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# CONTRIBUTIONS FOR EFFICIENT MICROALGAE BIOMASS CULTURE: PHOTOBIOREACTOR DESIGN, OPERATION AND HARVESTING

# CONTRIBUCIONES AL CULTIVO EFICIENTE DE MICROALGAS: DISEÑO DE UN FOTOBIORREACTOR, OPERACIÓN Y RECOGIDA DE LA BIOMASA

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*Siempre acabamos llegando a donde nos esperan*

Libro de los itinerarios, Jose Saramago.

**Abstract**

This thesis is focused on the microalgae biomass production efficiency through the intervention in several key steps of the two main parts of the process: culturing and harvesting. In spite of the wide range of applications of the microalgal biomass, this work targets its viability as oil producers for biofuel making; consequently the developed theoretical and experimental work was referred to the enhancement of lipid productivity and quality. Specifically, photobioreactor design and continuous operation, harvesting by means of auto-flocculation and medium reuse are the topics faced in this work.

**Chapter 1** introduces the topic of this thesis and places it within the context of the current scientific research. General and specific objectives are also stated in this Chapter.

**Chapter 2** describes the theoretical background in the field of microalgae culturing. Through several sections, the main parameters and variables involved in the culture of microalgae are reviewed. Regarding the photobioreactor design, an emphasis is placed on light distribution criteria and the effect of the light intensity over the biomass growth and related phenomena like photolimitation, photoinhibition and photosaturation. The main kinds of photobioreactors are also reviewed, summarizing their evolution, their advantages and disadvantages and the main current trends in the photobioreactor design field. Regarding photobioreactor operation and downstream processing, strategies to reduce the footprint of microalgae culturing facilities are examined. More detailed theoretical background and State of the Art for each chapter's field is specified in each one's introduction.

**Chapter 3** describes the materials and methods used in the experimental work. Although specific materials and methods are summarized in the two experimental chapters (**Chapter 6** and **Chapter 7**) some general issues about the selected species, the culture conditions and analytical methods deserve special attention and they are detailed in this Chapter.

In **Chapter 4**, the design process of a novel photobioreactor for outdoor cultivation of microalgae is tackled. The main goal of the new configuration is to improve the areal productivity of microalgae outdoor cultures by enhancing the distribution of incident sunlight over the culture avoiding oversaturating conditions in the external layers of the culture. This phenomenon occurs in both open and close photobioreactors and, for this reason, many recent technological advances in this field have been targeted to reduce irradiance over the photobioeactor surface by changing its orientation or inclination. If the light intensity falling over the culture surface can be diluted, oversaturation can be avoided and

light is used more efficiently. With this in mind, in this work, a photobioreactor design in which light enters into the culture volume through the introduction of transparent conical structures was conceived. This device can be used with solar or artificial light. When it is exposed to solar light, the conical structures remain with its longitudinal axis parallel to the solar direct beams by means of a solar tracking system. The shape of the cone allows for a dilution of the light over its internal surface, and this dilution effect can be regulated during the design process by varying the aperture angle of the cone. In order to check the viability of this idea, a model to predict the biomass areal productivity was applied to a unit of volume of the cited photobioreactor, being a unit of volume a light distribution device with its surrounding culture volume, which would be the minimum unit to be repeated when scaling-up the reactor. A Monod-type kinetic was used to express the relationship between growth rate and light intensity, being the light the limiting factor in the biomass growth, which supposes to assume that all the other factors are not limiting in the modelized scenario. Among the different kinds of models that can be applied, summed up in models using averaged parameters and models using local parameters, a model based on local light intensities and local growth rates was implemented, thus meaning that a cell experiments a growth depending on instant conditions.

The model was applied to a photobioreactor unit located in Santander and the main parameters of the cone were optimized. For a sole photobioreactor unit, a diameter of $0.30\,\mathrm{m}$ and an aperture angle of $10^{\circ}$ were decided. In order to compare the obtained results with a well known technology, the model was also applied to an open pond with the same ground surface occupancy that the novel photobioreactor unit. While in the open pond the exposed surface coincides with the occupied surface, in the photobioreactor it is multiplied by $11$. Furthermore, the occupied surface to culture volume ratio is reduced from $3.33$ to $0.78$. Areal productivities of $15.17\,\mathrm{g\,m^{-2}\,d^{-1}}$ and $34.57\,\mathrm{g\,m^{-2}\,d^{-1}}$ were predicted for the most unfavorable (January) and favorable (July) months respectively, both under monthly average cloud cover. These results are, in average, $2.72$ times higher than predicted values for an open pond under identical irradiance conditions. An average photosynthetic efficiency of $8.30\,\%$ is predicted in the photobioreactor, while in the open pond is estimated in $3.11\,\%$.

The dimensions and arrangement of the structures that distribute the light are studied in accordance with the solar position and the irradiance of the location of the photobioreactor. In order to evaluate the different distributions, the comparison parameter is the active volume (that receiving irradiance between the compensation point and the saturation point) per unit surface through the year. Three decision variables must be introduced in the model in order to obtain this result: the height of the pivot joint of the cone along its longitudinal axis, the maximum inclination angle in the South direction and the angle between

the South and the plane in which the cone is aligned with the contiguous one in South-East or South-West direction.

The most efficient configurations are proposed taking the city of Santander as model location. The base was identified as the most suitable location for the pivot joint. The floor view of the optimal distribution of the cones when scaling-up this technology in the location of Santander makes a regular rhomboid grid with diagonal of $0.47\,\mathrm{m}$.

The obtained results evidenced the potential of the conceived idea, and since it complied with the patentability requirements, it was applied for this protection obtaining.

**Chapter 5** consists on the translation of the Patent ES2356653 with title "*Fotobiorreactor para el cultivo de organismos fotótrofos*". The invention protected by this patent consists on a photobioreactor for phototrophic organisms culturing, comprising a tank that contains a culture medium and biomass in contact with said culture medium. The photobioreactor also comprises at least one conical or frustoconical transparent or translucent structure totally or partially placed within the tank through which light enters into said tank. This concept is included in the independent claim, while the dependent ones make reference to possible embodiments that may vary depending on the inclusion of the solar tracking system, the possibility to use this device under artificial light, the material of the cones, the carbon supply and the solid-liquid separation system. Six drawings accompany the description of the invention for a better understanding of its characteristics, representing the scheme of an elemental unit of the photobioreactor and several possible embodiments.

Two experimental set-ups were built to develop the experimental work. On the one hand, the laboratory scale microcosms, that consisted on $2\,\mathrm{L}$ flasks in a culturing chamber under controlled conditions. On the other hand, a bench scale pilot plant was constructed and operated indoors, also under controlled conditions.

**Chapter 6** is focused on the harvesting step, taking part of the overall microalgae cultivation process. Due to the high energy consumption associated with this step, a low energy consuming method was tested and evaluated: high pH-induced flocculation-sedimentation in comparison with centrifugation. Furthermore, the supernatant obtained after these two processes was utilized as the base for preparing new culture medium. The biomass growth, the lipid productivity and the fatty acid composition were compared as a function of the type of water used to prepare the media; being the types of water the supernatant of the centrifugation, the supernatant of the auto-flocculation, analytical grade water and tap water.

Flocculation-sedimentation assays for *Scenedesmus obliquus* and *Chlorella vulgaris* were carried out at biomass concentrations of $0.428\,\mathrm{g\,L^{-1}}$ and $0.450\,\mathrm{g\,L^{-1}}$ respectively and different pH values, using $Ca(OH)_2$ and NaOH as pH-increasing agents. No significant differences were detected between assays carried out with the same species but highly significant differences were seen when comparing assays with different microalgae, except in the comparison between *S. obliquus* with Na(OH) and *C. vulgaris* with $Ca(OH)_2$. Recovery efficiencies were always higher when using *S. obliquus* with $Ca(OH)_2$. Although $Ca(OH)_2$ proved to be an efficient precipitating agent, the formation of $CaCO_3$ precipitates that remain in the microalgal pellet causes trouble when lipids are extracted by means of acid hydrolysis using HCl due the reaction between $CaCO_3$ and HCl. For this reason, Na(OH) was used in the subsequent flocculation processes.

Regarding the effects of the type of water, highly significant differences were found in the biomass concentration achieved as dry weight between the cultures grown in medium prepared with tap water and recycled medium, the latter showing better results. Averaged doubling times were $1.94 \pm 0.60\,\mathrm{d}$ for cultures grown in medium prepared with analytical grade water, $2.05 \pm 0.60\,\mathrm{d}$ with tap water, $1.63 \pm 0.60\,\mathrm{d}$ with recycled medium via centrifugation and $1.66 \pm 0.60\,\mathrm{d}$ with recycled medium via auto-flocculation.

Although the highest lipid content appeared in analytical-grade water medium, the productivity was higher in the two reused media due to the higher biomass productivity in reused media. The highest lipid productivities were obtained for those cultures grown in medium with the supernatant of centrifugation ($26.367 \pm 0.697$ and $26.056 \pm 0.689\,\mathrm{mg\,L^{-1}\,d^{-1}}$ for samples collected by centrifugation and auto-flocculation respectively), followed by those grown in medium with the supernatant of auto-flocculation ($25.884 \pm 2.051\,\mathrm{mg\,L^{-1}\,d^{-1}}$ and $25.234 \pm 1.999\,\mathrm{mg\,L^{-1}\,d^{-1}}$), while the lowest were those grown in tap water medium ($21.591 \pm 0.354\,\mathrm{mg\,L^{-1}\,d^{-1}}$ and $10.840 \pm 0.178\,\mathrm{mg\,L^{-1}\,d^{-1}}$). Regarding the fatty acid composition, in all cases more unsaturated than saturated fatty acids were found (average values of $66\,\%$ and $34\,\%$ respectively) and polyunsaturated fatty acids (PUFAs) accounted for $23\,\%$.

**Chapter 7** deals with the continuous experimentation of a two-stage bench scale photobioreactor in series, maintaining the culture in steady-state conditions firstly in an exponentially growth reactor and afterwards in a reactor with stress conditions. This guaranteed light limited conditions in the first stage and N-stress conditions in the second stage. Four assays were carried out: three using medium based on fresh water and one using recirculated (supplemented with nutrients and neutralized by bubbling the photobioreactors with the $CO_2$ outlet current) medium.

This two-stage cultivation resulted in biomass productivity values at the best

dilution rate ($0.118\,\mathrm{d}^{-1}$) of $15.25 \pm 1.06\,\mathrm{g\,m^{-2}\,d^{-1}}$, slightly higher than that expected according to batch experiment ($12.90 \pm 0.75\,\mathrm{g\,m^{-2}\,d^{-1}}$). The dilution rate that maximized the lipid content was coincident with that for the maximum biomass productivity, resulting in an intensification of the lipid productivity.

Regarding the lipid content and lipid productivity, both were higher in the second stage than in the first one in all the assays. It was noted that both parameters tended to increase with the increase of the dilution rate. The lowest lipid content and productivity were related with the longest stress time. Lipid productivities among fresh water and recycled water were similar.

Analyzing the fatty acid composition, it was seen that in the second stage, saturated fatty acid productivity was similar between the different dilution rates, while polyunsaturated fatty acids decreased as the stress time increased. In terms of percentage, an increase in saturated fatty acids was observed, as the unsaturated fatty acids decreased with the reduction of the dilution rate. Microalgae flocculation with NaOH does not result in a variation of the obtained lipid profile in comparison with the harvesting by centrifugation.

Finally, **Chapter 8** presents the general conclusions of this work and guidelines for future works related to this topic.

## Resumen

Esta tesis aborda aspectos relacionados con la eficiencia de producción de biomasa microalgal en fotobiorreactores mediante la intervención en varios de los procesos clave de las dos partes principales que forman parte del proceso global: cultivo y recolección de la biomasa. A pesar de la amplia variedad de aplicaciones de la biomasa microalgal, este trabajo se centra en su viabilidad como productoras de aceites para la elaboración de biocombustibles; consecuentemente en el trabajo teórico y experimental desarrollado se hace referencia a la mejora de la productividad y la calidad de los lípidos. De forma específica, en este trabajo se afrontan los aspectos relacionados con el diseño de fotobiorreactores y su operación en continuo, la separación de la biomasa mediante autofloculación y la reutilización del medio de cultivo.

El **Capítulo 1** introduce el tema que trata esta tesis y lo sitúa en el contexto de la investigación científica actual. Además, se describen los objetivos generales y específicos.

El **Capítulo 2** describe el trasfondo teórico en el campo del cultivo de microalgas. A través de varias secciones, se revisan los principales parámetros y variables involucrados en el cultivo de microalgas. En referencia al diseño de fotobiorreactores, se hace especial énfasis en los criterios de distribución de la luz y los efectos de la luz sobre el crecimiento de la biomasa, como son la fotolimitación, la fotoinhibición y la fotosaturación. Se revisan también los principales tipos de fotobiorreactores, resumiendo su evolución, sus ventajas y desventajas y las tendencias actuales en el campo del diseño de fotobiorreactores. En cuanto a la operación del fotobiorreactor y el procesado de la biomasa, se examinan las estrategias para reducir el impacto negativo de las instalaciones de cultivo. En cada capítulo se presenta un trasfondo teórico más detallado y el Estado del Arte referido al tema que en cada uno se trata.

El **Capítulo 3** describe los materiales y métodos empleados en el trabajo experimental. Aunque en cada uno de los dos capítulos experimentales (**Capítulo 6** y **Capítulo 7**) se resumen los materiales y métodos empleados, algunos aspectos generales, como los relacionados con la selección de las especies a cultivar, las condiciones de cultivo y los métodos analíticos, merecen especial atención y por ello son detallados en este Capítulo.

En el **Capítulo 4** se aborda el proceso de diseño de un fotobiorreactor innovador para el cultivo de microalgas. El principal objetivo de la nueva configuración es mejorar la productividad por unidad de superficie de los cultivos en exterior mediante la mejora de la distribución de la luz solar incidente, evitando que se produzcan condiciones de fotosaturación en las capas externas del cultivo. Este

fenómeno se da tanto en sistemas abiertos como cerrados y por ello, muchos de los avances tecnológicos recientes en este campo están dirigidos a reducir la irradiancia sobre la superficie del fotobiorreactor variando su orientación o su inclinación. Si se consigue diluir la luz incidente sobre el cultivo, la fotosaturación se puede evitar y se consigue un uso más eficiente de la luz. Con esta idea, en este trabajo se ha concebido un diseño de fotobiorreactor en el cual la luz entra al volumen de cultivo mediante la introducción de estructuras cónicas transparentes. Este dispositivo puede ser utilizado tanto bajo luz solar como artificial. Cuando se va a exponer a la luz solar, estas estructuras cónicas transparentes permanecen con su eje longitudinal paralelo a los haces de luz solar directa mediante un sistema de seguimiento solar. La forma de los conos permite una dilución de la luz sobre su superficie interna, y este efecto de dilución puede ser regulado durante el proceso de diseño variando el ángulo de apertura del cono. Para evaluar la viabilidad de esta idea, se ha aplicado un modelo para predecir la productividad de biomasa por unidad de superficie a una unidad de volumen del fotobiorreactor, entendiendo como unidad de volumen un elemento de distribución de la luz y el volumen de cultivo circundante, que correspondería con la mínima unidad que se repetiría al escalar este reactor. Se aplicó una cinética tipo Monod para expresar la relación entre la tasa de crecimiento y la intensidad de luz, siendo la luz el factor limitante en el crecimiento de la biomasa, lo que quiere decir que el resto de factores no son limitantes en el escenario modelizado. Entre los diferentes tipos de modelos que se pueden aplicar, resumiéndolos en modelos que utilizan valores promedio y modelos que utilizan valores locales, se eligió un modelo basado en intensidades de luz y tasas de crecimiento locales, lo que significa que se asume que una célula experimenta un crecimiento que depende de sus condiciones instantáneas.

El modelo se aplicó a una unidad del fotobiorreactor situada en Santander y se optimizaron los principales parámetros del cono. Para una sola unidad, se eligió un diámetro de $0{,}30\,\mathrm{m}$ y un ángulo de apertura de $10^{\circ}$. Para comparar los resultados obtenidos con una tecnología conocida, se aplicó el mismo tipo de modelo a un reactor abierto conocido como *open pond* con la misma ocupación de suelo que el fotobiorreactor. Mientras que en el reactor tipo *open pond* la superficie iluminada coincide con la superficie ocupada, en el fotobiorreactor este valor se multiplica por 11. Además, la relación superficie ocupada por unidad de volumen se reduce de 3,3 a $0{,}78$ Los valores de productividad del fotobiorreactor por unidad de superficie según el modelo para el mes más desfavorable (Enero) y más favorable (Julio) son $15{,}17\,\mathrm{g\,m^{-2}\,d^{-1}}$ y $34{,}57\,\mathrm{g\,m^{-2}\,d^{-1}}$ respectivamente, ambos bajo nubosidad media mensual. Estos resultados son, de media, $2{,}72$ veces mayores que los predichos para el sistema *open pond* bajo idénticas condiciones de irradiancia. Para el fotobiorreactor se ha obtenido una valor de eficiencia fotosintética media de $8{,}30\,\%$ mientras que en el *open pond* el valor es $3{,}11\,\%$.

Las dimensiones y la colocación relativa de las estructuras cónicas que distribuyen la luz se estudiaron en función de la posición solar y de los valores de irradiancia del lugar de implantación del fotobiorreactor. Para evaluar las diferentes distribuciones, el parámetro de comparación es el volumen activo (aquel que recibe una intensidad de luz entre el punto de compensación y la intensidad de saturación) por unidad de superficie a lo largo del año. Para obtener este valor se ha desarrollado un modelo en el que es necesario introducir el valor de tres variables: la altura del punto de giro de los conos a lo largo de su eje longitudinal, la máxima inclinación en dirección sur y el ángulo entre la dirección sur y el plano en el que un cono está alineado con el siguiente en dirección sureste o suroeste.

Como resultado de este modelo, se obtuvieron las configuraciones más eficientes en cuanto a la captación de la luz, tomando como localización la ciudad de Santander. La base del cono fue identificada como la situación más adecuada para el punto de giro. La vista en planta de la distribución óptima para escalar esta tecnología en la ciudad de Santander conforma una red romboidal regular con diagonal de $0{,}47\,\mathrm{m}$.

Los resultados obtenidos evidenciaron el potencial de la idea concebida, y puesto que cumplió con los requisitos de patentabilidad, se propuso su protección bajo la figura de patente.

El **Capítulo 5** consiste en una traducción de la Patente ES2356653 con título «Fotobiorreactor para el cultivo de organismos fotótrofos». La invención protegida bajo esta patente consiste en un fotobiorreactor para el cultivo de organismos fotótrofos y comprende un tanque que contiene medio de cultivo y biomasa en su interior y en contacto con dicho medio de cultivo. El fotobiorreactor también comprende al menos una estructura cónica o troncocónica transparente o translúcida, total o particalmente introducida en el tanque a través de la cual la luz penetra en el tanque. Este concepto queda recogido bajo la reivindicación independiente, mientras que las reivindicaciones dependientes hacen referencia a posibles realizaciones que varían dependiendo de la inclusión de un sistema de seguimiento solar, la posibilidad de usar este dispositivo bajo luz artificial, el material de los conos, el aporte de carbono y el sistema de separación sólido-líquido. Seis dibujos acompañan a la descripción de la invención para una mejor comprensión de sus características, representando el esquema de una unidad elemental del fotobiorreactor y varias posibles realizaciones.

Para la realización del trabajo experimental, se construyeron dos instalaciones. Por un lado, los microcosmos a escala de laboratorio, que consistieron en matraces de 2 L en una cámara de cultivo bajo condiciones controladas. Por otro lado, una planta piloto a escala de bancada se construyó y se operó en interior, también bajo condiciones controladas.

El **Capítulo 6** se centra en el proceso de separación de la biomasa, formando parte del proceso global de cultivo de biomasa microalgal. Debido al elevado consumo energético asociado a este proceso, se probó y evaluó un método con menor consumo: floculación-sedimentación inducida por el aumento de pH en comparación con la centrifugación.

Además, el sobrenadante obtenido tras estos dos procesos se utilizó como base para preparar nuevo medio de cultivo. Según el tipo de agua utilizada para el medio de cultivo, se compararon el crecimiento de la biomasa, la productividad lipídica y el perfil lipídico; siendo los tipos de agua utilizados el sobrenadante de la centrifugación, el sobrenadante de la autofloculación, agua de grado analítico y agua de distribución.

Se llevaron a cabo ensayos de floculación-sedimentación con *Scenedesmus obliquus* y *Chlorella vulgaris* en concentraciones de $0{,}428\,\mathrm{g\,L^{-1}}$ y $0{,}450\,\mathrm{g\,L^{-1}}$ respectivamente y a diferentes valores de pH, usando $Ca(OH)_2$ y NaOH como agentes para el incremento de pH. No se observaron diferencias significativas entre ensayos llevados a cabo con la misma especie pero sí diferencias altamente significativas cuando se compararon ensayos con diferentes microalgas, excepto en la comparación entre *S.obliquus* con Na(OH) y *C. vulgaris* con $Ca(OH)_2$. Aunque se probó que el $Ca(OH)_2$ es un agente de precipitación eficiente, la formación de precipitados de $CaCO_3$ que permanecen en los pellets de microalgas causaron problemas durante la extracción de lípidos mediante hidrólisis ácida usando HCl, debido a la reacción entre $CaCO_3$ y HCl. Por este motivo, en los siguientes ensayos se utilizó NaOH.

En cuanto a los efectos del tipo de agua, se encontraron diferencias altamente significativas en la concentración alcanzada como peso seco en los ensayos en batch entre cultivos preparados con agua de distribución y los preparados con agua reutilizada, siendo mayores los resultados con ésta última. Los tiempos de duplicación medios fueron de $1{,}94 \pm 0{,}60$ días en los cultivos preparados con agua de grado analítico, $2{,}05 \pm 0{,}60$ días con agua de distribución, $1{,}63 \pm 0{,}60$ días con agua recuperada por centrifugación y $1{,}66 \pm 0{,}60$ días con agua recuperada por autofloculación.

Aunque el mayor contenido lipídico apareció en los cultivos con agua de grado analítico, la productividad fue mayor en los dos medios con agua reutilizada debido a la mayor productividad de biomasa. Las mayores productividades lipídicas se dieron en los cultivos con sobrenadante de centrifugación ($26{,}367 \pm 0{,}697$ $\mathrm{mg\,L^{-1}\,d^{-1}}$ y $26{,}056 \pm 0{,}689\,\mathrm{mg\,L^{-1}\,d^{-1}}$ para muestras recogidas por centrifugación y autofloculación respectivamente), seguidas de las muestras en medio con sobrenadante de auto-floculación ($25{,}884 \pm 2{,}051\,\mathrm{mg\,L^{-1}\,d^{-1}}$ y $25{,}234 \pm 1{,}999\,\mathrm{mg\,L^{-1}\,d^{-1}}$), mientras que las menores productividades se encontraron en los medios con agua de distribución ($21{,}591 \pm 0{,}354\,\mathrm{mg\,L^{-1}\,d^{-1}}$

y $10{,}840 \pm 0{,}178\,\mathrm{mg\,L^{-1}\,d^{-1}}$). En referencia a la composición de ácidos grasos, en todos los casos aparecieron más ácidos grasos insaturados que saturados (valores medios de $66\,\%$ y $34\,\%$ respetivamente) y los ácidos grasos poliinsaturados constituyeron el $23\,\%$.

El **Capítulo 7** trata la experimentación en continuo de un fotobiorreactor a escala de bancada en dos fases, manteniendo el cultivo en estado estacionario, primero en condiciones de crecimiento exponencial y después en condiciones de estrés. Esta configuración garantizó condiciones de luz limitantes en la primera fase y estrés por falta de nitrógeno en la segunda. Se llevaron a cabo cuatro ensayos: tres con medio elaborado con agua de distribución y uno con agua recirculada (suplementada con nutrientes y neutralizada mediante burbujeo con la corriente de salida de $CO_2$).

Este cultivo en dos fases dio como resultado un valor de productividad de biomasa en la mejor tasa de dilución ($0{,}118\,\mathrm{d^{-1}}$) de $15{,}25 \pm 1{,}06\,\mathrm{g\,m^{-2}\,d^{-1}}$, ligeramente mayor que lo esperado de acuerdo con los ensayos en batch ($12{,}90 \pm 0{,}75$ $\mathrm{g\,m^{-2}\,d^{-1}}$). La tasa de dilución que dio el mayor contenido lipídico coincidió con la máxima productividad de biomasa, resultando en una intensificación de la productividad lipídica.

En cuanto al contenido lipídico y la productividad lipídica, ambos fueron mayores en la segunda fase que en la primera en todos los ensayos. Se observó que los dos parámetros tendieron a incrementar con el aumento de la tasa de dilución. El menor contenido lipídico y productividad se dieron con el mayor tiempo de estrés. Las productividades lipídicas fueron similares entre los medios con agua de distribución y con agua recuperada.

Analizando la composición de los ácidos grasos, se observó que en la segunda fase la productividad de ácidos grasos saturados fue similar entre las distintas tasas de dilución, mientras que los ácidos poliinsaturados descendieron con el aumento del tiempo de estrés. En términos de porcentaje, se produjo un incremento de ácidos grasos saturados con la reducción de la tasa de dilución. La separación de biomasa mediante floculación con NaOH no produjo variaciones en el perfil lipídico obtenido en comparación con la centrifugación.

Por último, el **Capítulo 8** presenta las conclusiones generales de este trabajo así como recomendaciones para futuros trabajos desarrollados en este campo.

## List of Publications

A patent, one contribution in the form of a poster and a communication to an international congress and two articles in an international journal have emerged from this work.

- Tejero, I., Castrillo, M., Díez, R., Moreno-Ventas, X.E. Fotobiorreactor para el cultivo de organismos fotótrofos. ES2356653
- M. Castrillo, R. Díez, I. Tejero. Design of a novel photobioreactor with enhanced incident solar light utilization. 4th International Congress on Energy and Environment Engineering and Management. Mérida, Spain. May 2011. School of Industrial Engineering of the Extremadura University. ISBN 8499780148, 9788499780146
- Castrillo, M.; Lucas-Salas, L.M.; Rodríguez-Gil, C.; Martínez D. High pH-induced flocculation–sedimentation and effect of supernatant reuse on growth rate and lipid productivity of *Scenedesmus obliquus* and *Chlorella vulgaris*. Bioresour Technol 128 (2013) 324–329
- Lucas-Salas, L.M.; Castrillo, M.; Martínez, D. Effects of dilution rate and water reuse on biomass and lipid production of *Scenedesmus obliquus* in a two-stage novel photobioreactor. Bioresour Technol 143 (2013) 344-352

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# 1

# Background and scope of this thesis

In the last decades the culture of microalgae has awakened scientific and commercial interest since these microorganisms have been seen as an attractive source of valuable biomass. A wide variety of applications have been attributed to this biomass and its subproducts. Its utilization with environmental purposes like bioremediation and $CO_2$ fixation, as well as with commercial purposes in different industrial sectors, has been reported (Mata, Martins, and Caetano 2010). Moreover, a new interest in microalgae culturing has grown in the last few years since the lipids that they produce are viewed as a good alternative for fossil fuel (Chisti 2007), however they are not yet cost-efficient enough to be produced at large scale (Wijffels and Barbosa 2010). A recent study estimated in $4.95\,€$, $4.15\,€$ and $5.96\,€$ the cost of producing $1\,\mathrm{kg}$ of biomass in raceway ponds, tubular reactors and flat panels respectively ($100\,\mathrm{ha}$ plants), which could be reduced to $1.28\,€\,\mathrm{kg\,biomass^{-1}}$, $0.70\,€\,\mathrm{kg\,biomass^{-1}}$ and $0.68\,€\,\mathrm{kg\,biomass^{-1}}$ by implementing improvements in the location, the mixing, the photosynthetic efficiency and the source of $CO_2$ and water (Norsker et al. 2011). Another economic analysis identifies the cost of the photobioreactor as the major factor in the production cost. The cost reduction, as well as the productivity improvement and the reduction of the cost of the growth medium and the $CO_2$ source are needed to be competitive with other energy sources (Acién et al. 2012).

The initial investment, the operational costs, and the environmental negative impacts, need to be reduced in order to contribute to the sustainability of large-scale microalgae culturing. The initial investment is related with building costs and equipments. This cost is high and could be hardly reduced. Regarding the operational costs, the intervention in every step of the overall process is essential to make it affordable. Two basic subprocesses can be distinguished within the overall process: the biomass cultivation and the biomass harvesting. A third subprocess may be considered, that is the target products extraction but it is out of the scope of this work. These two subprocesses, together with the identification of preferable conditions for high oil productivity, have been recognized as key challenges for producing microalgal biofuels (Chen et al. 2011).

Regarding the intervention in the two aforementioned subprocesses, steps to reduce the economical and environmental footprint are being taken in two fields: biology and technology. Referring to the biology, the genetic and metabolic engineering has as main objectives to stimulate the productivity of target products and to enhance the tolerance to oversaturating conditions. With regard to the technology, several challenges are nowadays being faced by the scientific community.

Firstly, the development of efficient large-scale facilities —that is, using sunlight, achieving high areal productivity and being inexpensive and energy efficient— is

needed to make them competitive with other energy sources. There is no general agreement within the scientific community about which kind of technology could guarantee the future of energy from microalgae. Traditionally, the main drawback of photobioreactors using solar light has been the requirement of large areas in order to provide large illuminated surfaces, especially when talking about open ponds. More recently, systems with increased illuminated surface-to-volume ratios with the aim of reducing the light path inside the culture and excessive irradiance over the culture surface have been proposed, including vertical columns, tubular photobioreactors (horizontal or tilted) or flat panels among others (Ugwu, Aoyagi, and Uchiyama 2008). Although nowadays open ponds followed by tubular systems seem to be the most successful types, more efficient systems should be designed and developed in order to avoid limitations produced by exposure to excessive light intensities and highly heterogeneous light fields through the culture. The design of light distributing systems with low energy requirements has been concluded as a promising strategy (Wijffels and Barbosa 2010). According to Posten 2009, the answer of process engineering resides in vertically mounted photobioreactors with a large illuminated surface area. As a guidance value, it is said that the relation between illuminated area and ground area should be in the range of 10 or higher.

Besides an efficient light utilization, the productivity of the target products must be optimized. Regarding oil accumulation it has been observed to be very low under standard growth conditions and to increase under N-deprivation (Illman, Scragg, and Shales 2000). Nutrient stress conditions compromise the biomass growth, therefore cultivation strategies must be developed in order to maximize the lipid production.

Since microalgae culturing implies a high freshwater usage, measures in order to save water should be adopted. For example the use of closed photobioreactors in contrast to open ponds allows for more concentrated cultures, thus generating more microalgae biomass per volume of water. Furthermore, evaporation losses are reduced. However, since open ponds continue to be the most economically favorable technology, other actions in order to reduce the water footprint must be carried out. The reuse of the supernatant of solid-liquid separation operation has been seen as a feasible way to save water and nutrients, thus contributing to make the process more economically viable (Kim et al. 2011). The search for sustainability in the water usage is one of the most recent addressed issues in the microalgae culturing field, and by the moment, little is known about the feasibility of water recycling, and less about its effects on lipid productivity and fatty acids composition. The times that the water can be recycled or how can dilution with freshwater contribute to it is still uncertain.

Finally, among the challenges related to the downstream processing of microalgal

cultures, improvements in the harvesting techniques are being developed. Downstream processing needs for a solid-liquid separation operation and it is a high energy consuming step. Sedimentation, filtration and centrifugation are the most common ways to separate solids from liquid, and sometimes a chemical coagulation or flocculation step is needed before. In the way to search for a more sustainable method, flocculation induced by pH adjustment is attracting considerable interest (Vandamme et al. 2012). However, not all the harvesting methods can be applied to all the cultures, on the contrary the selection of the harvesting technique is subjected to the quality of the target product and the physiological status of the microorganisms in the moment to be harvested. For this reason, the applicability of an economical method for microalgae harvesting for lipid production is addressed in this work.

The scope of this Thesis is to address the constrictions found in the key process on microalgal biomass production with the aim of contributing to the development of new knowledge and understanding in this field. The objectives of this study can be stated as follows:

- Design and feasibility evaluation of a novel area efficient photobioreactor, via modelization.
- Optimization of a continuous biomass culture operation to enhance lipid productivity.
- Evaluation of the viability of a low cost harvesting method in comparison to a conventional one.
- Reduction of inputs to the production system through the recycling of the culture medium and study of its influence on biomass growth, lipid production and fatty acid composition.

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# 2

# State of the art

## 2.1. Aim of microalgae cultivation

Microalgae cultivation has created interest for scientists since mid-20th century, due to their various economic and scientific possibilities. Firstly they were viewed mainly as source of nutritional compounds like proteins, vitamins, pigments, etc (Becker 1983; Delanoue and Depauw 1988). Nowadays other applications such as animal feed, fertilizer, wastewater treatment and raw material for biofuel production are being studied as sole or combined objectives for microalgae large-scale cultivation (Ahmad et al. 2011; Becker 1994). Since 1970, when evidences of petroleum scarcity and the rise of its price alerted the population to the need for other energy sources, biological applications of solar energy, like hydrogen or methane, began to attract interest. Raw materials for biofuel production coming from microalgae cultures belong to the third generation of biodiesel feedstocks. The first generation is composed by feedstocks like rapeseed, soybeans, palm oil and sunflower and the second generation is composed by non-food feedstocks like jojoba oil, tobacco seeds, salmon oil, etc. (Ahmad et al. 2011). Among the advantages that have been attributed to the biofuel from microalgae as raw material, it is worth identifying the following:

- High photosynthetic efficiency to produce biomass and their higher growth rates and productivity compared to conventional crops.
- Fast reproduction, being easier to cultivate than many other types of plants and producing a higher yield of oil for biodiesel production.
- Relatively lower harvesting and transportation costs compared to those of other biomass materials such as trees and crops.
- They do not directly affect the human food supply chain and do not compete for land with crops used for food production.
- Microalgae can be grown in a number of environments that are unsuitable for growing other crops.
- Microalgae produce valuable co-products or by-products such as biopolymers, proteins, carbohydrates and residual biomass, which may be used as feed or fertilizer. In addition, cultivation of microalgae does not require herbicides or pesticide.

Microalgae biofuel is seen as technically feasible since several microalgae strains have been demonstrated to produce fatty acids suitable for biofuel production (Gouveia

and Oliveira 2009), however efforts in the fields of culture efficiency, harvesting and extraction must be invested in order to make this option economically viable.

A recent review (Chisti 2013) identifies the major constraints to commercialization of microalgae derived fuels. They can be summarized in economic constraints (high demands on certain key resources like $CO_2$, nutrients and water) and technical and biotechnological constraints that by the moment revert on a high cost of microalgae culturing, making the derived biofuels not feasible for replace petroleum based fuels. According to that work, some fields still need more attention to make microalgae culturing more sustainable from the energetic and the economic point of view; among them the improvement of light penetration in dense cultures and the improvement of microalgae harvesting by means of autoflocculation are found. These issues are addressed in the present work.

## 2.2. Microalgae growth

Microalgae growth is usually divided in several phases, whose length depends on environmental factors like temperature, light intensity, composition of the culture medium, etc. The most common approach when a medium is inoculated with organisms is to consider that microalgae growth begins with the *lag phase*, in which their concentration in the medium is low and the nutrients consumption is hardly noticeable. After the acclimatization of the microalgae cells to the medium, the biomass (expressed as dry weight, cell number, optical density, etc.) begins to exponentially increase over time. During the *exponential phase* the cells divide at a constant rate that depends on intrinsic characteristics of the organism and the culture conditions. Knowing the growth rate is very important to know the state of the culture. Calling $td$ the doubling time or mean generation time, it can be calculated as follows:

$$t_d = \frac{t}{n}$$

being $t$ the time needed to produce $n$ cells.

Having into account the exponential growth of microorganisms in this phase, the specific growth rate ($\mu$) can be calculated as:

$$\mu = \frac{0.69}{t_d}$$

When something is limiting the cells reproduction, the culture begins to decrease its growth rate, and the increase of algal biomass over time becomes almost linear. After this intermediate step, the culture enters into the *stationary phase*, characterized by a net growth equals to zero. In this phase, microalgae cells undergo biological changes, depending on the factor that makes them to enter in this phase. For example, the lack of nitrogen in the medium is associated with the accumulation of oils (Converti et al. 2009; Rodolfi et al. 2009; Illman, Scragg, and Shales 2000). Finally, if the metabolism can no longer be maintained; the culture goes into the *death phase*, characterized by the decreasing of the biomass until the breakdown of the algal population. The Figure 2.1 shows a typical growth curve, with the parameter representing the population size in ordinate axis and time in the abscissa axis.

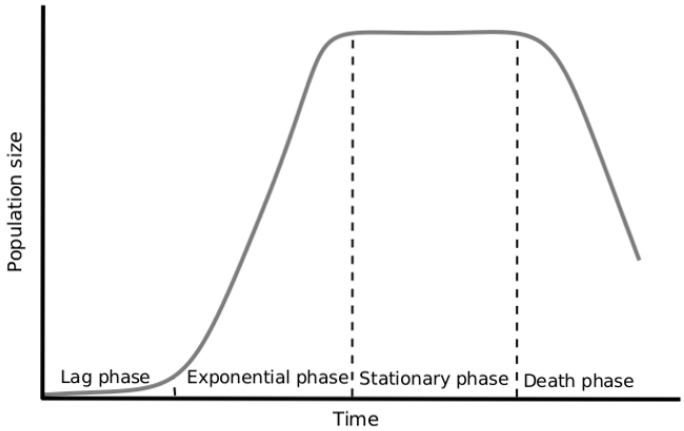


Figure 2.1: Typical growth curve.

Among the factors controlling the microalgae cultures, nutrients availability, light, pH, salinity and temperature are the most important. When culturing microalgae outdoors, all of them, except light, can be optimized by means of control methods like chemical reactants adding, $CO_2$ diffusing, etc., as it is usually done in reactors operation. The light has a different behavior, since it is independent of the dilution rate and cannot be homogeneously distributed through the culture medium because its intensity is exponentially decreased as it goes through the culture. Only highly diluted cultures can maintain a quasi-homogeneous light intensity, however such a diluted medium will probably have a low productivity.

## 2.3. Considerations about the light

Since they are photosynthetic organisms, microalgae need the light to grow and it is the main factor to have into account when designing mass cultivation devices for photoautotrophic organisms. Simplifying, the photosynthesis is the conversion process of light energy to organic matter and can be expressed as a redox reaction driven by light energy:

$$n\mathrm{CO_2} + n\mathrm{H_2O} + \text{light} \longrightarrow (\mathrm{CH_2O})_2 + n\mathrm{O_2}$$

In the oxygenic photosynthesis, that takes places in two stages called light stage and dark stage, carbon dioxide and water are converted to carbohydrates and oxygen. The fraction of the light spectrum utilizable in photosynthesis is the so called PAR (Photosynthetic Active Radiation), that corresponds to the wavelegths of visible light, ranging from the violet of about $380\,\mathrm{nm}$ to the far red at $750\,\mathrm{nm}$. The photosynthesis needs a minimum of $8\,\mathrm{moles}$ quanta to produce $30\,\mathrm{g\,biomass}$. Furthermore, photosynthesis efficiencies range from $0.1\,\%$ to $8\,\%$ of total irradiance (Grobbelaar 2009).

The SI unit of radiant energy flux is the watt ($\mathrm{W}$). There is no SI unit for photon flux, but it is usually measured in lumens ($\mathrm{lm}$) and the intensity of illumination is expressed in $\mathrm{lux}$ ($\mathrm{lm\,m^{-2}}$). However, in photobiology light energy is usually expressed per unit surface, i.e irradiance, using units of power per area ($\mathrm{W\,m^{-2}}$) as well as Photosynthetic Photon Flux Density ($\mu\mathrm{E\,m^{-2}\,s^{-1}}$). Irradiation is used to consider the amount of solar energy falling on unit area over a stated time interval ($\mathrm{Wh\,m^{-2}}$). The photosynthetic Photon Flux Density is defined as the photon flux density of photosynthetically active radiation (PAR) incident per unit time on a unit surface. An Einstein has $6.022 \times 10^{23}$ photons. On a sunny day, average direct solar irradiance reaching the earth's surface is between $1000\,\mu\mathrm{E\,m^{-2}\,s^{-1}}$ and $2000\,\mu\mathrm{E\,m^{-2}\,s^{-1}}$, being only about the $40\,\%$ the PAR radiation (Richmond 2008).

The conversion factor of PAR from $\mathrm{W\,m^{-2}}$ to $\mu\mathrm{E\,m^{-2}\,s^{-1}}$ can be calculated making use of the Planck relation:

$$\epsilon = \frac{hc}{\lambda}$$

being $\epsilon$ the energy of a quantum of light, $h$ the Planck constant ($6.62 \times 10^{-34}\,\mathrm{J\,s}$), $c$ the speed of light ($3 \times 10^{8}\,\mathrm{m\,s^{-1}}$) and $\lambda$ the wavelength, considering $550\,\mathrm{nm}$ the average PAR wavelength.

Then:

$$1\,\mathrm{W\,m^{-2}} \approx 4.6\,\mu\mathrm{E\,m^{-2}\,s^{-1}}$$

## 2.4. Use of light

When designing a photobioreactor it is very useful to know the light-response (P/I) curve of the species to cultivate, since the higher is the light utilization efficiency the lower is the area needed to cultivate a given quantity of biomass (Simionato et al. 2013). The P/I curve represents the general kinetic response of an algal cell to light intensity, when light is the only limiting factor in the culture (nutritional requirements are supposed to be satisfied and temperature is optimal).

The dependency of the growth rate on the available light may be assumed to follow a Monod type kinetics. The use by first time of the Monod model for light-limited cultures is attributed to Tamiya 1951 (Kurano and Miyachi 2005). After that other models based on light limitation has been proposed in order to account for photoinhibition in the modellings, as reported by Grima et al. 1999.

The P/I curve (Figure 2.2), in a first part, represents a net growth equals to zero, which takes place when the received and absorbed light is balanced by decay. Equilibrium between photosynthesis and respiration occurs at that point. The light intensity below which this occurs is called compensation point ($C_p$). Over this light intensity, growth is higher than decay. The initial slope of the curve (generally denoted as $\alpha$) represents the maximal efficiency of growth response to light intensity. It is the light-limited region in which photosynthesis increases with increasing irradiance. This part of the curve may differ in its slope from one strain to another, in the degree of deviation from a straight line and in the position where it achieves the saturation. The light utilization efficiency in the lower part of the curve is of ecological importance since it affects the survival possibilities under shaded or deep water (Sorokin and Krauss 1958).

At higher values of light intensity the maximal growth rate is achieved, which can occur gradually or abruptly, but above this point no further growth is noticeable. The light saturation irradiance ($I_k$) is that that produce a response equals to $P_{\max}$ and is located in the light-saturated region. Over $I_k$ photosynthesis becomes less efficient until it reaches a plateau (at $I \geq I_s$) and even a photoinhibited region in which photosynthesis decreases with increase in irradiance (at $I \geq I_h$), even damaging the photosynthetic apparatus in extreme cases. It is worth mentioning that most of the microalgae species have its $I_k$ between $100\,\mu\mathrm{E\,m^{-2}\,s^{-1}}$ and $200\,\mu\mathrm{E\,m^{-2}\,s^{-1}}$,

which represents about $5\,\%$ to $10\,\%$ of full daylight irradiance ($2000\,\mu\mathrm{E\,m^{-2}\,s^{-1}}$) (Fallowfield and Osborne 1985).

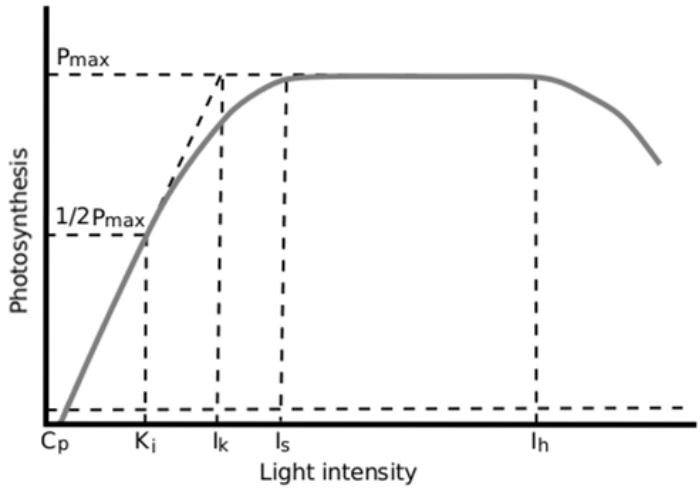


Figure 2.2: Light-response curve of photosynthesis (P/I curve).

Determining the light saturation efficiency for a microalgal strain, even when there is no other limiting factor than light, is not easy due to the shape and situation of the light source in most of the experiments that are usually carried out in laboratories. Light intensity is exponentially attenuated as it goes through a dense culture, then not all the culture receives the same light intensity. As a result, in any culturing device we can establish two zones: the outer illuminated volume, in which light is sufficient to support photosynthesis and the dark volume, in which net photosynthetic productivity cannot take place. The light intensity that determines the extent of these two volumes is the compensation point. Furthermore, not all the regions of the spectra penetrate in the same grade. The pigments content of a certain microalgal species determines the regions of the spectra that will be absorbed with higher or lower intensity (Yun and Park 2001). The knowledge of this phenomenon is important when experimentally measuring the light irradiance, since most of the available photometers outputs the value of the total visible spectra.

Unless the culture is confined in a very thin layer, this curve is not applicable to most of the microalgae cultures due to the complex light distribution through the bulk liquid. The internal shading in the suspension makes the microalgal cells to be

exposed to intermittent light. The illumination cycles can last from milliseconds to a few seconds, depending on the mixing on the culture, on the cell density and on the size of the different areas with different light intensities. Big efforts are being invested nowadays to elucidate the effect of the duration and the ratio between the light and the dark period within a cycle over the microalgae growth (Park and Lee 2000; Lunka and Bayless 2013).

The classification of the culture volume into light and dark areas has been commonly made in order to simplify the complexity of the light in the photobioreactor although it is known that several parameters related to the light have an influence over the microalgae growth and productivity: dark/light residence times, cycle time, frequency of exposition, average light, etc. All of them have been included in a phenomenon called *light regime* (Brindley, Fernández, and Fernández-Sevilla 2011) and has attracted considerable attention during the last years, specially the so called *flashing light effect* phenomenon, which has been demonstrated to have an influence over the overall productivity. This phenomenon is produced when microalgae are subjected to alternating light and dark periods (L-D cycles) due to the turbulence in the photobioreactor, so the duration of the cycles depends on the mixing rate of the culture. In this situation the cells are supposed to process the accumulated intermediate products in the dark. The extent of time that the cells continue to grow in the dark determines how long the cycles can be. Reactor productivity could decrease if the duration of the dark period becomes too long (Ogbonna, Yada, and Tanaka 1995; Simionato et al. 2013). In general, it has been noted that the higher the frequency of the L-D cycles, the more efficient strong light may be used for photosynthesis (Richmond 2008; Simionato et al. 2013). Several reasons may be attributed to this phenomenon. According to Lunka and Bayless 2013 one reason may be the further penetration of intense light into the water column due to the exponential attenuation of light, effectively reaching more algae in comparison with lower light intensity supplied continuously (Park and Lee 2000). The second possible factor contributing to the increase in growth has to deal with the photochemistry of the photosynthesis (Simionato et al. 2013). According to Carvalho et al. 2011, what seems to be clear is that it allows for an optimization of light use efficiency for biofuel production in algae, but high intensity pulses as well as high cell densities are needed to achieve the benefits of the *flashing light effect*.

## 2.5. Microalgae culturing

The design of microalgae culturing devices and processes mainly depends on the final desired product. When the end-product belongs to the field of pharmaceutics or

human feeding or nutrition, sophisticated and clean processes (meaning using clean water and high quality mineral nutrients and carbon sources) must be implemented. Only the high cost of the end-product can justify the high economic and technical investment. On the other hand, the use of wastewater and industrial carbon emissions, in which are nowadays known as hybrid-technologies (Maity et al. 2014), making the process more economically sustainable, can be used for end-products like raw materials for biofuels or when the waste water treating is the main goal itself. When the product of the microalgae culture has a low value in the market, no other source of light than the Sun should be considered. Although indoor culturing technology is being developed and must be taken into account to guarantee the stability of the culture, the value of the final product must balance the energy invested in maintaining the light sources,

Several operating modes are usually employed for microalgae culturing:

**Batch culture:** this method makes the culture to growth until it reaches its maximum density for the provided conditions. In this kind of cultures the properties of the medium change with time, since the suspension becomes more turbid avoiding the light to arrive to the deeper region and the nutrients are depleted. When the medium reaches the desired concentration, it is harvested except a small part that remains as inoculum for the next cycle. This is the most common way to culture microalgae at laboratory scale. Simple flasks are usually used as reactors, where a $CO_2$ enriched air stream is provided by means of diffusers and the light source is situated only in one side of the culturing chamber. Since conditions vary with time, several factors are usually affecting the changes observed in the biomass and comparisons become difficult. The typical growth curve showed in Section 2.4 is applicable to batch cultures, where firstly the availability and afterwards the limitation by one or more factors drive the culture along the mentioned phases.

**Semi-continuous culture:** the biomass suspension is regularly diluted, in such a way that the microalgae population reaches a given density, and then it is partially harvested by removing a certain volume. The same volume of fresh medium is then supplied.

**Continuous culture:** the culture is feed with fresh medium at the same flow rate that the suspension is removed, having a constant volume of culture. This method allows for a continuous exponential phase, which is only possible when the growth rate is equals to the dilution rate of the system. In spite of this, the

study of microalgae culturing continuous systems have been emphasized only during the last few years (Sforza, Enzo, and Bertucco 2013).

The increase of biomass in the culture is given by the difference between the production of organic matter during the photosynthesis and the removal due to cell death and the outlet stream.

$$\text{Net increase of biomass} = \text{Growth} - \text{Biomass removal}$$

Since experimentally obtained growth rates take into account the net biomass growing (productivity minus decay due to death), the net increase of biomass can be simplified to the difference between growth and removal.

For an infinitely time interval $dt$, the balance can be written as follows:

$$V\,\mathrm{d}x = V\mu\,\mathrm{d}t - FX\,\mathrm{d}t$$

being $V$ the culture volume (L), $\mathrm{d}x$ the change in biomass concentration ($\mathrm{g\,L^{-1}}$), $\mu$ the specific growth rate ($\mathrm{h^{-1}}$), $X$ the biomass concentration ($\mathrm{g\,L^{-1}}$) and $F$ the flow rate ($\mathrm{L\,h^{-1}}$).

Simplifying,

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \left(\mu - \frac{F}{V}\right) X$$

The term $F/V$ is the dilution rate ($D$) of the culture and for reaching the steady-state $F/V$ must be equals to $\mu$. The inverse of $D$ should be equivalent to the hydraulic retention time in the field of reactor designing.

At large-scale, continuous operation is preferred since this way provides a better control and growth rates can be easily modified only by varying the flow rate. However, there are some reasons that make difficult the implementation of continuous cultures with certain products as end-products. Some compounds need a progressive or a sudden change to be produced, since their production is usually a consequence of metabolic or physiological changes in the cells. Furthermore, usually stress conditions needed to produce some compounds are detrimental to achieve high biomass productivity. In the case of oil accumulation, it has been observed to be very low under standard growth conditions and to increase under N-starvation (Illman, Scragg, and Shales 2000). For this reason, two-stage cultures are usually considered, being

the first stage dedicated to the cell reproduction and the second to the end-product accumulation.

The change from nutrient-rich conditions to stress conditions may be done in a sudden way, which implies harvesting the biomass and transferring to a new culture medium (Mujtaba et al. 2012). Similar lipid productivity was found when comparing a sudden starvation strategy to a progressive one, both with cultures in batch (Pruvost et al. 2009). Little information has been found about N-starvation strategies in continuous cultures.

## 2.6. Types of photobioreactors

There is a wide variety of photobioreactors for microalgae culturing regarding their architecture, their configuration, the way to supply the light, etc. In this work, only photobioreactors using sunlight are considered since any other source of energy hardly could justify the development of photobioreactors for low value target products. It is known that the use of solar light may be a limiting factor due to the diurnal cycles and the seasonal variations, constricting the viability of commercial production. For this reason, achieving a proper distribution of light and profiting the maximum of the irradiated surface are the main challenges in photobioreactor designing. One of the main limitations of large-scale microalgae cultures is the low efficiency of the existing technology, specially related to the occupied surface (Wijffels and Barbosa 2010).

Usually photobioreactors are classified in two types: open and closed. A third type has been recently included, known as hybrid photobioreactors and consisting on the combination of open ponds with closed photobioreactors (Wang 2009). This last type profit from the two-stage cultivation strategy by making use of the closed photobioreactor for cell growth and open ponds for the lipids production stage. The coupled system has been seen as a good choice to take advantage of the benefits of both photobioreactors and open ponds, while avoiding their disadvantages, achieving fast growth rates in the first reactor and with low control needed in the second one, characterized by high cell density and depletion of nitrogen (Huntley and Redalje 2007).

The most common commercial technology for microalgae biomass is the raceway type that consists on shallow paddle wheel mixed ponds. It is the most representative technology within the open type. Their main characteristics are that they are flexible and of low cost, however its scalability only can be reached by increasing the occupied surface, ranging from a few ha. to several hundreds (Benemann 1997). They typically consist on a closed loop generally between $0.2\,\mathrm{m}$ to $0.5\,\mathrm{m}$ deep, with mixing devices

and circulation required to stabilize algae growth and productivity. The paddlewheel is in continuous operation to prevent sedimentation. The microalgae $CO_2$ requirement is usually satisfied from the surface air, but submerged aerators may be installed to enhance $CO_2$ absorption (Brennan and Owende 2010). Although they occupy large land surfaces, they do not necessarily compete for land with agricultural crops, since they can be implemented in areas that are not viable for feeding crops. In comparison with most of the closed systems, they are reported to be less energy consuming and easy to maintain, however they are less efficient due to the difficulty to control several factors like evaporation losses, temperature fluctuations, etc. but especially due to the low light utilization efficiency. In southern Spain areal productivity values ranging from $2\,\mathrm{g\,m^{-2}\,d^{-1}}$ to $14\,\mathrm{g\,m^{-2}\,d^{-1}}$ have been reported (Jimenez, Cossio, and Niell 2003).

Generally, any other kind of photobioreactors are included in the closed type and the term photobioreactor is reserved for this kind of technology. Depending on the specific design they have different advantages or disadvantages in comparison to the open ponds. In general it is accepted that they allow for a better control over the culture conditions, specially that it is possible to modulate the light falling over its surface (Morweiser et al. 2010) and other parameters like pH, temperature, mixing, $CO_2$, $O_2$, contamination, etc. Furthermore, they usually allow higher cell concentrations and higher volumetric productivities (Mata, Martins, and Caetano 2010). Closed photobioreactors usually looks for high surface to volume ratios, having short light paths in order to increase the availability of light to each cell. The most popular photobioreactor configurations are tubular, vertical or column and flat plate reactors.

**Tubular photobioreactors** consist on arrays of thin and long transparent tubes (diameter about $10\,\mathrm{cm}$) which are usually configured horizontally or near horizontally following different patterns: straigh, spiral, etc. Its main advantage is that they have large illuminated surface to volume ratio, but as disadvantages they present poor mass transfer and $O_2$ accumulation. The scale-up of this photobioreactors cannot be reached by increasing the diameter of the tubes; otherwise its main advantage of high illuminated surface to volume ratio would disappear. A balance between volumetric productivity and areal productivity should be achieved to profit from the advantages of this kind of photobioreactor. The other possibility to scale-up is to increase the length of the tubes, which would increase gradients of $O_2$ and $CO_2$ transfer along the tubes. Finally, temperature is difficult to maintain and photoinhibition is very common (Ugwu, Aoyagi, and Uchiyama 2008). In spite of its disadvantages, this has been one of

the most studied improved photobioreactor design and a wide number of pilot and industrial facilities are currently found around the world.

**Vertical columns** are vertical configurations that have been proposed to overcome the problems related to horizontal systems (pumping through the tube length, oxygen accumulation, fouling, etc), generally in the form of transparent airlifts reactors. They allow for a better gas exchange and oxygen inhibition of photosynthesis is avoided. A better mixing due to the turbulence created by the bubbles and definitely a more efficient exposure of cells to light can be achieved (Camacho et al. 1999). As an disadvantage, the difficulty to be scaled-up can be remarked.

**Flat-plates** consist on narrow panels that are usually placed with a certain tilt angle to optimize light capture. They have as its main advantage the high illuminated surface to volume ratio and their easiness to modulate the received irradiance varying the orientation of the plates respect to the solar beams (Slegers et al. 2011; Qiang, Faiman, and Richmond 1998). As the tubular photobioreactors, they have a suitable light path, shorter enough to have a near-homogeneously illuminated culture. Flat panel photobioreactors have been widely used with research purposes due to the easiness of controlling and measuring the irradiance over them (Richmond 2008). They have been also seen as a promising technology to be scaled-up since high reactors can be oriented and tilted at optimal angles according to the season. The tilt angle can be calculated to make the direct solar beams to hit the photobioreactor surface with high angles, thus avoiding photoinhibition and increasing the efficiency or light capturing per unit surface. Scalability can be done in two dimensions, although the higher is the size of the plates, the higher is the difficulty to operate. An accurate design of the culturing platforms must be done in order to avoid the shadowing among the plates.

Due to their initial high investment and their sometimes expensive or difficult maintenance, closed photobioreactors are usually focused on monocultures with high value end-products. Enclosed photobioreactors productivity usually ranges from 20 $\mathrm{g\,m^{-2}\,d^{-1}}$ to 30 $\mathrm{g\,m^{-2}\,d^{-1}}$ (Cuaresma et al. 2011). According to Mata, Martins, and Caetano 2010, photobioreactors still present some limitations that should be addressed and solved. These limitations are mainly: overheating, oxygen accumulation, the difficulty to scale-up, the high cost of photobioreactors building and maintenance and the cell damage due to shear stress. A more detailed overview of photobioreactors and recent designs is made in Chapter 4 and Chapter 5

Finally, to establish comparisons among photobioreactors based on results, several parameters are used:

**Volumetric productivity:** is the productivity per unit volume, generally expressed as $\mathrm{g\,L^{-1}\,d^{-1}}$.

**Areal productivity:** is the productivity per unit of ground surface, generally expressed as $\mathrm{g\,m^{-2}\,d{-}1}$.

**Illuminated surface productivity:** is the productivity per unit of illuminated photobioreactor surface, generally expressed as $\mathrm{g\,m^{-2}\,d^{-1}}$.

However, since the problem of microalgae cultures is the difficulty to distribute irradiance through the culture volume, and irradiance is limited by the illuminated surface, when designing a competitive photobioreactor is not worth comparing the volumetric activity. The volumetric productivity of the cultures depends strictly on the light energy input per unit volume and gives no information on how efficiently the incident irradiance is being used (Tredici and Zittelli 1998).

## 2.7. Optimizing light utilization

The availability as well as the intensity of light are the major factors controlling productivity of photosynthetic cultures. In a given geographical location, the amount of light that a culturing device can receive is determined by the surface exposed to solar irradiance. Cultivation facilities should be designed in a way that allows for a maximization of the light conversion efficiency. This has been usually reached by the use of very dense cultures. However, light is highly attenuated and only a very thin layer of culture is exposed to it, resulting in an overexposure of the upper layer, leading to a final low efficiency. There are two technological strategies to overcome this problem: on the one hand a possibility is to maintain the medium highly mixed in order to prevent saturation during long times. On the other hand, the design of photobioreactors with special geometries to improve light distribution has gained a lot of interest. With regard to the first strategy, the difficulty to find mixing devices able to induce light-dark cycles shorter enough to prevent from saturation and its energy consumption are the main bottlenecks. Regarding to the developing of new designs; one of the biggest challenges of this strategy is to develop large-scale —industrial level— photobioreactors with appropriate dilution effect of the incident irradiance.

In the last years much effort has been invested in increasing photosynthetic efficiency under oversaturating light conditions. There is no general agreement

about the cells behavior in dense cultures, where they move among a wide gradient of irradiances. The response of the photosynthetic efficiency to fast variations of irradiance is not clear, but it seems to be influenced by the total radiation and the frequency of the variations, as stated before.

With the purpose of getting an appropriate distribution of solar energy, light harvesting and distributing methods have been proposed, especially by making use of Fresnel lenses to harvest light and then distribute it by means of optical fiber (Ogbonna, Soejima, and Tanaka 1999) or vertical plastic light guides (Zijffers et al. 2008). There are also some recent studies on systems that try to guide the light to a deeper area of the photobioreactor by means of transparent chambers receiving the light and spreading it out to the culture through their lateral walls, avoiding the use of optical devices like lenses (Hsieh and Wu 2009). The design of light distributing systems with low energy requirements has been proposed as a promising strategy (Wijffels and Barbosa 2010). Also for scaling-up reasons, internally lightened photobioreactors are seen as the only way to easily scale-up reactors (Cornet 2009).

Most of the developed studies with solar tracking systems have been applied to flat panels. It has been shown that using solar tracking systems enables a higher irradiance in winter days by facing the panel perpendicular to the solar beams, thus increasing the overall productivity. On the contrary, at low cell densities or at high irradiances it is possible to provide lower irradiance over the culture adjusting the tilt angle of the panel. According to the work of Wijanarko et al. 2006, the main advantages of using solar tracking systems are:

1. The possibility to decrease photoinhibition of photosynthesis in a microalgal culture of low density, by reducing the irradiance.
2. Enhancing the irradiance beyond $100\,\%$ of the horizontal irradiance in high cell density cultures by exposure of the reactor perpendicular to the sun light.
3. Regulating culture temperature by adjusting the irradiance or cooling to avoid heat stress.

## 2.8. Harvesting

Algal harvesting consists on biomass recovery from the culture medium, and may contribute to $20\,\%$ to $30\,\%$ of the total biomass production cost (Grima et al. 2003). Harvesting of microalgae is seen as one of the major challenges of using microalgae for the production of biodiesel (Rawat 2012). In order to remove large quantities of

water and process large algal biomass volumes, a suitable harvesting method may involve one or more steps and be achieved in several physical, chemical, or biological ways, in order to perform the desired solid-liquid separation. Biomass concentration of microalgal suspension may be low, values under $1\,\mathrm{g\,L^{-1}}$ are not strange and this makes the harvesting process quite difficult.

Centrifugation, filtration and gravity settling are current harvesting methods. These processes may be preceded by a flocculation step. Centrifugation is the most rapid and reliable method, but due to its high cost, its implementation at large-scale is not considered (Christenson and Sims 2011). Harvesting of biomass using membrane filtration has been also addressed and anti-fouling strategies are being studied in order to maintain the flux across the membranes (Rossignol et al. 1999; Zhang et al. 2010). Flocculation-sedimentation is assumed to be more effective than centrifugation and gravity settling, since it allows treating large culture volumes and does not consume much energy. It can be also considered as a step for improving centrifugation or filtration yields (Lee et al. 2012).

However chemical flocculation implies reactive consumption and other mechanisms like auto-flocculation or bio-flocculation have been seen as promising alternatives from the environmental and economical point of view (Lavoie and Delanoue 1987). The first one makes reference to the flocculation induced by pH-increasing in presence of divalent cations like $Mg^{2+}$ or $Ca^{2+}$. At high pH values calcium or magnesium compounds form positively charged precipitates that are adsorbed on the negatively charged microalgal cells inducing to flocculation and sedimentation (Vandamme et al. 2012; Leentvaar and Rebhun 1982). The pH increase may be achieved by adding basic species or just in the absence of $CO_2$ input. The second one refers to the secretion of extracellular products, mainly polysaccharides that are produced under stress conditions —i.e. nutrient deprivation— and contribute to increase the size of the aggregates (Yang et al. 2010; Lee, Lewis, and Ashman 2009).

## 2.9. Medium reuse

Since microalgae need light and warm temperatures to grow, low latitudes are the most appropriate for their culture. These zones are just those which most suffer from water scarcity making a sustainable use of this resource a key issue. Furthermore, evaporation losses are higher exactly were radiation and temperature are also high.

The water consumption for culturing *C. vulgaris* in an open pond under the conditions similar to the summer in California has been estimated as $3726\,\mathrm{kg\,(water)\,kg\,(biodiesel)^{-1}}$. This can be reduced to $591\,\mathrm{kg\,(water)kg\,(biodiesel)^{-1}}$

if water is recycled (Yang et al. 2011). As a solution to avoid high water consumption, the use of wastewater, especially secondary effluents, has been proposed. However, if the target of the process is the biomass production, nor the water treatment, a proper pre-treatment like filtration or UV-radiation should be applied to remove competing microorganisms (Cho et al. 2011).

Recycling culture media can help to minimize water and nutrient consumption, and it is therefore, a recent highlighted challenge in the development of large scale facilities. It needs for a previous separation process and, since chemicals remains in the water, the kind of process may affect the subsequent cultures. Among the several studies that have attempted to test the viability of medium reuse, major constraints have been found when using alum as flocculating agent, since it has made the subsequent culture to decrease its growth yield in comparison to the use of bioflocculants. The use of bioflocculants has been demonstrated to be an effective method for the harvesting of high density cultures. Furthermore, when the reused medium is supplemented with nutrients and increase in biomass growth yield was seen in comparison with the culture in fresh medium (Kim et al. 2011). The influence of consecutive reuse cycles is not yet clarified, since conductivity, fungus or bacteria may accumulate in the medium.

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