- Water soluble proteins, e.g. BSA.
- Soluble polysaccharides: e.g. alginate, hyaluronate, carrageenans, chitosan or starch.
- Detergents, e.g. small molecular surfactants: e.g. tween, sodium dodecyl sulphate (SDS), cetyltrimethylammonium bromide (CTAB).
- Microparticles of biological origin, e.g. hydrophobic cellulose particles, microalgae cells.
- Microparticles of non-biological origin: e.g. hydrophobic silica-based nanoparticles.

Combinations of different compounds, as exemplified above, may be used and are very effective in forming foams.

There are instances where the microalgae or cyanobacteria alone in the presence of growth medium and without the addition of specific foaming agents form foams in accordance with the invention. The average skilled person will be able to determine the optimal amounts of foaming agent, or whether or not any particular foam agent is needed for a particular species or microalgae or cyanobacteria, by way of simple testing involving bubbling of gas into a growth medium containing the algae.

When detergents (e.g. small molecular surfactants) are used, these quickly form foams because of the lowering of surface tension. However, these foams are often unstable and the water in these foams quickly drains away. Proteins and
polysaccharides can be added to stabilize the foam because they limit drainage of water. Also, certain hydrophobic microparticles can be used to “stabilize” foams. When required, the person of average skill in the art will be able to select useful combinations of foam forming components for use in the invention.

One way of forming a suitably stable foam in accordance with the invention comprises the passing of appropriately sized gas bubbles through the liquid growth medium containing the algae and/or foaming agent. The size of the gas bubbles formed may fall in the range 1 – 1000 μm; preferably 10 – 500 μm; more preferably 10 – 250 μm or 10 - 100 μm in average diameter. The gas bubbles formed may be no more than 10 μm; no more than 20 μm; no more than 30 μm; no more than 40 μm; no more than 50 μm; no more than 60 μm; no more than 70 μm; no more than 80 μm; no more than 90 μm; no more than 100 μm; no more than 150 μm; no more than 200 μm; no more than 250 μm; no more than 300 μm; no more than 350 μm; no more than 400 μm; no more than 450 μm; no more than 500 μm; no more than 600 μm; no more than 700 μm; no more than 800 μm; no more than 900 μm; or, no more than 1 mm in average diameter.

The design of the foam generator, usually a sparger, is such that sufficiently small bubbles may be produced. The gas may be passed through a microporous substrate which is in contact with the liquid to be foamed and the average diameters of the pores of the microporous substrate are in the micron ranges noted above for the gas bubbles. The microporous substrates may be uniform in their average pore size diameter or they may be heterogenous and have a range of pore size diameters present.

The algal nutrient solutions contain suitable bioavailable forms of nitrogen (N), phosphorus (P), potassium (K), sulphur (S), magnesium (Mg) and calcium (Ca). Silica and/or iron or other trace elements will be known to be needed where certain marine microalgae need to be grown. Algal nutrients and culture media are available from commercial sources, e.g. Varicon of Malvern, Worcs, UK, or alternatively fertilizers can be used which are used in agriculture an horticulture, e.g. Yara.
In other embodiments of the invention, the surface characteristics of microalgae can be altered such that the microalgae themselves act as foam stabilizing agents.

So long as a photobioreactor is able to contain a liquid foam and has an appropriate surface area to volume ratio, then it can be of any suitable design. A good example is a flat panel photobioreactor. The surface to volume ratio should be such that at the biomass density required all light can be absorbed efficiently by the microalgae culture.

The height of the reactor is variable and will depend on the strength and stability of the foam. The foam is created at the bottom where there is a limited volume of liquid containing the foam stabilizing agent(s) and the microalgae. Over the complete cross sectional area of the bottom of the photobioreactor small gas bubbles are continuously created by a gas distributor resulting in a rising foam bed (see Figure 2). The gas distributor can be of varying design such as membrane diffusers, or gas distributors based on tubes with small holes in combination with the use of meshes to create homogenous bubbles. Due to continuous foam break up the foam needs to be re-created continuously. The rate of gassing determines the rate of foam formation and therefore should be balanced to the rate of foam break up. This balancing is even more important when considering that gassing of photobioreactors (see Clarenst et al., (2010); Norsker et al., (2011)) is one of the major energy inputs for the large-scale cultivation of microalgae and it should be minimized.

At the top of the photobioreactor the foam flows over into a foam breaking device. In the laboratory system (figure 1) a stirred tank reactor equipped with two turbine stirrers is used to break the foam but several other designs exist. In the foam breaker the gaseous phase is separated from the liquid phase containing the algae. The liquid phase can be partially harvested but the larger fraction is recycled to the photobioreactor where the foam is regenerated and the algae can commence growth. Optionally the foam is allowed to rise to high levels that the liquid films drain by itself and the foam breaks up spontaneously. Reactor design could even be adopted such that the cross sectional surface increases at a certain height leading to foam stretching and breaking.
A flat panel photobioreactor in accordance with the invention is shown in figure 1. There is a reactor space enclosed between two sheets of glass and side fixing plates. Growth medium is introduced into the reactor space from the base and passes slowly upwards. Figure 2 shows how the gas is distributed in the bottom in such a way that finely dispersed and homogenous foam is created. In this example an array of needle spargers is used to create the foam.

On the right hand side there is a foam breaker where the gas is separated from the liquid phase of the foam. The resultant liquid containing the microalgae is pumped back into the photobioreactor. The foam breaker consists of a stirred tank reactor mixed by two turbine stirrers. The gas is withdrawn from the zone between the two turbines which is free of foam.

In a photobioreactor in accordance with the invention, tubes may be used rather than a flat panel or panels; or a combination of tubes and panels may be used. The tubes can be acrylic tubes, but also appropriate are polycarbonate, polythene, PVC or glass. There is also a plastic film photobioreactor which may be used in accordance with the invention.

The methods and foams of the invention can be applied to many existing types of photobioreactor. For example, a photobioreactor as described in WO2009/040383 (PROVIRON HOLDING) or as described in WO2011/113006 (SOLIX) may be operated using a foam and a method in accordance with the present invention.

Other techniques for filtering algae from foam may include membrane filtration and/or centrifugation.

Referring in more detail to the drawings, figure 3 shows a diagram of a panel photobioreactor (1) made of opposed flat panels of glass. In use, the panel of the bioreactor defines an internal space which contains liquid foam containing growing microalgae. The panels are held apart by spacer elements along each edge which also act in part as sealing elements to create a chamber (2) for holding the algae in foam. The edges of the panels are liquid and gas tight sealed. There is a liquid input (3) feeding into the base of the chamber (2). There is a pump (4) for pumping
liquid in line with the liquid input (3). The base of the chamber acts as a liquid reservoir (5).

There is a gas input (6) for air, CO₂ or flue gas (arrow shows input flow). This supplies a gas chamber (7) beneath and separated from the chamber (2). There are valves and control means (not shown) the construction and operation of which will be well known to a person of average skill. The gas chamber (7) and the chamber (2) are separated by a silicone rubber membrane (8). There is a foam generation zone (F) at the base of the photobioreactor. (G) highlights the gas distributor, e.g. sparger. Spanning the membrane and held transversely to it are a multiplicity of needle jets (9). The needles (9) provide gas supply into the fluid reservoir (5). The assembly of gas chamber (7), membrane (8) and needles (9) accessing the liquid reservoir (5) provides a gas distributor/sparger.

At the opposite end of the chamber (2) is an outlet (10) for foam. The outlet (10) is connected to a pipe (11) which feeds into a separator unit (12). The arrow between (10) and (11) shows the direction of flow of foam containing microalgae.

Separator unit (12) consists of a generally tubular and closed body (13) with a concave base (14). Within the concave base in use is a foam-free suspension of microalgae in liquid. Mounted for rotation at the top of the body (13) and in line with the central axis of the separator (12) is a shaft (15) with blades (16) forming a turbine stirrer. The shaft is connected to a rotational drive and power source and control systems (not shown) and which will be familiar to a person of skill in the art. (F) shows the foam-free zone in the separator unit.

A bleed pipe (17) is positioned to allow gas to escape from the central portion of the separator volume near to the turbine stirrer. The arrow from the open end of the pipe shows the direction of gas flow. The gas is enriched in O₂ from the photosynthetic activity of the algae. The bleed pipe (17) passes through the top of the separator body. The inlet pipe (11) is connected to the chamber. There is a recirculation pipe (18) with inlet located at the base of the separator chamber (13). The recirculation pipe (18) is connected to the pump (4) and there is
a valve (not shown). (L) shows the direction of flow in use of liquid containing microalgae.

There is also a harvest pipe (19), the collector of which extends into the base of the separator body. The harvest pipe is under the control of a valve (not shown). The arrow shows the direction of flow of the harvested algae. In setting up the photobioreactor for use, a mix of growth medium and water is pumped via liquid pump (4) into the base of the panel (2) where a reservoir of liquid (5) forms. Gas under pressure is then turned on to supply the gas distributor /sparger. Gas escapes from the needles (8) into the liquid and forms bubbles. The bubbling of the liquid in the reservoir forms a foam. Algae in the foam are carried up into the chamber (2) that it fills. Continuous pumping of liquid and gas into the base of the chamber causes a flow of foam and algae which when in sufficient volume flows into outlet pipe 11. A dynamic steady state can be reached whereby the input of liquid, algae and foam at the base results in a flow of foam and algae from the top of the chamber.

Foam and algae are fed into the separator (12). Once sufficient foam fills the separator (12) then the turbine stirrer is rotated at speed. This flings the foam creating a foam free gas pocket in a central volume of the separator body. The outlet of the gas bleed pipe (17) is situated in this zone and gas is bled off. When carrying out this bleed, more oxygen-rich gas is removed from the system and helps maintain optimal growth environment for the algae in the chamber (2). Also, liquid containing algae forms in the base of the separator body. The algae and liquid is drawn into the recirculation pipe (18) and pumped back to the inlet of the chamber (2) to allow for foam formation with a new supply of CO₂ rich gas.

When required, algae and water are drawn from the base of the separator chamber (13) through the harvest pipe (19). The algae and water are concentrated to a concentration in the range 20 – 50 g/l whereby the algae is readily filtered off, dewatered and dried, and subjected to any desired further processing steps, depending on the strain of algae concerned and the product being made.
Illumination of the flat panel is from one side with two 300 W tungsten-halogen lamps.

The combination of panel photobioreactor and separator is run on a continuous basis, whereby the input materials of water, algae, nutrients, CO₂–rich gas, temperature and illumination are controlled and regulated to generate a dynamic steady state of foam and algae production.

**Example: Growth of microalgae in foam**

This experiment was carried out using the apparatus of figures 1 and 2 (also as shown diagrammatically in figure 3) and as described above with the green alga *Chlorella sorokiniana*. The dimensions of the panel photobioreactor are height x width x depth 0.6 m x 0.2 m x 0.03 m. The photobioreactor was gassed with an air-CO₂ mixture at a flow rate of 1.5 L min⁻¹ composed of 2 % v/v CO₂.

The algae were grown in water enriched with the following nutrients (composition expressed in mol L⁻¹): KH₂PO₄, 5.4x10⁻³; Na₂HPO₄·2H₂O, 1.5x10⁻³; MgSO₄·7H₂O, 1.6x10⁻³; CaCl₂·2H₂O, 0.9x10⁻⁴; CO(NH₂)₂, 60x10⁻³; EDTA ferric sodium salt, 0.3x10⁻³; Na₂EDTA·2H₂O, 0.1x10⁻³; H₃BO₃, 1.0x10⁻⁶; MnCl₂·4H₂O, 0.7x10⁻⁴; ZnSO₄·7H₂O, 0.1x10⁻⁴; CuSO₄·5H₂O, 0.7x10⁻⁵. The pH was adjusted to 6.7 with a concentrated solution of NaOH. As a foam agent, bovine serum albumin (BSA) was used at a concentration of 5 g L⁻¹.

The light intensity applied was 350 μmol PAR photons per square meter per second. The final cell concentration reached of 20x10⁶ μm³ per ml of culture liquid corresponds to a dry weight concentration of 8-10 g L⁻¹. During the first day of growth an average specific growth rate of 0.11 h⁻¹ was attained.

In figure 4 microalgae growth in a foam bed reactor measured in the laboratory is shown. These data clearly illustrated that rapid microalgae growth is possible within the foam and this is a surprising discovery.
CLAIMS

1. A photobioreactor comprising a chamber for culturing microalgae or cyanobacteria under illumination, characterised in that at least a portion of the illuminated culture chamber contains a foam comprising microalgae or cyanobacteria.

2. A photobioreactor as claimed in claim 1, further comprising a foam generator; preferably wherein the foam generator comprises at least one aperture leading from a supply of gas; more preferably the aperture is a hole in membrane or tubing; or a sparger; e.g. a needle sparger.

3. A photobioreactor as claimed in claim 1 or claim 2, wherein the gas supply is pressurised.

4. A photobioreactor as claimed in any of claims 1 to 3, wherein the gas is air, CO₂-enriched air, flue gas/combustion gas, or CO₂.

5. A photobioreactor as claimed in any preceding claim, further comprising a foam breaker.

6. A photobioreactor as claimed in any preceding claim, which is a flat panel photobioreactor.

7. A method of culturing microalgae or cyanobacteria comprising culturing the microalgae or cyanobacteria when carried by the liquid phase of a foam, the foam comprising a gas and a liquid growth medium.

8. A method as claimed in claim 7, further comprising exposing the microalgae or cyanobacteria suspended in foam to light.

9. A method as claimed in claim 7 or claim 8, wherein foam is introduced into the growth chamber of a photobioreactor.
10. A method as claimed in claim 9, wherein foam is introduced into the growth chamber of the photobioreactor at the base thereof.

11. A method as claimed in any of claims 7 to 10, wherein the foam is generated by passing gas through a liquid growth medium comprising a foaming agent and/or the microalgae or cyanobacteria.

12. A method as claimed in claim 11, wherein the foaming agent is selected from one or more of a protein, e.g. BSA; soluble polysaccharides: e.g. alginate, hyaluronate, carrageenans, chitosan or starch; detergents, e.g. small molecular surfactants such as Tween, sodium dodecyl sulphate (SDS), cetyltrimethylammonium bromide (CTAB); microparticles of biological origin, e.g. hydrophobic cellulose particles, microalgae or cyanobacteria cells; microparticles of non-biological origin, e.g. hydrophobic silica-based nanoparticles.

13. A method as claimed in any of claims 7 to 12, wherein microalgae or cyanobacteria are introduced into the liquid prior to foam generation.

14. A method as claimed in any of claims 7 to 13, wherein microalgae or cyanobacteria are introduced into the foam.

15. A method as claimed in any of claims 7 to 14, wherein after a period of time of exposure to light and culture of the microalgae or cyanobacteria, at least a portion of foam is extracted from the growth chamber.

16. A method as claimed in any of claims 7 to 15, wherein gas is extracted from the foam extracted from the growth chamber and microalgae or cyanobacteria are thereby recovered in liquid medium.

17. A method as claimed in claim 16, wherein the microalgae or cyanobacteria in liquid medium are dewatered and optionally dried.
Figure 2
# INTERNATIONAL SEARCH REPORT

**International application No**
PCT/EP2013/073065

## A. CLASSIFICATION OF SUBJECT MATTER

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According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N  C12P  C12M  A01G

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, FSTA, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>US 4 654 305 A (BARNETT STANLEY M [US] ET AL) 31 March 1987 (1987-03-31) the whole document column 1, line 44 - column 2, line 8 column 4, lines 3-18 -----</td>
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* Further documents are listed in the continuation of Box C.

* Special categories of cited documents:
  * "A" document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search

27 January 2014

Date of mailing of the international search report

03/02/2014

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2
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Fax. (+31-70) 340-3016

Authorized officer
van de Kamp, Mart

Form PCT/ISA/210 (second sheet) (April 2005)
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