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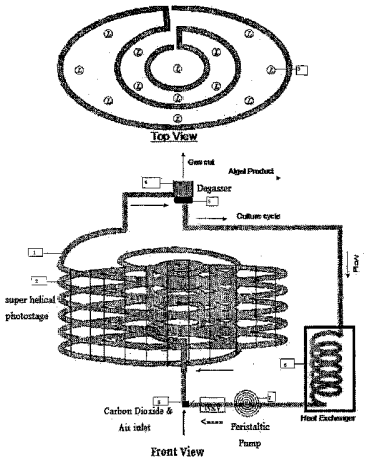
(54) Title: A PHOTO BIO-REACTOR FOR CULTIVATING AND HARVESTING A BIO-MASS AND A METHOD THEREOF



(57) Abstract: The present invention proposes to meet the long standing need for Photo Bio-reactors for the laboratory scale and mass cultivation of photosynthetic organisms such as spirulina and many other micro algae. The photo bio reactor comprises a system of coaxial helical transparent autoclavable tubular coils (1) for flow of a culture medium containing micro algae to be cultivated. The annular spaces between the adjacent coils and the space enclosed by innermost coil are provided with means (3) of providing predetermined alternate periods of light and darkness on the inner and outer surfaces of each of said coil and for temperature control of the medium to improve photosynthetic performance. The tubular coils have high surface area to volume ratio for extensive dissolution of the carbon-dioxide gas injected into the media. For cultivating biomass suitable for producing hydrocarbon, the organism used is the blue-green micro algae Botryococcus braunii.

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"A PHOTO BIO-REACTOR FOR CULTIVATING AND HARVESTING A BIO-MASS AND A METHOD THEREOF"

Field of Invention

This invention relates to a PhotoBioreactor for cultivation of *Botryococcus braunii* and a method for efficiently growing the microalgae. More particularly, the invention relates to a PhotoBioreactor and method for cutivation of biomass for producing hydrocarbon. The bioreactor and the method can be adapted to production of other microalgae including *spirulina.*

Botryococcus braunii, is a highly rich renewable source of hydrocarbons. The high cost of known microalgal culture systems relates to the need for light and the relatively slow growth rate of the algae. The photobioreactors must be designed keeping in mind that light is needed continuously for microalgal cultures. Since the intensity of light decreases rapidly with the depth of the culture, the geometry of the reactor is equally important. Important concern is to reduce the costs of these systems further to make them economically competitive.

Bioreactor System design depends on organism property, media property and system kinetics. The property of the organism is important because we need the organism to grow in the desired fashion so that the acclaimed metabolic route is followed to yield us the preponderance of desired product. Factors such as Oxygen / Carbon Dioxide dependency play crucial role, hence the importance of the size of the bubble and the time it spends in the liquid column. We also need to take care of the hydrodynamic shear since the growth may decrease sharply after increase of gas flow rate beyond certain value due to cell damage. Cell damage is dependent on the strain used, the bubble size based on the nozzle because smaller nozzle, which produce smaller bubbles, are more detrimental. But there is advantage of having smaller nozzle as it gives in more diffusion of the gas in the medium, and hence we need to make an optimum choice. Thus there is a need of proper sparger. In general superficial air velocity of 0.085 m/s can be considered the upper limit beyond which organism without cell wall can undergo damage. In case of scale up, when more quantity of gas is required, we must assure either increasing the number of nozzles or increasing the diameter does not exceed this velocity.

Algae use light as an energy source and obtain all the carbon they need from inorganic sources (CO_2) and are thus photoautotroph. Micro algal biotechnology has the potential to produce a vast array of products including foodstuffs, industrial chemicals and compounds with therapeutic applications, bioremediation solutions and hydrocarbons.

Background of the Invention

Oak Ridge National Laboratory and Ohio University have been demonstrating biomass production in photobioreactors using sunlight. Large solar collectors on the roof track the sun, collect sunlight, and distribute it through large optical fibers to the bioreactor's growth chamber. The fibers function as distributed light sources to illuminate cyanobacteria (algae). Each growth chamber consists of a series of illumination sheets containing the optical fibers and moist cloth-like membranes on which the algae grow. By stacking the membranes vertically and better distributing the light, more algae can be produced via photosynthesis in a smaller area. Ohio University photobioreactors use sunlight to sequestor carbon from coal-fired power plants as they produce biomass. A drawback of this system is that in horizontal cultivator systems, light penetrates the suspension only to 5 cm, leaving most of the algae in darkness. The top layer of algae requires only about 1/10th the intensity of full sunlight to maximize growth, so the remaining sunlight is wasted.

James C. Ogbonna and Hideo Tanaka have developed a prototype photobioreactor consisting of four units built with 0.5-cm-thick transparent Pyrex glass for the cultivation of C. pyrenoidosa. Each unit was equipped with a centrally fixed glass tube into which the light source was inserted. Transparent glass tubes were used as housings for the lamps, so the reactor was illuminated by simply inserting the lamps into the glass tubes (no mechanical fixing). Any light source could be used. Either 4-W fluorescent or halogen lamps with controllable light intensity were used as the illuminating system. Because the lamps are not mechanically fixed and can easily be replaced, the same reactor can be used for efficient cultivation of various cells by using a light source with controllable light intensity or by simply replacing the light source with one that gives the desired light intensity. For mixing, an impeller

modified in shape is used so that it did not touch the glass housing unit during rotation.

United States Patent 5,614,378 (Yang , et al, March 25, 1997) discloses a photo bio reactor comprising a hollow, cylindrical irradiation chamber, wherein said chamber comprises a top sealed to a cylindrical side wall; a bottom sealed to said cylindrical side wall; said top, bottom and cylindrical side wall together enclosing a cylindrical space; a first cylindrical light irradiator disposed in said cylindrical space and attached only to said top; and a second cylindrical light irradiator disposed in said cylindrical space and attached only to said bottom; wherein all cylindrical light irradiators and said cylindrical side wall are coaxial; the distance between said cylindrical side wall and cylindrical light irradiator closest to said side wall is within 1 mm to 20 cm, and the distance between each adjacent cylindrical light irradiator is within 1 mm to 20 cm, for a time sufficient to produce said biologically-active compound, while irradiating said cells with said first light cylindrical irradiator and said second light cylindrical irradiator. This photo bioreactor may also be used in a method to fix carbon dioxide, to produce, e.g., fuels and/or chemical feed stocks. In this application, the cultured cells are suitably, e.g., Botryococcus braunii.

United States Patent 4,952,511 (Radmer , August 28, 1990) teaches a photo bio reactor which comprising a tank for containing a liquid microbial culture; a high-intensity light source whose light is substantially entirely directed into a light compartment; said light compartment having at least one transparent wall extending into said tank; and said light compartment containing a tube of internally reflective prismatic sheet, said tube extending substantially from said light source to an end wall of said light compartment opposite said light source and said tube having transverse dimensions sufficient to substantially surround said light source, said tube further including a mirror at the end thereof opposite said light source oriented to reflect light back into said tube, wherein the light source, the tube and the mirror are arranged, so as to distribute light from said high-intensity light source substantially uniformly across the interior surface of the transparent wall of said light compartment.

United States Patent 5,137,828 (Robinson., et al. August 11, 1992) teaches an apparatus comprising an upstanding substantially cylindrical support structure; a

substantially transparent tube supported by the support structure and wound helically on the outside thereof so that, in use, the exterior of the wound tube is exposed to natural light, said tube containing at least living plant matter; a header tank at the top of the support structure, the upper end of the transparent tube being connected to the header tank; a pipe extending from the header tank to the bottom of the support structure, said pipe being connected to the lower end of the transparent tube; means for causing a synthesis mixture to flow under turbulent conditions through the tube, the header tank and the pipe; and means for withdrawing a biomass synthesis product from at least one of the header tank and the wound tube.

United States Patent 4,724,214 (Mori February 9, 1988) discloses an apparatus for photosynthesis comprising bath means containing a photosynthetic reaction bath, a plurality of tubular photoradiators arranged upright in said bath means in parallel array, upper support means and lower support means in said bath means for supporting the upper and lower end portions respectively of said tubular photoradiators, said tubular photoradiators being spaced from one another so as to define a plurality of upright passages between said tubular photoradiators, said upper support means closing off the upper ends of said upright passages, said bath means having a lower chamber underlying said lower support means, said lower support means having a flow-through portion which provides communication between said chamber and a first plurality of upright passages and a stopped up portion which blocks communication between said chamber and a second plurality of upright passages, conduit means leading to said chamber for supplying CO_2 -containing air, said flow-through portion and said stopped up portion of said lower support means being constructed and arranged such that said air passes from said chamber through said flow-through portion into said first plurality of upright passages, said air passing upwardly in said first plurality of upright passages and subsequently being directed generally laterally by said upper support means such that the air then passes downwardly in said second plurality of upright passages to subsequently again be directed generally laterally by said stopped up portion of said lower support means to once again pass upwardly in said first plurality of upright passages, whereby the air circulates in said bath means between said tubular photoradiators.

United States Patent 4,676,956 (Mori June 30, 1987) teaches an apparatus for photo synthesis comprising a reaction bath means for causing a photosynthetic reaction therein, said reaction bath means comprising an inner transparent wall and an outer transparent wall surrounding and spaced from said inner transparent wall to define an annular space between the inner and the outer transparent walls, the inner and outer transparent walls being generally vertically disposed, and a light source positioned radially inwardly of the inner wall; a plurality of narrow tubular photoradiators arranged upright in said annular space parallel to each other, each of said photoradiators being constructed to radiate light which propagates therethrough; and a disc rotatable in a horizontal plane below the reaction bath means and disposed perpendicular to the photoradiators to eject jets of carbon dioxide-containing air into the annular space.

Japanese Patent JP7023767 provides a culture tank opened at one end provided with a fixing plate having plural holes to close the open end of the culture tank. Plural protection tubes made of a transparent material and each having an insertion opening at one end and closed at the other end are fixed to the fixing plate parallel to each other by bonding the circumference of each hole to the outer circumference of the insertion opening. The fixed tubes are put into the culture tank. A rod light-source is removably inserted into each protection tube and a lid is applied to the fixing plate at the side opposite to the side holding the protection tubes. The circumference of the opening of the culture tank 10 is detachably fixed to the circumference of the fixing plate in liquid-tight state with an engaging means to provide this photo- bioreactor.

Japanese Patent JP9009953 teaches a method to obtain a new Botryococcus braunii B race strain producing hydrocarbons mainly consisting of 30-33C hydrocarbons and containing above a specific weight ratio of a 30C hydrocarbon to the total of hydrocarbons. Planktons on the surface of Ippeki lake (Shizuoka prefecture) are collected by using a Kitahara type plankton net with 25μ m mesh. The prepared specimen is observed by a microscope and a colony of an alge of the genus Botryococcus is collected. The colony is cultured in a test tube, a single colony of the alga of the genus Botryococcus is raked by a platinum wire while observing characteristic properties of the alga of the genus Botryococcus in a colony state, etc.,

by using a stereoscopic microscope and cultured in a float plate medium to give a new Botryococcus braunii B race strain which produces hydrocarbons consisting mainly of 30C to 33C hydrocarbons and containing ≥ 5wt.% 30C hydrocarbon based on the total of hydrocarbons and is Botryococcus braunii SI-1 strain.

Japanese Patent JP9234055 recites a method for efficiently culturing the strain of fine algae belonging to the genus Botryococcus braunii A race capable of producing a 33C hydrocarbon. A hydrocarbon produced by the alga of this strain contains a 33C hydrocarbon. The new strain of the fine alga belonging to the genus Botryococcus braunii. A race is cultured under intermittently irradiating the strain with an artificial light preferably once daily for 5-15 hours.

Japanese Patent JP9173050 teaches a method for efficiently culturing a microalgae belonging to green algae, which has ability to fix CO_2 by light energy and converts it into useful substances such as fuel comprises culturing microalgae (e.g. Botryococcus braunii CCAP807/1, etc.) belonging to such as Botryococcus, Chlorella, or Haematococcus, which belongs to green algae, while adding intermittently a disinfectant such as hypochlorous acid, hypochlorite, hydrogen peroxide and ozone to the culture medium intermittently each in a quantity of 0.01-200ppm so as to attain an effective quantity of the disinfectant in an open system under conditions of light illuminance and aeration of 0.05-5vvm air containing CO_2 in concentration of 0.03-30% at culturing temperature of 10-4^{O}C for about 10 days.

Accordingly, various types of Biorectors known in the art are described along with draw backs in the following paragraphs:

A common *CSTR type reactor*, which is externally illuminated by light source. This has a poor surface to volume value and since the illumination is from outside, a major part of light energy is wasted and inefficiently utilized.

A *common lab scale reactor* provides high light supply capacity and can be illuminated by both artificial and solar light. However, this has a poor surface to volume value and so a large number of light tubes would be required and thus would be energy consuming, a factor very detrimental for scale up.

A *Flat Plate reactor* derives the laminar concept from plants. If light energy has to be available continuously to the cells, a lamination of photobioreactor directed to the light source seems to be the best solution. Apart from the high surface/volume value offered in case of tubular reactors, plate-type reactors have some advantages with respect to compactness. The advantages of a flat plate photobioreactor include easy introduction of turbulence, easy approachability of inner walls, providing easy control of wall growth and fouling and they may be tilted towards sun, ensuring higher absorption of incident energy. However, there is a major demerit of problems faced when attempting to scale-up since a corresponding increase in width would lead to a reduction of light intensity in the inner section of the reactor.

In *a typical airlift reactor*, mass transfer is high and short liquid circulation time can be obtained. Further there is insignificant hydrodynamic stress. The riser can comprise of the dark zone and the down comer as photic zone. Airlift operation uniformly suspends microplantlets and improves exposure to light. Also there is continuous addition of nutrient medium and high cell density would be obtained in matter of time such that biomass is retained within vessel. Possibly modification include introducing alternating dark and transparent section in the inner column to take into account of organism specific flashing light effect. However, if the light source is from outside then there would be major loss in the energy.

One of the most promising closed tubular photobioreactors design is the *helical reactor*. Tubular systems are generally arranged in a Horizontal serpentine form and made of glass or plastic tubes. Recirculation of culture suspension can be obtained by airlift technology or by means of pump. Floating or submerging the tubes on or in a pool of water controlled temperature, oxygen degassing being guaranteed by flexible tube elements. Biocoil is an arrangement of coiled polyethylene tubes of about 30-60 mm diameters around an open circular framework.

Helical tubular photobioreactor is advantageous because it allows a larger ratio of surface area to culture volume to receive illumination effectively, thereby reducing the self-shading phenomenon. Thus there is improved light transfer since the tube diameter has short light path to reduce light attenuation through culture suspension

However this reactor has a drawback that upscaling involves large area for a given biomass production.

Objects of Present Invention

The principle object of the present invention is to propose a photo bioreactor for efficiently growing the microalgae *Botryococcus braunii* with an enhanced growth rate than obtained by bioreactors known in the art.

Another object of the present invention is to propose a method efficiently growing the microalgae *Botryococcus braunii* with an enhanced growth rate than obtained by the methods known in the art.

Yet another object of the present invention is to propose a bioreactor and method which is simple and economical to implement.

Other objects and advantages of the present invention will be clear from the description, examples, claims and drawings which follow.

Statement of Invention:

According to one aspect of the invention there is provided a photo bio-reactor for cultivating and harvesting a bio-mass suitable for producing hydrocarbon comprising: (i) a helical tubular system having at least two substantially coaxial helical transparent autoclavable tubular coils for flow of a culture medium containing micro algae to be cultivated, said coils being at least a first coil and at least a last coil, each coil being hydraulically connected to its adjacent coaxial coil, said coils having annular spaces interposed between the adjacent coils and a space enclosed by the inner diameter of innermost coil, the first and the last coil each having a free end; (ii) a means of providing periods of light and darkness on each point alternately for predetermined time periods on the inner and outer surfaces of each of said coil; (iv) a de-gasser chamber having hydraulic connection with said free end of first coil for removing unwanted gases from said culture medium; (v) optionally, a stirrer provided in said de-gasser chamber for keeping the bio-mass in suspension in said culture medium; (vi) said de-gasser chamber having hydraulic connection with a heat exchanger for controlling temperature of said culture medium; (vii) a means for causing flow

of said culture medium in said coils without said means coming into direct contact with said medium; and (viii) a gas injection means connected to said de-gasser chamber on one end and said free end of last coil on the other end.

According to another aspect of the invention there is provided a method of cultivating and harvesting a bio-mass in a photo bio-reactor, comprising the steps of: (a) circulating a culture medium containing a micro algae in said reactor at a predetermined flow rate; (b) providing alternately period of light and darkness for predetermined time periods on each point along the flow path of said circulating culture medium; (c) removing unwanted gases from said circulating culture medium; (d) controlling temperature of said culture medium between 25° and 36° C; (e) optionally, stirring said circulating culture medium to keep said micro algae in suspension; (f) injecting CO_2 and air into said circulating culture medium to ensure a predetermined gas flow rate; (g) providing continuous darkness for a predetermined time period on each point along the flow path of said circulating a culture medium; and (h) harvesting cultivated micro algae from said circulating culture medium.

Brief Description of Accompanying Drawings

Figure 1. shows the photobioreactor.

Figure 2. shows the cage around which the helical coil is wound.

Figure 3. shows the growth profile of *B. braunii* at 31°C

Figure 4. shows the hydrocarbon content profile of of *B. braunii* at 31°C

Figure 5. shows the growth profile of *B. braunii* at 27°C

Figure 6. shows the hydrocarbon content profile of of *B. braunii* at 27°C

Detailed Description of the Invention

The ability of photoautotrophic microbes to make use of solar energy for metabolism is dependent upon the effective surface area of the rays to which it is subjected, which reduces with increase in population. As opposed to heterotrophic microorganisms where mixing solves the distribution of the organic molecules as energy carriers by mixing, the supply of photons is based on the surface area. Light gradient would always tend to occur due to mutual shading of the cells and light absorption. The light

intensity in a photobioreactor decreases exponentially with the depth. Since the intensity of light decreases rapidly with the depth of the culture, the geometry of the reactor is equally important.

High alteration between subjecting to light regime and dark regime in the order of microseconds to up to a second results in enhancement of photosynthetic efficiency. It has been observed that turbulence in an optically dense culture increases the efficiency of light utilization by photosynthesis. This effect is denoted as a flashing light effect. This effect is due to the fact that photosynthesis does not cease at the instant when light is removed but continues until the energy absorbed in the prior light period has been used and chemically stored. Short cycle time flashing light effect could be a result of fast effect of electron acceptors associated with the photo-system II followed by their oxidation in the dark period. In turbulence, individual cells are subjected to a pattern of light and dark periods. The flashing light effect could thus increase photo-accepting capacity. The subjection of Light & Dark regime should not be in the order of seconds, as it would then lead to reduction in photosynthetic activity resulting in reduced biomass yield. But the exact order of the Light/Dark value depends on the organism of choice and the product required. However, it is evident that this Light/Dark cycle will determine the efficiency and productivity that we desire for a given compound. Algal culture is in light demand through antenna pigments in the cell. Antennas that are damaged need a recovery time.

Helical tubular bioreactor facilitates temperature control and restricts contaminants because it is a closed bioreactor. There is better CO_2 transfer from the gas stream to the liquid culture medium due to the extensive CO_2 absorbing pathway. Possibly, a modification may include introducing alternating dark and transparent section in the inner column to take into account of organism specific flashing light effect. Its disadvantages include requirement of a large land area for a given volume. Another aspect is that the system is suitable only for disperse culture suspension as pump action disperses cell clumps.

Some factors that influence the light requirement of an algae culture are:

1. *Type of algae culture and optimum wavelength:* The Light requirement of algae depends on the major pigment present on the algae. Different pigments absorb light in

different range of wavelengths; chlorophyll a in the range of 400-450 nm (between Violet and Blue) and around 680nm(Red), β-carotene 400-500 nm (Violet to Green), chlorophyll b (400-500nm). Further we also need to remember that energy delivered is inversely proportional to the wavelength, but the penetration is directly proportional to the wavelength. In Green algae Chlorophill a, Chlorophill b and β-carotene are light harvesting pigments which have their absorption wavelength maxima in the range 400nm-500nm and 620-680nm.

2. *Type of Light source:* Some of the efficient light sources from the electric consumption and wavelength requirement point of view are Light Emitted diodes (LEDs), Fluorescent lights, and incandescent/halogen lamps. Fluorescent lamps are used most frequently for the cultivation of phototrophic organisms. The emission wavelength emitted from the mercury vapor can be converted to continuous radiation by modifying the composition of the fluorescent material, which allows for an optimum spectrum of photosynthesis. LEDs are one of the most efficient one in converting electricity to light with desired wavelength. However, if broader wavelength is required then combination of LEDs has to be used. We also need to keep into account the increase in wavelength due to absorption of rays as Heat energy.

3. *Intensity of Light source:* The light intensity at which the cell growth begins is called the compensation intensity (Ic) and the light intensity after which no further increase in growth takes place with increased light intensity is called the saturation intensity (Is). After a certain more increased value of light intensity, decrease in specific growth rate starts to takes place. This phenomenon is called photoinhibition and the intensity is denoted (Id). Further, the values of Ic, Is and Id is strain and temperature dependent. The specific growth rate of algae increases linearly with light intensity up to the saturation light intensity, thereafter-light inhibition is observed. Generally, the Is increases as the temperature is increased resulting in increased specific growth rate.

In general, the helical system bioreactors prove to be better than the stirred system. This can be attributed to large dark volume characterized by cylindrical reactors. This dark volume can be taken care by inserting transparent pipes into the cylinder from the top through holes in the lid, holding fluorescent tubes, such that it can easily be

removed during sterilization. T-shaped multifunctional stirrer is used to agitate the suspension and to supply sterile air.

The denser is the culture of micro algae, the more problematic is the light penetration, and extremely limited. This restricts the commercial production to just flat plate photobioreactor and narrow bore tubular reactor. In spite of the efficient performance of thin channel flat plate types of photobioreactors, there is difficulty in scaling it up for production of significant quantities of product. This further limits our choice to tubular photobioreactor. The major disadvantage of tubular reactors is that for the same amount of volume, the area required is much more in comparison to other reactors. Other possibilities comprise of bubble column and airlift reactors that are more compact than tubular devices and can be deployed if a certain loss of productivity is acceptable.

From Commercial point of view, a PBR must have high productivity, large volume, low maintenance and building expenses, and flexibility and ease to control culture parameters.

Use of Immobilized Algal Cells

Immobilized cell culture offers important advantages over free suspension in particular when the cells are slow growing. Extra-cellular products can be recovered continuously with ease. Since micro algae are shear sensitive, immobilization can protect cells against hydrodynamic shear forces. Immobilization by Calcium Alginate beads results in cells with enhanced chlorophyll content in attempt to capture more of the available light. Gel-entrapped cells are thus protected from photoinhibition, a condition in which intense irradiance actually causes a loss in photosynthetic performance. However, Alginate gel-immobilized cells have a lower growth rates and lower biomass production relative to free controls, possibly because immobilization can reduce availability of substrate to the cells. It is to be noted that Immobilized cells retain the ability to produce hydrocarbons, whose structure and relative abundance are not affected by immobilization. PUF (Polyurethane Foams) support matrices have broad range of porosity and mechanical strength. However, the exposure to light to the cells drops down noticeably and so does the growth. Cotton gauze – immobilized B. braunii cells show higher levels of hydrocarbon production, biomass growth, and

photosynthetic activity when compared with cells immobilized in PUF. Despite advantages, the practicability of immobilized algal culture remains questionable unless the cells can be cultured in long-duration continuous culture and the hydrocarbons can be extracted continuously, say by doing some protein engineering for post-translational modification in the corresponding DNA sequence, or by selectively subjecting the beads to extracting solvent such that the hydrocarbon is extracted and the cell remains as it is inside the beads.

B. braunii has a disperse culture growth and growth into clump-form is not an advantage to the organism, rather causes reduction in the supply of oxygen to the inner cells. Hence application of Tubular coiled reactor is advantageously is the best for the purpose. High surface to volume value is desired for more or less equal distribution of light intensity. Hence application of Tubular coiled reactor serves best for the purpose.

In a preferred embodiment, to introduce proper Flashing light effect for the organism *B. braunii* dark strips of appropriate width are introduced at regular intervals, such that the order is not more than a second. For maximum utilization of energy, light is provided from inside and not from outside, such as inside the coiled helix in the Tubular coiled reactor. To ensure better distribution of light, straight tube-lights may be preferred over bent 'U' shaped tube-lights. Light of appropriate wavelength must be used which is optimum for *B. braunii.* Typically in Green algae Chlorophill a, Chlorophill b and ß-carotene are light harvesting pigments which have their absorption wavelength maxima in the range 400nm-500nm and 620-680nm. Either single fluorescent light can be deployed covering the entire wavelength of 400-700nm, or 2 LEDs can be made use of, one with the emission bandwidth of 400-500nm and another with 600-700 nm as LEDs have a narrow bandwidth. The choice would be governed by comparative economics. Light intensity is kept to be around saturation intensity, as an increase further would lead to decrease in growth rate and wastage of energy. Use of Solar-Light and optical fibre can be deployed since in a Tropical country such as India there is abundant supply of solar energy.

Care is taken to ensure appropriate and more-or-less uniform temperature as the saturation intensity changes with change in temperature, and would thus lead to errors

in observation. In general superficial air velocity of 0.085 m/s can be considered the upper limit beyond which organism without cell wall can undergo damage. Since each cell of *B. braunii* has its own cell wall, we can operate at values above this numerical figure, but at the same time the value should not be very high as micro algae are known to be shear sensitive.

The Carbon-Dioxide percentage of 5% can be considered to be optimum for microalgae growth though the exact optimum value would be dependent on other factors such as the strain & temperature.

Tubular Helical PBR takes into account of most of the above considerations. The only demerit is the large land area required for scale up as for a given volume the coiled tube would be very long. The present invention nullifies this effect and thereby reduces the problem of large land area.

In an embodiment, a peristaltic pump is provided it is not harmful for the shear-sensitive organism such as the *B. Braunii*. Usage of pump also reduces the chances of reverse flow. Additionally, the pump action also disperses cell clumps and thereby facilitates more of absorption of nutrients and light by individual cells. Care should be taken that pressure of the medium being pumped does not exceed the osmotolerance pressure which is 1 atmosphere (guage).

The present 'Flash Light Super Helical Photo Bio Reactor' takes care of the scalability issue of traditional helical photobioreactor and also introduces increase in the photosynthetic activity of the microalgae by Flash-Light effect.

A preferred embodiment of the bio-rector comprises a helical tubular system having at least two substantially coaxial helical transparent autoclavable tubular coils(1) for flow of a culture medium containing micro algae to be cultivated, each coil being hydraulically connected to its adjacent coaxial coil, said coils having annular spaces interposed between the adjacent coils and a space enclosed by the inner diameter of innermost coil, the first and the last coil each having a free end. The number of coaxial coils may be 2 or 3 or more. The end of each coil is hydraulically connected to the starting point of adjascent coil.

The primary requirements of the tube forming superhelical structure is that it should be transparent and autoclavable. Further, it should be non-fragile and economical.

One possibility was that of Glass. Though glass satisfies the first two criteria extremely well, it is highly delicate and mishandling or accidental touch can destroy the entire setup. Further, the cost of fabrication using Glass for the above scheme turns out to be extremely high. More importantly, the brittle and fragile nature of glass makes it incapable to tolerate high guage pressure over an extended period. Lastly, there is constraint on the fabrication in limiting the gap distance between any two adjacent rings to be not less than a centimeter, which reduces the compactness and thereby the scalability.

In a preferred embodiment, pipe material used which is resistant to heat and is autoclavable is transparent type Silicone Tube. Though the transparency of Silicone is not as good as that of a Glass, silicone is flexible, non-fragile, autoclavable and costs almost half of what it costs for fabrication using Glass. The photostage of present invention comprises said transparent autoclavable tubular coils made from silicon polymer material.

The bio reactor has a means(2,3) of providing Flash-Light effect i.e. periods of light and darkness on each point alternately for predetermined time periods on the inner and outer surfaces of each of said coil. In a preferred embodiment means(3) for providing periods of light is a plurality of tubelights uniformally placed in the annular space between two adjascent coils and the space enclosed by inner diameter of the innermost coil. In another embodiment, a plurality of light emitting diodes is used for this purpose. An optic fiber coil may be used to provide solar illumination in another embodiment. In another embodiment, a combination of these means are used. In a preferred photo bio-reactor means(2) of providing period of darkness comprises a plurality of opaque strips or wires of predetermined width or diameter which obstruct the light from means of illumination falling on various points along the surface of the helical tubular coils and thus provide periods of light and darkness for the culture medium circulating in the helical tubular coils. In a preferred photo bio-reactor said alternating light and darkness time periods on each point along the flow path of said circulating culture medium are in the ratio 1:0.1 to 1: 0.2. In an embodiment, these coils are wound around a 'wire cage'(2) as illustrated in Figure 2, which would provide strips of shadow or less light intensity zone at regular intervals to result in

alternating light and darkness time periods on each point along the flow path. Vertical strips of black-paper at the opposite face of the cage after the tube may be provided to further ensure reduction in light intensity in that zone. In another preferred embodiment of photo bio-reactor said means of providing periods of light and darkness comprises an electronic flashing device. In order to get best results, there is preferably a cycle comprising alternately continuous darkness time period of 8 hours after each period of 16 hours of said alternating light and darkness time periods.

The bio reactor has a de-gasser chamber(4) having hydraulic connection with said free end of the first coaxial coil for removing unwanted gases from said culture medium. Optionally, a stirrer(5) is provided in said de-gasser chamber for keeping the bio-mass in suspension in said culture medium. In a preferred embodiment, the stirrer is a magnetic stirrer or a small air-lift reactor for suspending the culture. The de-gasser chamber has hydraulic connection with a heat exchanger(6) for controlling temperature of said culture medium preferably between 25° C and 36° C. The heat exchanger may comprise a water bath for heating and/ or a chilling unit for cooling.

The water bath may comprise copper coils for flow of said culture medium, fitted inside a container for water circulation.

The bio reactor has a means(7) for causing flow of said culture medium in said coils without said means coming into direct contact with said medium. In a preferred embodiment this means is a perislatic pump(7), which acts on the outer surface of the tube to cause pumping of the culture medium without coming in contact with the medium. A gas injection means(8) is connected to said de-gasser chamber of the bio reactor on one end and said free end of last coil on the other end. In a prferred embodiment the gas injection means may be a Y-shaped junction(8) having two inlet arms and one outlet arm with said outlet arm connected to said free end of last coil and said two inlet arms connected to a CO_2 source and an air source respectively.The flow of the air plus carbon-dioxide and the pump together are kept such that the relative superficial velocity of the gas with respect to the liquid does not exceed the value 0.085 m/s. We can obtain such value by taking air flow rate to be < 0.170 m/s and the pump flow rate to be 0.085 m/s. In a preferred embodiment flow rate of said

circulating culture medium is from .085 m/s to 0.10 m/s and said gas flow rate is between 0.170 m/s to 0.200 m/s.

A preferred method of cultivating and harvesting a bio-mass in the photo bio-reactor of present invention comprises the steps of:

(a) circulating a culture medium containing a micro algae in said reactor at a predetermined flow rate;

(b) providing alternately period of light and darkness for predetermined time periods on each point along the flow path of said circulating culture medium;

(c) removing unwanted gases from said circulating culture medium;

(d) controlling temperature of said culture medium between 25° and 36° C;

(e) optionally, stirring said circulating culture medium to keep said micro algae in suspension;

(f) injecting CO_2 and air into said circulating culture medium to ensure a predetermined gas flow rate;

(g) providing continuous darkness for a predetermined time period on each point along the flow path of said circulating a culture medium; and

(h) harvesting cultivated micro algae from said circulating culture medium.

The micro algae used is *Botryococcus braunii.* The method may also be adapted for cultivating and harvesting *spirulina* or any other algae.

In the preferred embodiment, the modified Chu-13 medium is used to culture *B. braunii.* This medium has the following composition (Kgm^{-3}) at four-fold normal strength:

KNO_3	(0.2)	Copper	(0.02ppm)
K_2HPO_4	(0.04)	Cobalt	(0.02ppm)
$MgSO_4.7H_2O$	(0.1)	Molybdenum	(0.02ppm)
$CaCl_2.6H_2O$	(0.08)	Manganese	(0.5ppm)
Ferric Citrate	(0.01)	Boron	(0.5ppm)
Citric Acid	(0.1)		

The production of hydrocarbons in B. braunii appears to be growth associated, irrespective of the specific culture conditions and the nutrients used. The production

of hydrocarbon is energetically demanding and thus it leads to slow growth rate of algae and so is not dominated by the amount of nutrients present. The color of algal colonies is dependent on the carotenoid-to-chlorophyll ratio, which is affected by the intensity of light. Carbohydrates concentration, cellular nitrogen, and phosphorus content of *B. brauni* are decreased by extended exposure to a light intensity.

Colony size increase with increased light intensity initially when the cell concentration is low and sufficient light for photosynthesis was available. As the cell concentration increase, the average light intensity within the photobioreactor decreased because of mutual shading, and thus the production rates of extracellular polysaccharides and hydrocarbons decrease with decreasing average light intensity. The equilibrium colony size is determined by a dynamic balance between the mechanical strength of colonies and the hydrodynamics stress due to turbulence in the reactors.

The pH of the culture medium is generally adjusted to between 7.4 and 7.6 before inoculation. A regular increase in pH is observed during active growth followed by a slight decline later. The increase in pH is partly due to the consumption of dissolved CO_2 for photosynthesis. Similar changes in pH are observed in CO_2 enriched culture during exponential growth.

Potentially domestic sewage that has been pretreated by activated sludge treatment can be used as a medium for hydrocarbon production by *B. braunii*. Sewage can reduce the cost of producing the hydrocarbons. Secondary stage-treated sewage (STS) has been characterized, and found to contain a large amount of nitrogen (as nitrate) and phosphorus (as phosphates). Botryococcus cannot grow on industrial wastewater containing a high concentration of inorganic ions. Potentially, the productivity of continuous algal culture can be improved by optimizing the dilution rate. The optimal dilution rate is expected to depend on the strength of wastewater and the intensity of illumination.

The pH is adjusted to 7.5 before sterilization. Optimum temperature for growth is 25-30°C. Reviews of the different techniques available (flocculation, filtration, centrifugation and air flotation) have concluded that centrifugation is possibly the most reliable technique and only slightly more expensive than other techniques.

Here we have assumed that the goal of microalgal biotechnology efforts to recover a high value product from the microalgal biomass. Thus, the high value product needs to be separated from the biomass. Depending on the process, the microalgal cells may need to be physically disrupted. Both ball mills and high pressure homogenisers have been used successfully to disrupt microalgal cells to enhance recovery.

Depending on the product to be recovered, in the process it might entail reducing the water content of the microalgal biomass. Absence of water in the biomass enhances the recovery of lipid soluble components . Microalgal mass can be dehydrated in spray dryers, drum dryers, freeze dryers and sun dryers. In the case of Heat sensitive compounds commercial producers have developed technologies that limit exposure to conditions known to cause degradation.

In some cases the biomass may not need to be dehydrated, and the extraction and fractionation can be carried out on the wet biomass. Further downstream processing may be needed to isolate the active compound depending on the intended final product.

Use of solvents and enzymes might help with cellular disruption and product recovery but care must be taken regarding what aids are used if the product is intended for human consumption.

NIES–N-836. *B. braunii,* sourced from Japan (NIES culture collection centre) was obtained from Central Food Technological Research Institute, Mysore, India and was used for evaluation of the performace of the reactor and other studies.

Example 1

In order to inroduce flash light effect, a wire strip is intermittently interposed between light source and tube. In an embodiment, for every tubular length of 2.3 cm the wire strip has a thickness of 0.3 cm.

Thus *Flash-Light effective ratio* = time in darkness / time in light = 0.3/2.0 =1: 0.15

Example 2

The Biomass estimation is carried out by the following steps:

1. 25ml. medium is harvested.

2. Take eppendorf and pre-weigh it.
3. Transfer the pellet into eppendorf and lyophilize.
4. Weigh the eppendorf again. The difference in weights is the weight of the biomass.

Hydrocarbon estimation by gravimetric method:

1. Sonicate known quantity of biomass in n-hexane for 30 min.
2. Centrifuge and take the supernatant in pre-weighed vials and evaporate the solvent under nitrogen to complete dryness.
3. Repeat the extraction 2 more times, pool the solvents and evaporate to complete dryness with nitrogen.
4. The weight of hydrocarbons is calculated by the difference in the weight of vial.

Determination of growth of B. braunii:

The doubling time for biomass growth rate in the exponential phase is found to be nearly 8-9 days at 27°C and 14-15 days at 31°C as shown in the Figure 5 and 3 respectively. The hydrocarbon accumulation was found to be growth associated and comprised 50-55% of the cell mass as shown in Figures 4 and 6.

I claim:

1. A photo bio-reactor for cultivating and harvesting a bio-mass suitable for producing hydrocarbon comprising:

(i) a helical tubular system having at least two substantially coaxial helical transparent autoclavable tubular coils for flow of a culture medium containing micro algae to be cultivated, said coils being at least a first coil and at least a last coil, each coil being hydraulically connected to its adjacent coaxial coil, said coils having annular spaces interposed between the adjacent coils and a space enclosed by the inner diameter of innermost coil, the first and the last coil each having a free end;

(ii) a means of providing periods of light and darkness on each point alternately for predetermined time periods on the inner and outer surfaces of each of said coil;

(iii) a de-gasser chamber having hydraulic connection with said free end of first coil for removing unwanted gases from said culture medium;

(iv) optionally, a stirrer or 'perfusion air-lift reactor' provided in said de-gasser chamber for keeping the bio-mass in suspension in said culture medium;

(v) said de-gasser chamber having hydraulic connection with a heat exchanger for controlling temperature of said culture medium;

(vi) a means for causing flow of said culture medium in said coils without said means coming into direct contact with said medium; and

(vii) a gas injection means connected to said de-gasser chamber on one end and said free end of last coil on the other end.

2. A photo bio-reactor according to claim 1, wherein said transparent autoclavable tubular coils comprise silicone polymer material.

3. A photo bio-reactor according to claim 1, wherein said means of providing light comprises incandescent lighting device selected from a plurality of tube-lights, light emitting diodes and optical fibers or a combination thereof.

4. A photo bio-reactor according to claim 1, wherein said means of providing period of darkness comprises a plurality of opaque strips or wires of predetermined width or diameter.

5. A photo bio-reactor according to claim 1, wherein said means of providing periods of light and darkness comprises an electronic flashing device.

6. A photo bio-reactor according to claim 1, wherein said stirrer is a magnetic stirrer or a 'perfusion air-lift reactor'.

7. A photo bio-reactor according to claim 1, wherein said heat exchanger comprises a water bath for heating and/ or a chilling unit for cooling.

8. A photo bio-reactor according to claim 7, wherein said water bath comprises copper coils for flow of said culture medium, fitted inside a container for water circulation.

9. A photo bio-reactor according to claim 1, wherein means for causing flow of said culture medium comprises a peristaltic pump or any pump maintaining sterile condition.

10. A photo bio-reactor according to claim 1, wherein gas injection means comprises a Y-shaped junction having two inlet arms and one outlet arm with said outlet arm connected to said free end of last coil and said two inlet arms connected to a CO_2 source and an air source respectively.

11. A method of cultivating and harvesting a bio-mass in a photo bio-reactor according to claim 1, comprising the steps of:

(a) circulating a culture medium containing a micro algae in said reactor at a predetermined flow rate;

(b) providing alternately period of light and darkness for predetermined time periods on each point along the flow path of said circulating culture medium;

(c) removing unwanted gases from said circulating culture medium;

(d) controlling temperature of said culture medium between 25° and 36° C;

(e) optionally, stirring said circulating culture medium to keep said micro algae in suspension;

(f) injecting CO_2 and air into said circulating culture medium to ensure a predetermined gas flow rate;

(g) providing continuous darkness for a predetermined time period on each point along the flow path of said circulating a culture medium; and

(h) harvesting cultivated micro algae from said circulating culture medium.

12. A method according to claim 11, wherein said micro algae is *Botryococcus braunii.*

13. A method according to claim 12,wherein said culture medium is a modified Chu-13 medium.

14. A method according to claim 11, wherein said alternating light and darkness time periods on each point along the flow path of said circulating culture medium are in the ratio 1:0.1 to 1: 0.2.

15. A method according to claim 11, wherein flow rate of said circulating culture medium is from .085 m/s to 0.10 m/s.

16. A method according to claim 11, wherein said gas flow rate is between 0.170 m/s to 0.200 m/s.

17. A method according to claim11, wherein said continuous darkness time period is 8 hours after each period of 16 hours comprising said alternating light and darkness time periods.

18. A method according to claim11 as and when used for cultivating *spirulina.*

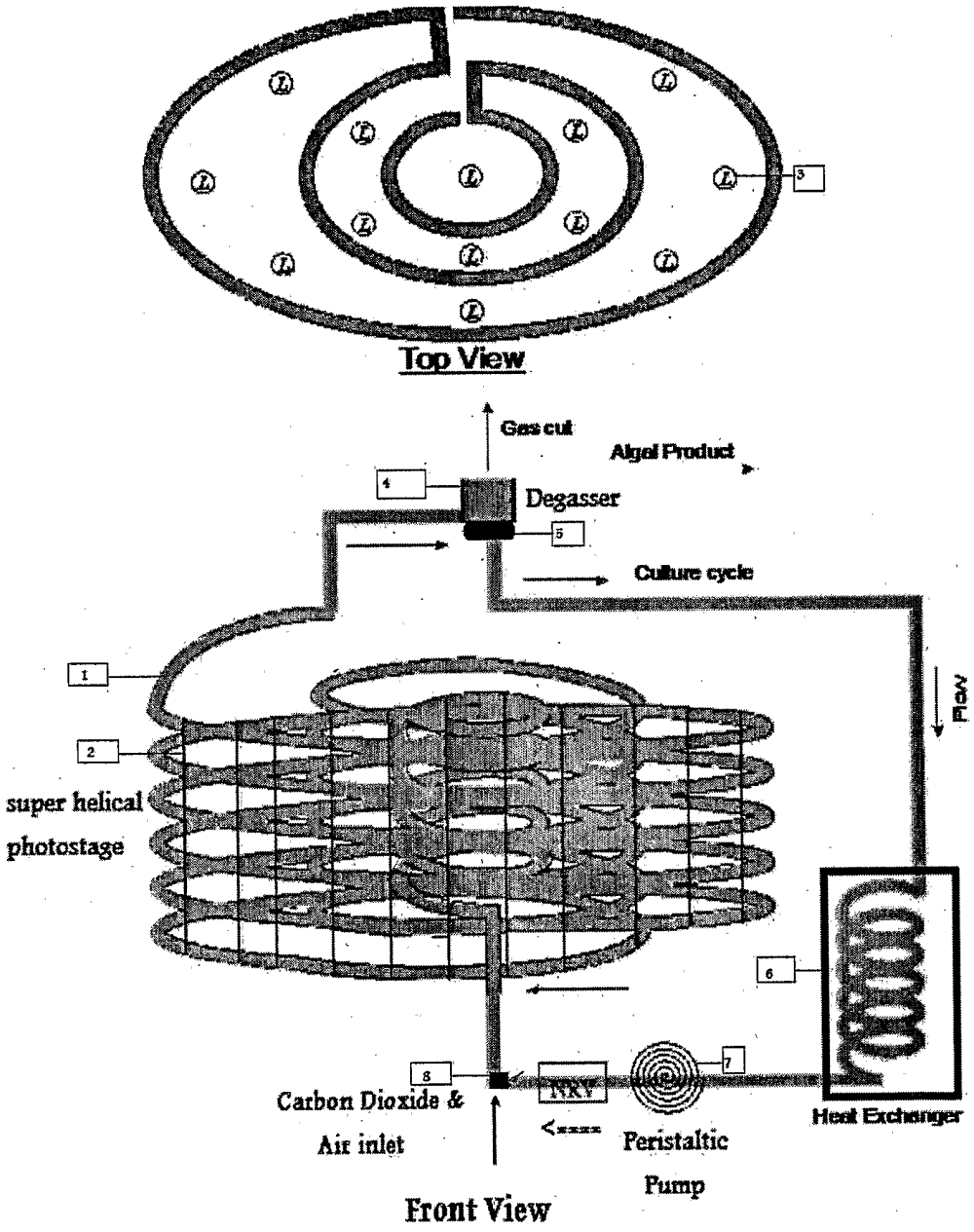
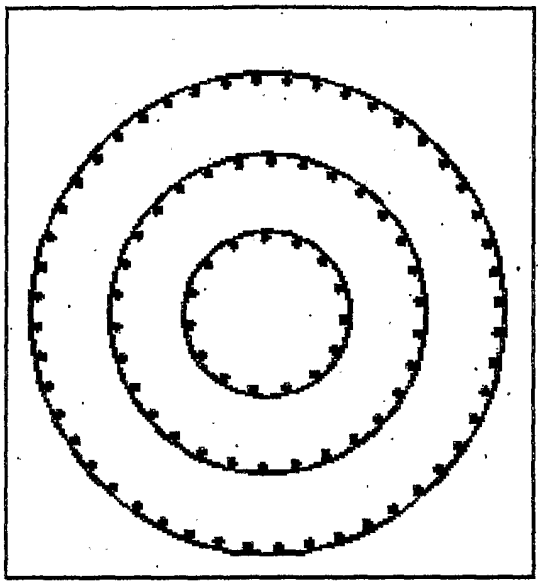
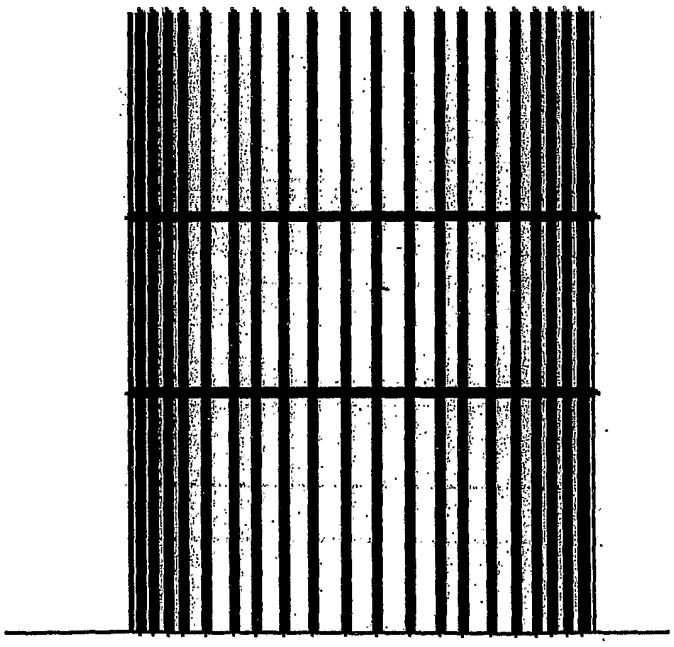

Top View
Gas out
Algal Product
Degasser
Culture cycle
Flow
super helical
photostage
Carbon Dioxide &
Air inlet
Peristaltic
Pump
Heat Exchanger
Front View

Fig 1.



Top View



Front View

Fig 2.

Operated at 31°C
Doubling time 14-15 Days

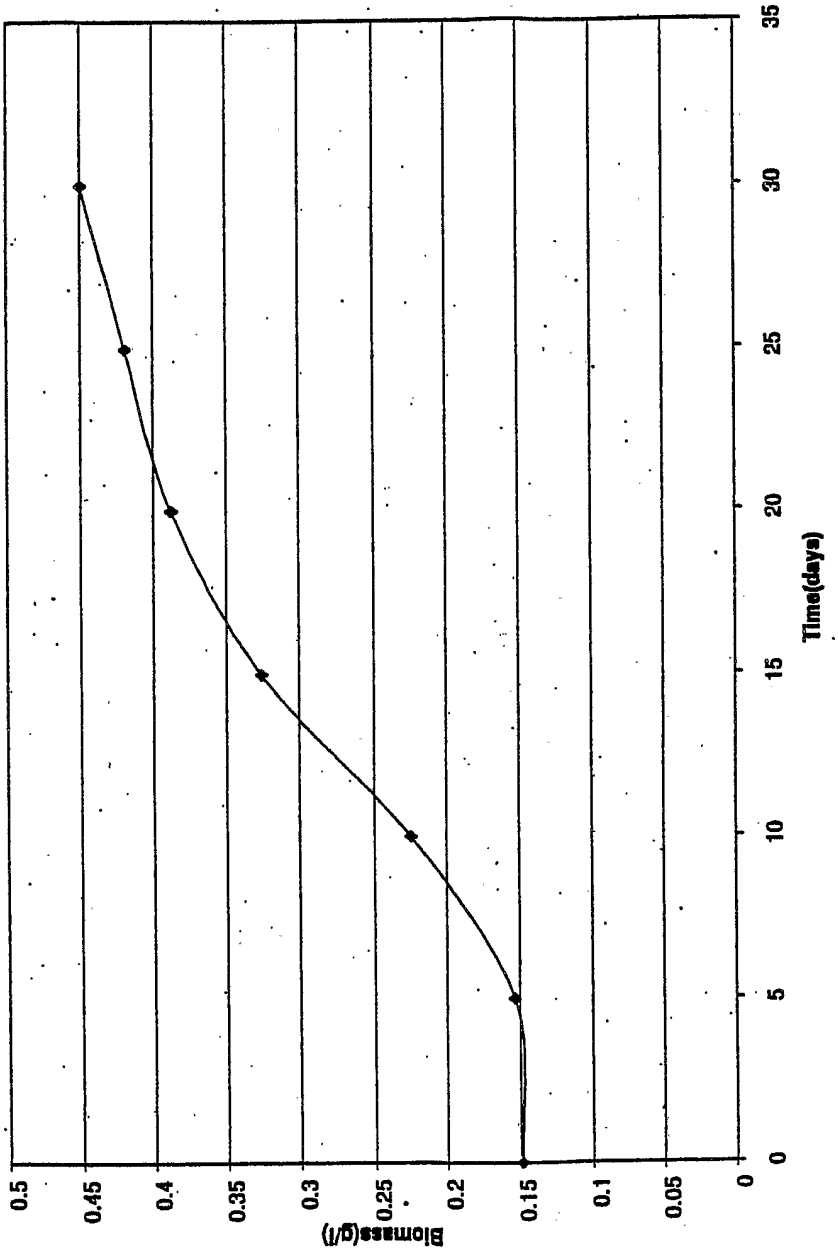


Fig. 3

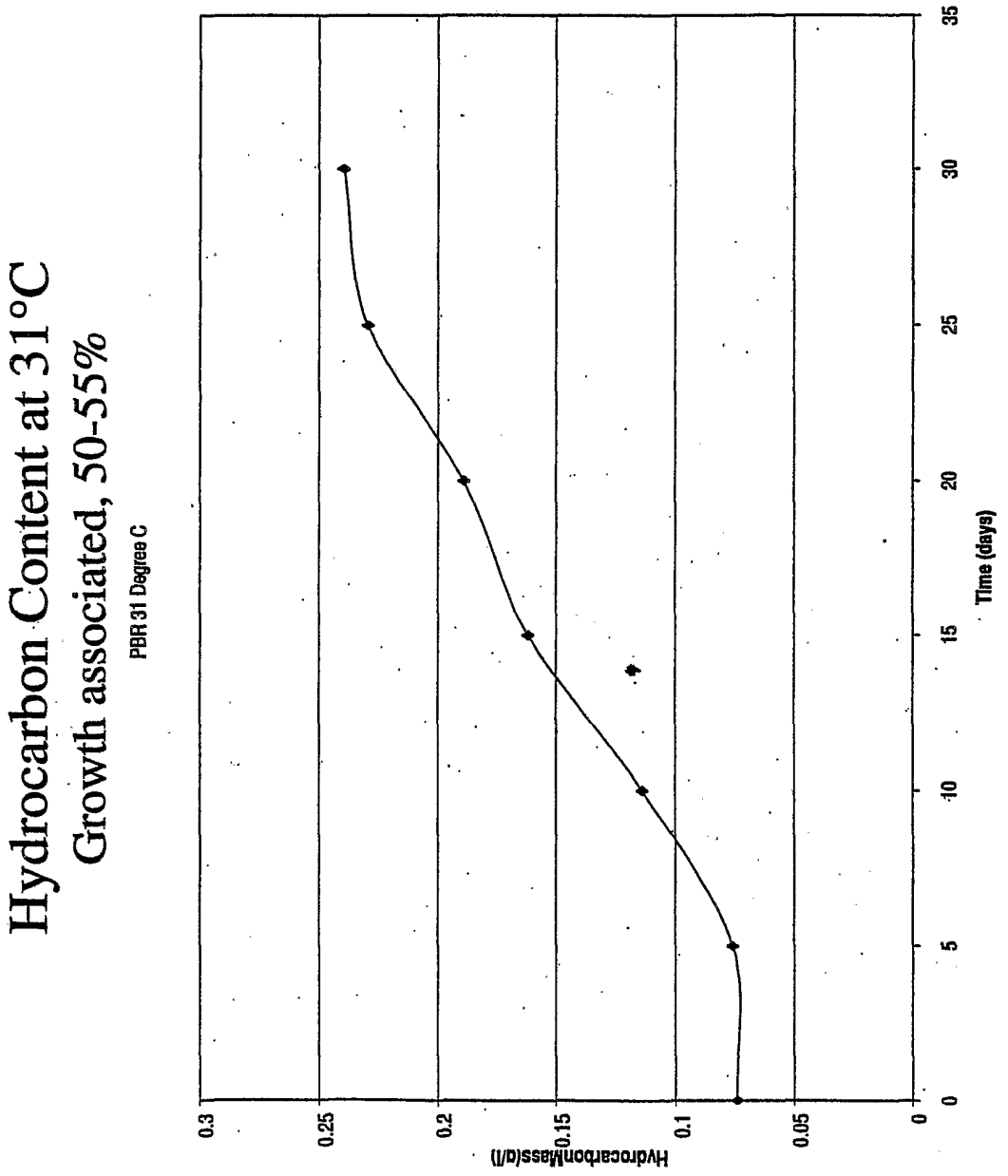

Hydrocarbon Content at 31°C
Growth associated, 50-55%
PBR 31 Degree C
HydrocarbonMass(g/l)
0.3
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
20
25
30
35
Time (days)

Fig. 4

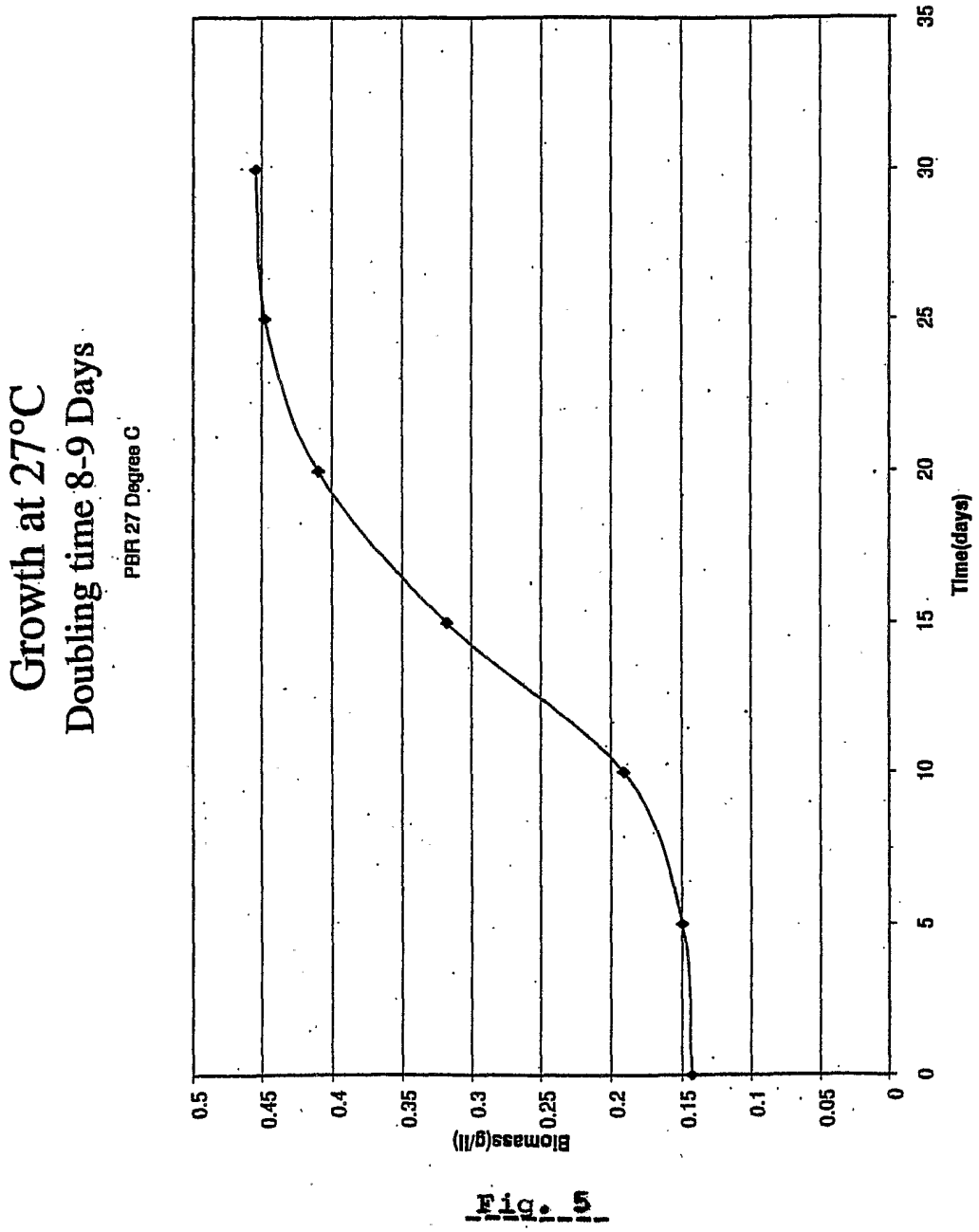

Growth at 27°C
Doubling time 8-9 Days
PBR 27 Degree C
Biomass(g/l)
Time(days)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
20
25
30
35

Fig. 5

Hydrocarbon Content 27°C
Growth Associated, 50-55%

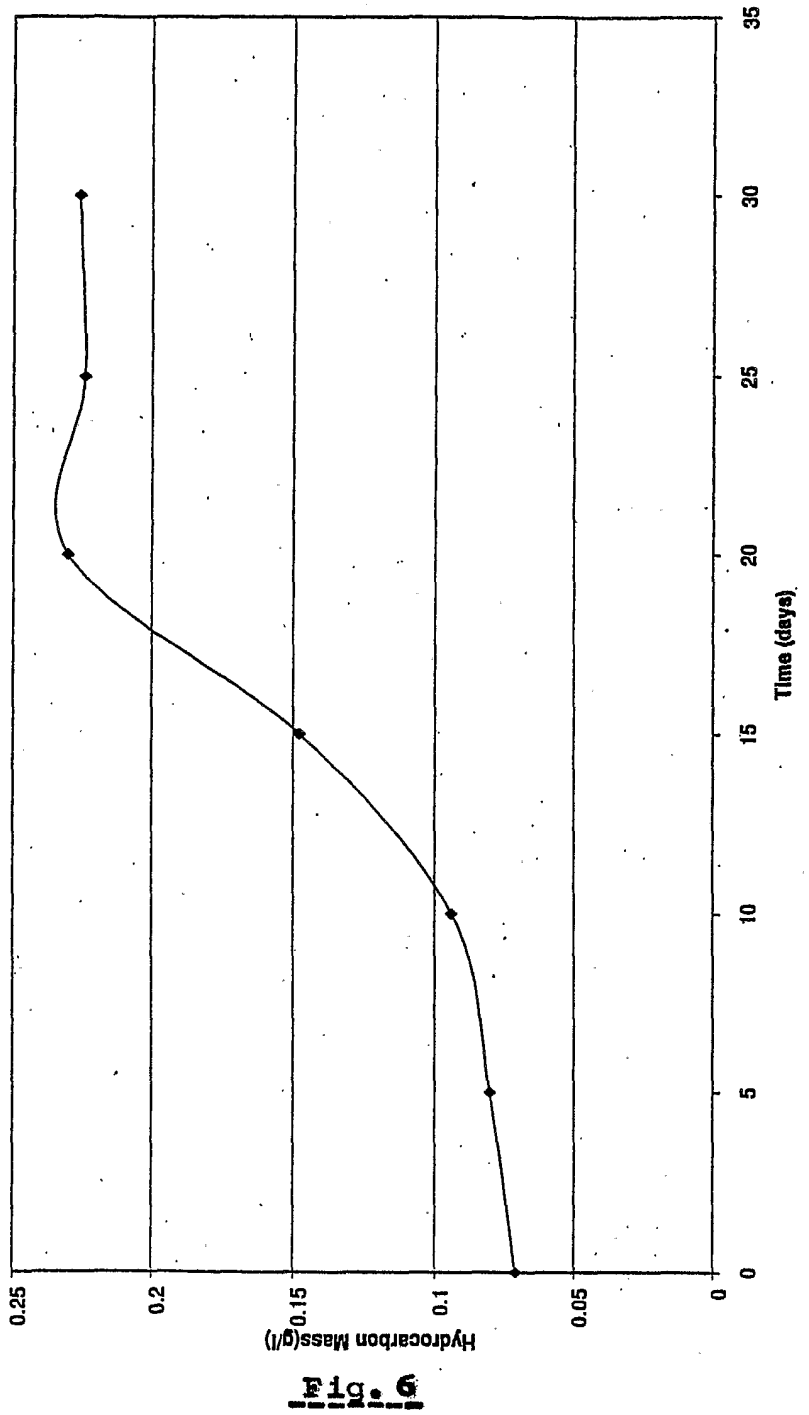


Fig. 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2006/000407

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12M1/00

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)
C12M B01J

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 practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 402 496 A1 (INST GETREIDEVERARBEITUNG [DD]) 19 December 1990 (1990-12-19) column 1, line 1 - line 13 column 2, line 38 - column 3, line 6 column 3, line 53 - column 5, line 37 figures 1-3	1-18
Y	US 5 137 828 A (ROBINSON LEE F [GB] ET AL) 11 August 1992 (1992-08-11) cited in the application column 3, line 62 - column 7, line 34 figures 1-3	1-18
A	GB 2 118 572 A (QUEEN ELIZABETH COLLEGE) 2 November 1983 (1983-11-02) claims 1-17 figure 1	1-18

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 December 2006	02/01/2007

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cubas Alcaraz, Jose

1

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2006/000407

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 614 378 A (YANG VICTOR C [US] ET AL) 25 March 1997 (1997-03-25) cited in the application column 11, line 53 - line 56 claim 1	1-18

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IN2006/000407

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0402496 A1	19-12-1990	AT 92954 T	15-08-1993
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