Towards increased microalgal productivity in photobioreactors

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abstract

Currently there is much interest to cultivate microalgae for the production of bulk products like lipids for biodiesel or as feedstock for industrial chemical processes. To make the production economically feasible, it is essential to develop cultivation systems in which algae convert the light with a high photosynthetic efficiency, to obtain microalgae with specific production characteristics, to optimize the culture medium, to develop cheap harvesting methods and finally professionals to implement and develop the technology. For the development of better algal cultivation systems, insight is needed on the efficiency of light conversion by these algae. Two concepts of light dilution in photobioreactor design are presented to reflect how potentially higher photosynthetic efficiencies can be achieved. Also, fast screening methods for microalgae are discussed, which can be applied to select strains with better production characteristics, to optimize the medium, to compare cultivation conditions and to investigate the toxic effects of chemicals. Apart from obtaining enhanced algal productivities a low-cost harvesting process is required. For harvesting of bulk products, as preconcentration step, controlled auto-flocculation is worthwhile investigating. Beside these research lines, more and better education for biochemical engineers should be developed to push microalgal biotechnology ahead.

Keywords: **bulk products, education, harvesting, medium optimization, microalgae, photobioreactor, photosynthetic efficiency, productivity, screening method**

Mejora de la productividad de microalgas en fotobiorreactores

Actualmente existe un gran interés en el cultivo de microalgas para la obtención de productos a gran escala como lípidos para biodiesel, o por su utilización como materia prima en procesos químicos industriales. La consecución de una producción económicamente viable necesita el desarrollo de sistemas de cultivo en los cuales se utilice la luz con una alta eficiencia fotosintética; la aplicación de condiciones de producción específicas; la optimización del medio de cultivo; el desarrollo de métodos de recolección baratos; y finalmente profesionales para poner en práctica y desarrollar la tecnología. Para una mejora de la productividad de los sistemas de cultivo de algas es fundamental profundizar en la eficiencia de la conversión de la luz. En este sentido se muestra la potencial mejora en la eficiencia fotosintética que podría alcanzarse mediante el uso de dos conceptos diferentes de distribución de la luz dentro de los fotobiorreactores. Por otro lado se discute el uso de métodos de detección rápida de microalgas, los cuales pueden ser utilizados en la selección de especies con interesantes características de producción, para optimizar el medio de cultivo, para comparar condiciones de cultivo, y para investigar el efecto tóxico de sustancias químicas. Además de la mejora en la productividad, un sistema de recolección de bajo coste es también requerido. Para ello la auto-floculación controlada, como paso de preconcentración, merece ser objeto de estudio. Junto a estas líneas de investigación, una mayor y mejor formación de los Ingenieros Bioquímicos ha de ser desarrollada para poder impulsar la biotecnología de microalgas.

Auf dem Weg zur erhöhten Mikroalgen-Produktivität in Photobioreaktoren

Derzeit besteht großes Interesse an der Kultivierung von Mikroalgen zur Produktion von Bulk-Produkten wie Lipiden für Biodiesel oder als Ausgangsmaterial für chemische Industrieprozesse. Um die Produktion wirtschaftlich realisierbar zu machen, ist es wichtig, Kultivierungssysteme, bei denen Algen das Licht mit hoher photosynthetischer Effizienz verwandeln, zu entwickeln, Mikroalgen mit speziellen Produktionscharakteristiken zu erlangen, das Kulturmedium zu optimieren, billige Erntemethoden zu entwickeln und schließlich Fachkräfte zur Implementierung und Entwicklung der Technologie zu finden. Um bessere Algenkultivierungssysteme entwickeln zu können, sind Einsichten zur Effizienz der Lichtkonvertierung dieser Algen nötig. Zwei Konzepte der Lichtverdünnung in Photobioreaktor-Designs werden präsentiert, um darüber zu reflektieren, wie möglicherweise höhere photosynthetische Effizienzen erreicht werden können. Ebenfalls diskutiert werden Schnell-Screening-Methoden für Mikroalgen, die dazu benutzt werden können, Stämme mit besseren Produktionsmerkmalen zu selektieren, das Medium zu optimieren, Kultivierungsbedingungen zu vergleichen und die toxischen Wirkungen von Chemikalien zu untersuchen. Neben der Erreichung verbesserter Algenproduktivität ist zudem ein billiges Ernteverfahren erforderlich. Zur Ernte von Bulk-Produkten lohnt sich als Vorkonzentrierungsschritt die Untersuchung kontrollierter Auto-Flockulation. Neben diesen Forschungslinien sollte zur Vorantreibung der mikroalgischen Biotechnologie auch die Ausbildung für biochemische Ingenieure ausgebaut und verbessert werden.

Aumento da productividade de microalgas em fotobioreactores

Existe presentemente muito interesses no cultivo de microalgas para a produção de produtos bulk como por exemplo lípidos, para a produção de biodiesel ou como matéria prima para a industria quimica. Para tornar a produção de microalgae economicamente viável é essencial: desenvolver sistemas onde as microalgas convertem a luz a uma taxa fotossintética elevada, obter microalgas com características específicas de produção, optimisar o meio de cultura, desenvolver processos económicos para recolha de biomassa e finalmente educar profissionais para implementar e desenvolver a tecnologia. Para o desenvolvimento de sistemas de cultivo mais eficientes é necessário ter conhecimento sobre a eficiência da conversão de luz pelas algas. Dois conceitos de diluição de luz dentro de fotobioreactors são apresentados neste trabalho, de forma a reflectir como potencial elevadas eficiências fotossintéticas podem ser obtidas. Em adição, são discutidos métodos para a rápida selecção de microalgas que podem ser aplicados para seleccionar estirpes com melhores caracteristicas de produção, para optmisar o meio de cultivo, para comparar condições de cultivo e para investigas os efeitos tóxicos de químicos. Além de obter productividades de biomassa mais elevadas, um processo para a recolha de biomassa com baixos custos é necessário. Para a recolha de productos bulk, o estudo de autofloculação como um passo de pre-concentração da biomassa vale a pena ser estudado. Em adição a estas linhas de investigação, a educação de engenheiros bioquimicos nesta àrea deverá ser desenvolvida de forma a poder avançar a biotecnologia de microalgas.

Introduction

Microalgae are regarded as promising organisms for the large-scale production of bulk products like proteins, polysaccharides and lipids as feedstock for industrial chemical processes and biofuel production. However, to make commercial production of such products with microalgae economically feasible, still many bottlenecks in the algal process should be overcome (Figure 1). This paper reflects on the bottlenecks photobioreactor design, light conversion, fast screening, harvesting and education.

Sun light as substrate for algae in photobioreactors

To produce bulk products with microalgae sunlight should be used as light source. Artificial illumination is much too costly; only the energy cost for the illumination (based on LED technology) is already 23 € kg biomass^{-1} (Appendix I), which is much more than the actual production costs of 5 to 10 € and 3 to 30 € kg biomass^{-1} [1] that were reported for open ponds and photobioreactors, respectively.

To reduce the costs of microalgal production much higher areal as well as volumetric productivities of the microalgae should be realized in the photobioreactor. This should be done by bridging the gap between the real and the theoretical maximum photosynthetic efficiency (9%) [2]. The photosynthetic efficiency (PE) represents the amount of biomass formed per light energy (complete spectrum) provided. In current commercial algal production systems PE are recorded ranging from 1% in open pond systems up to 3% in closed tubular photobioreactors (Table 1).

The light intensity of sunlight on a cloudless day can easily exceed 1500 µmol $m^{-2}s^{-1}$. However, this intensity is much more than the algae can handle. In order to achieve higher PE the incident light intensity should be decreased so that microalgae experience light intensities below the saturating light intensity. This reduction can be achieved in two ways; by light integration or by light dilution.

If the light integration approach is used, it is assumed that intensive mixing ensures that the microalgae convert all absorbed light with a photosynthetic yield based on the average light intensity that they experience [3]. Especially at high light intensities above photosaturation level, high turbulence is advantageous; if mixing is fast enough, the algae may experience an average lower light intensity below the photosaturation level and the productivity will thus be increased [4-6]. However, while beneficial effects are reported, complete light integration has never been shown in mass cultivation and only partial light integration is achieved [1,7].

Another way to increase the PE and thus the overall areal productivity is by diluting the amount of light that falls on a certain ground area by spreading it over a much larger illuminated area [8,9]. To achieve this, several vertically mounted photobioreactors should be placed in north/south orientation in order to reflect the light between the photobioreactors (Figure 2). The light intensity that an alga receives from the incident intensity in this way is reduced to below the saturating light intensity, thereby increasing the biomass yield on sunlight.

Figure 1. Bottlenecks to be overcome in order to achieve commercial production of microalgae in photobioreactors. The bottlenecks in grey areas are addressed in this paper

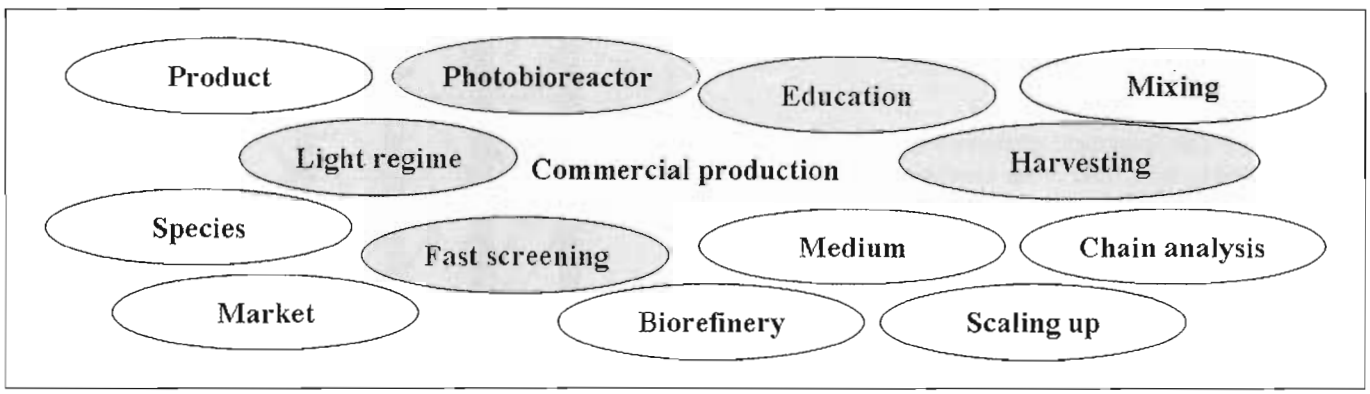


Table 1. Areal productivities of commercial photobioreactors for Huelva, Spain (37°15'56" North) deduced from data provided in literature

Reactor	Approach	Optical path (cm)	PE (%)	Areal productivity ton ha^{-1} yr^{-1}	References
Open pond	Growth integration	30	0.9	27	[57]
Thin-layer pond	Light integration	0.6	2.6*	78	[11]
Tubular reactor	Light integration	5	3	90	[7] [58]
Flat panel	Light dilution	1-2	6**	179	[8]
Theoretical maximum in photobioreactors			7	200	

* Photosynthetic efficiency (PE) measured

**Estimated from [23], assumed that light was diluted to an uniformly two-sided illumination of 115 µmol $m^{-2}s^{-1}$.

Higher PE possible by adequate photobioreactor design

Preferably, the photobioreactor is placed in countries close to the equator where much light is available. Photobioreactors should be designed to minimize reflection of light by the photobioreactor material and in addition a material should be chosen that has highest transmittance of PAR (Photosynthetic Active Region, 400-700 nm) light, so that the microalgae receive most light. However, transmission curves show that at least 10% will be lost on transmission and even more light will be lost when the angle of incidence of the sunlight gets higher than 45° [10]. During the night also biomass will be lost due to dark respiration of storage compounds in the microalgae. Night biomass losses (NBL) range from 2-14% [11-13] and can be minimized by decreasing the culture temperature after sunset. In addition, energy is needed for maintenance of the cells, which is expected to be proportional to the biomass density of the culture and is known to be significant [14]. If we assume 10% loss of the PAR light due to transmission and 5% loss of biomass due to photorespiration during the night and 10% of the energy absorbed is used for maintenance, the maximum photosynthetic efficiency using sunlight for algal production in photobioreactors will be about 7% as shown below:

Theoretical maximum photosynthetic efficiency
9%
10% of light lost by transmission x
0.90
5% of biomass lost during the night x
0.95
10% of energy used for maintenance x
0.90
Maximum photosynthetic efficiency in photobioreactors:
7%

To reach this photosynthetic efficiency (PE), productivity should only be limited by light. Most of the commercial photobioreactors that have been developed so far are based on either the concepts of light integration or on light dilution. Table 1 shows the reported or calculated photosynthetic efficiencies reached in several existing photobioreactors and the calculated corresponding areal productivities based on daily natural sunlight data for Huelva in Southern Spain (Appendix II).

Open ponds show the lowest productivity because the algae convert the light with a low photosynthetic efficiency due to a long optical path, slow mixing and lack of control.

The tubular reactor (Figure 3A) and the thin-layer pond (Figure 3B) show higher efficiencies due to a shorter optical path and higher turbulence combined with better control. However, light/dark cycles are still too long to obtain much light integration and also gradients (pH, nutrients, oxygen) occur in both systems [15]. The biggest disadvantage of these systems is that oxygen produced in these systems by the microalgae can easily exceed 300% oxygen saturation, leading to growth inhibition of the algae and a severe loss of productivity [16-18]. This can be solved by increasing liquid velocities, but only at the expense of higher energy costs [19]. Using the light integration approach for photobioreactor design leads to high demands of energy for mixing (334 W m^{-3}), required to achieve short light/dark cycles [20]. In addition, Zijffers found that increased turbulence does not necessarily lead to higher observed efficiencies for *Chlorella sorikiana* in a flat panel photobioreactor (optical path

Figure 2. Schematic overview of light dilution

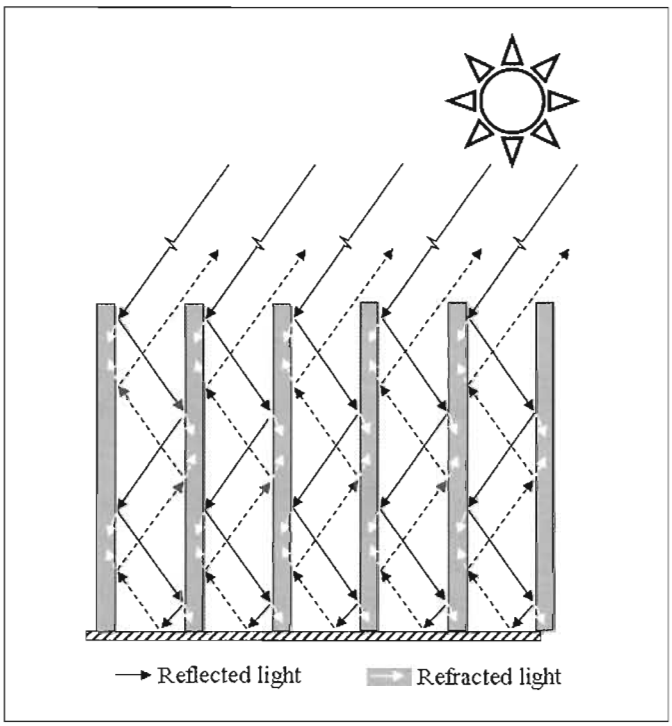


Figure 3. (A) Tubular reactors in Ritschenhausen (GMBH). **(B)** Thin-layered pond (courtesy of J. Doucha, Třeboň laboratory, Czech Republic) **(C)** Flat panels also described as green wall panel (courtesy of M.R. Tredici, University of Florence, Italy)

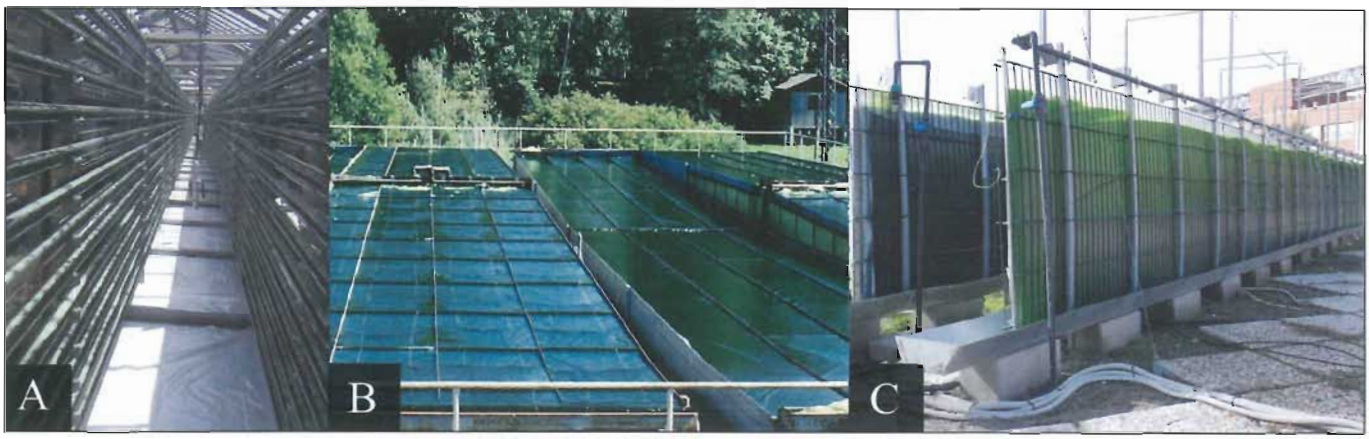
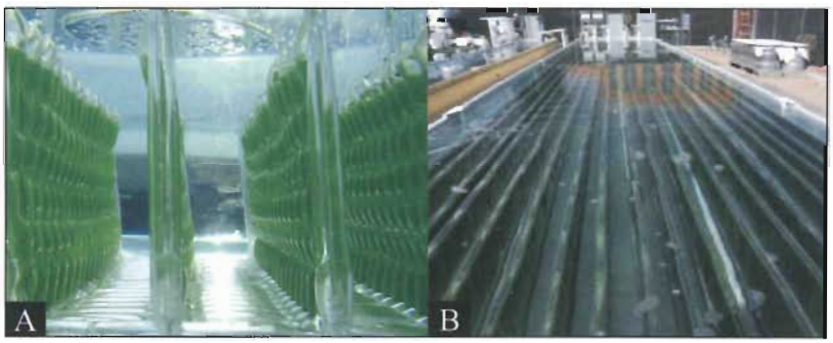


Figure 4. (A) Flat panel photobioreactor of Proviron (courtesy of M. Michiels, Proviron, Belgium) **(B)** Flat panel photobioreactor of Solix Biofuels (courtesy of B. Willson, Colorado State University)



1.25 cm) since the maintenance requirements of the high density culture of the microalgae will decrease the observed photosynthetic efficiency on light [21]. Also dissolved oxygen levels will be high and probably will limit productivity [22]. Altogether, it is better not to pursue the light integration approach in photobioreactor design, since this will lead to lower photosynthetic yields and thus high production costs of the biomass.

Innovative photobioreactors can better use the approach of light dilution to reduce the incident light intensity to 100 µmol $m^{-2}s^{-1}$. In this case, the lower amount of light that is absorbed by the microalgae can be converted with a much higher photosynthetic yield. Zittelli also showed that in a modular flat panel photobioreactor (example, Figure 3c) illuminated by a light intensity of 115 µmol $m^{-2}s^{-1}$ in which *Nannochloropsis sp.* was cultivated, a PE of 6% could be reached [23]. With this efficiency, an areal productivity of 179 ton ha^{-1} yr^{-1} is possible in Southern Spain (Table 1).

As building block, a thin-layered flat panel (optical path < 2 cm) film can be used best. Advantage of flat panels is that they have, like other vertical systems, a high mass transfer coefficient (0.006 s^{-1}) and require less power (53 W m^{-3}) compared to tubular photobioreactors (2400-3200 W m^{-3}) for adequate mass transfer [19].

Figure 5. (A) Relative growth rate of *C. vulgaris* (CCAP 211-118) in M8-A medium at different pH. **(B)** Relative growth rates of *Monodus* at different disinfectant (Virkon®) concentrations (Bosma *et al.* 2008)

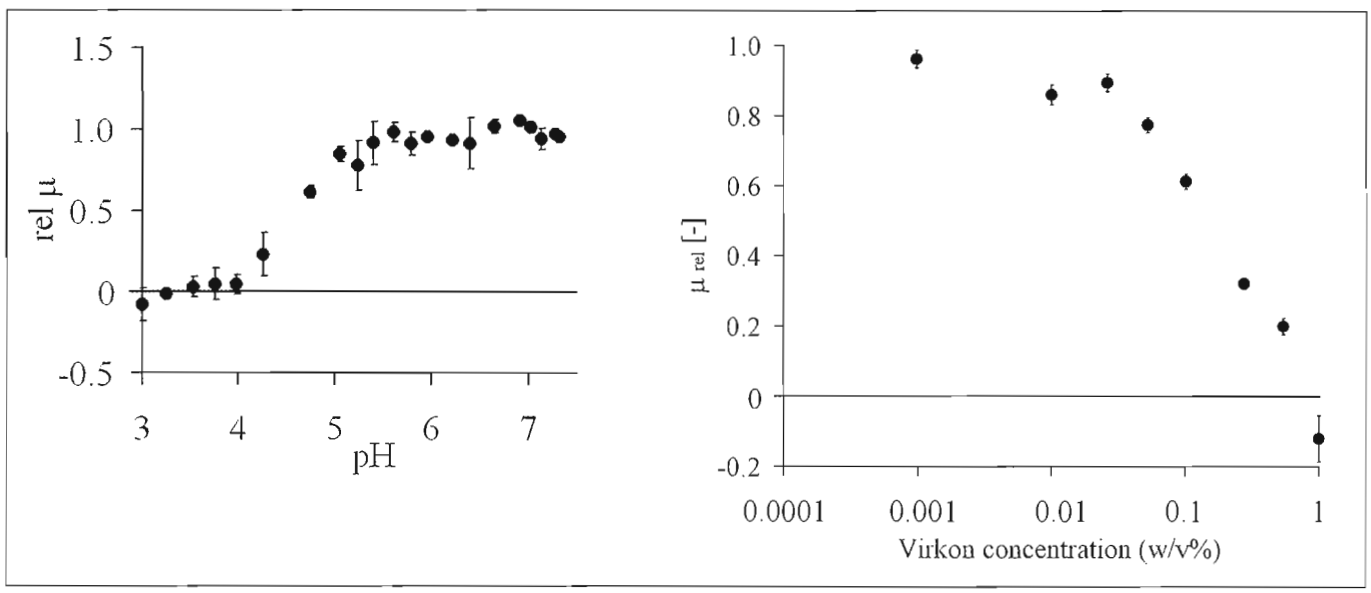
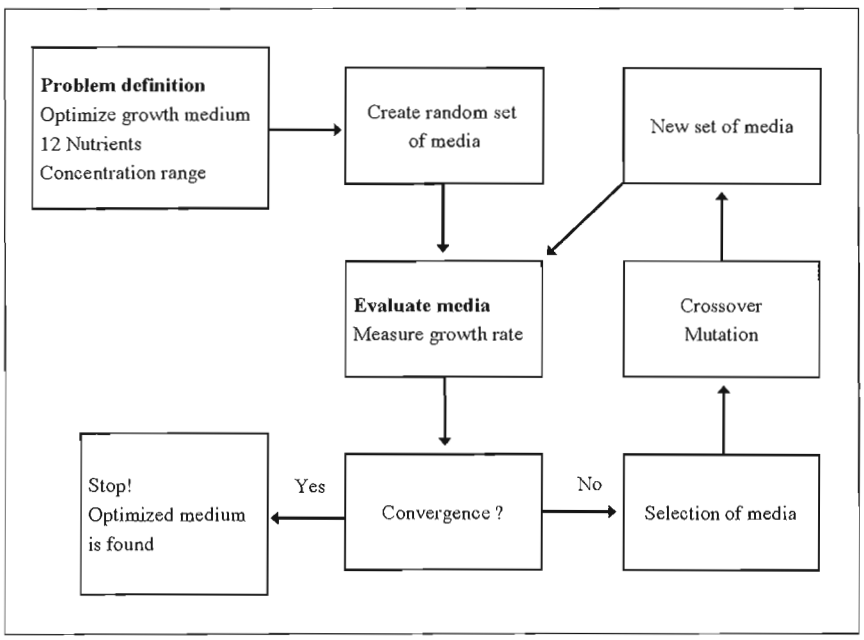


Table 2. Compositions of media used to cultivate *Chlorella vulgaris*

	BG-11	BBM	M8-A	Mod. Fitz	Sorokin/ Krauss
Macronutrients (µmol L^{-1})					
N	17.6	2.9	29.7	5.0	12.4
P	0.2	1.7	6.9	0.3	9.2
S	0.3	0.4	2.2	0.6	4.2
Mg	0.3	0.3	1.6	0.5	4.1
Fe	0.03	0.02	0.49	0.03	0.2
Ca	0.24	0.17	0.09	0.13	0.3
Trace elements (µmol L^{-1})					
Zn	0.8	30.7	11.1	1.0	0.31
Cu	0.3	6.3	7.3	0.0008	0.06
Mn	9.1	7.3	65.6	10.1	0.07
Co	0.2	1.8	-	0.6	0.02
Mo	1.6	4.9	-	0.6	0.05
B	46	185	-	50.1	1.84
Al	-	-	28.7	1.0	-
EDTA	2.3	171	27	32	1711
Salts (µmol L^{-1})					
Na	18.0	3.5	2.9	5	1.7
K	0.4	1.3	35.1	0.5	21.5
Cl	0.5	0.8	0.3	0.3	0.5
Organics (µmol L^{-1})					
Citric acid	0.06	-	-	-	-
Reference	[61]		[59]	[60]	[15]

Figure 6. Schematic overview of a genetic algorithm to obtain an optimized growth medium



Photobioreactors with longer optical paths should not be used because the light is not used efficiently, resulting in voluminous reactors with low volumetric productivities and consequently high costs of downstream processing.

Total costs of the cultivation system should be less than 15 € m^{-2} to make production costs of energy products with microalgae commercially feasible [8]. In our opinion, thin-layered inflatable plastic films should be used to reduce the costs of the material needed. Typical examples of photobioreactors using these types of films are presently developed (Figure 4). In the Proviron flat-panel type photobioreactor, the cultivation chambers are surrounded by water containing bags for extra support and temperature control (patent EP 2 039 753 A1). In the Solix Biofuels photobioreactor the plastic bags are hanging on a support device and the whole construction is placed in a water basin. The ground on which the cultivation is placed should be white-washed to reflect 64% of sunlight to otherwise shaded parts [21].

Fast screening

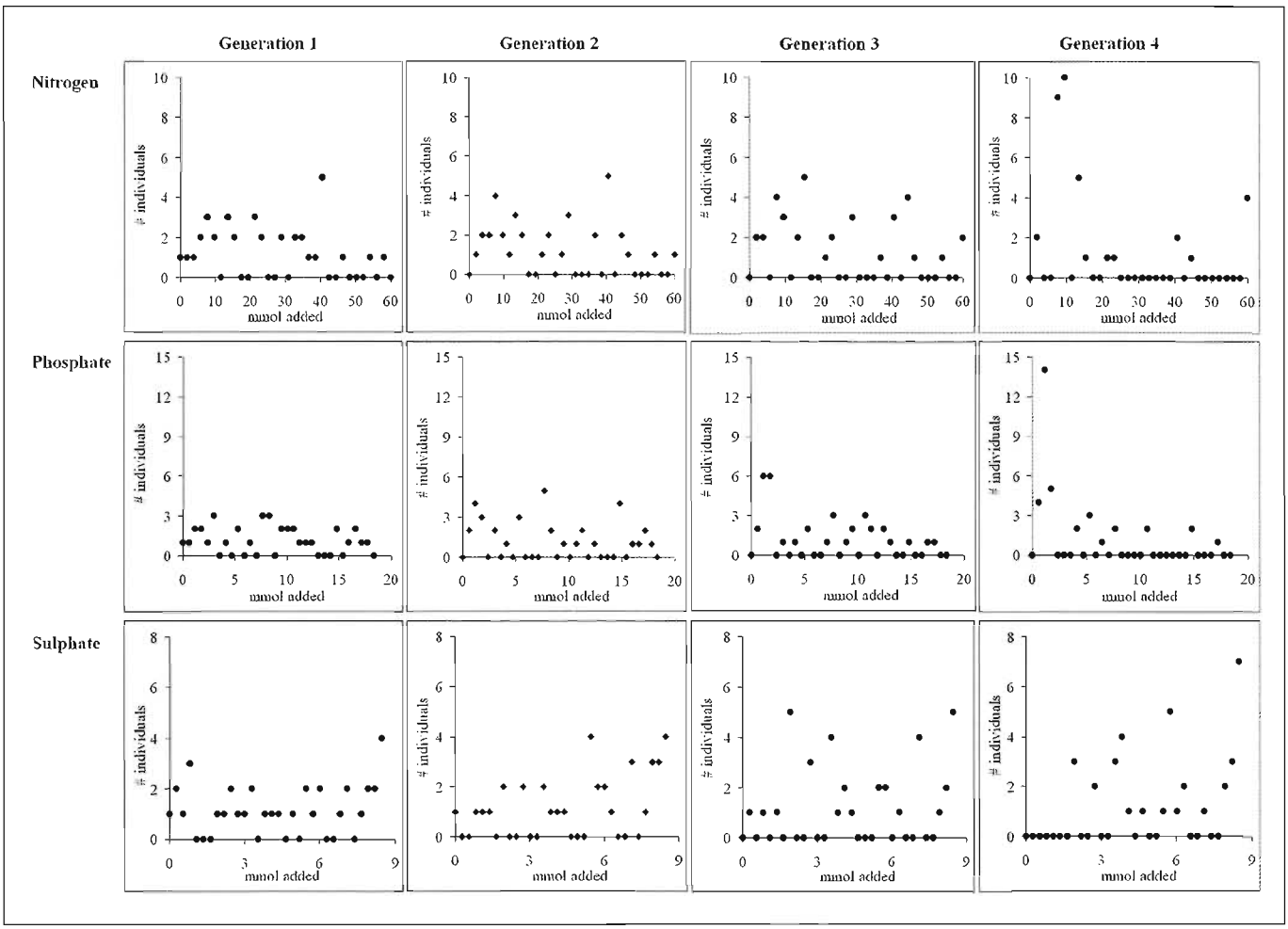
For commercial production it is important to select the best algal strains and to grow them under optimal conditions in optimized growth media. With high throughput screening methods, microalgae can be screened on desired product formation and on required stress conditions to obtain the highest amount of product per biomass [9]. We developed a screening method in which 24-wells plates are inoculated with microalgae that are subsequently grown under controlled conditions. Then, by measuring the optical density in time with a well reader, specific growth rates of the microalgae can be determined. This bioassay was used to determine the growth inhibition effects of free fatty acids in *Monodus subterraneus* [24].

The developed bioassay can also be used to study the effect of process conditions in a short time. Figure 5A for example, shows the effect of pH of the medium on the growth of *C. vulgaris*. To measure it, only one day was needed. Figure 5B shows the toxic effects of a disinfectant on the growth of *M. subterraneus*, determined in a time span of three days. These results show that a bioassay can be a powerful tool for optimization conditions and as troubleshooting device when commercially producing microalgae.

Medium optimization

High throughput growth measurements methods can be combined with rapid medium optimization using genetic algorithm as optimization tool. Often, algae are cultivated in media that were

Figure 7. Amount of media per generation that contained a certain concentration of a nutrient. In the first generation, the concentration of the nutrients is chosen randomly (most left figures). In the second generation, for nitrogen and phosphate still no convergence takes place. For sulphate, some convergence can already be seen in this generation. In the last generation (4), many media show convergence to a certain concentration of the main macronutrients (N, P, S)



developed for a range of species and not optimized for a specific species resulting in suboptimal productivities [25]. For example, several media are suggested for cultivation of *Chlorella* (Table 2). This table shows that nutrient concentrations vary widely; the macronutrients nitrogen and phosphate vary with a factor 10 and 35, respectively, and trace elements like boric acid, cobalt and aluminum are used in some media, while in other media they are omitted. Using media optimization techniques, essential nutrients can be identified and their optimal concentration for growth or product formation can be determined.

To optimize such a medium, efficient optimization methods like experimental designs and genetic algorithms are available. Experimental designs are systematical optimization tools that heavily rely on logic and statistics. They need a design and an optimization technique to describe the results of well-designed experiments [25, 26]. The design specifies the concentration ranges of the parameters (e.g. nutrients) that are chosen. These parameters are varied simultaneously and a response factor is chosen. Then, a mathematical model, depending on the chosen optimization technique, is used to optimize the parameters to the chosen response factor. When only a few parameters (e.g. macronutrients) are optimized, this method has the advantage that the design space is modeled and insight is obtained how parameters interact and the chosen response factor can be predicted [3, 27].

However, mostly media contain many nutrients (>6) and these have to be optimized. Then experimental designs are not efficient because still many experiments are needed to find the true optimum. In those cases, a genetic algorithm (GA) can better be used. It is inspired on evolution and can deal with larger sets of parameters in a highly directed way and has proven to be a powerful tool for optimization studies in several scientific fields [28, 29]. A schematic overview of a GA to optimize a growth medium to obtain highest specific growth rate of a microalgae is given in Figure 6. First, the problem should be defined and the number of variable nutrients as well as a concentration range of these nutrients should be chosen; this defines the parameter space. From this parameter space a random set of media (first generation) is generated by the GA program. The algae are then cultivated in these media at defined conditions and the specific growth rates are determined. Media in which algae show a high growth rate are selected by the GA program to generate a new set of media via cross-over and mutation, after which the alga is cultivated in this new set of media. Again growth of the alga on the tested media is evaluated. If the media all show convergence to one optimal value for the growth rate, the optimum medium for

growth is found. If not, the process of selection, crossover, mutation and evaluation of media is repeated until an optimal solution is found [29, 30, 31]. Several media for bacteria, yeast and insect cells were already successfully optimized using GA's yielding improvements up to 87% compared to a control medium[31,32]. However, optimization of nutrients for algae with a genetic algorithm was not done before, because a reproducible screening method to determine specific growth rates was not available.

Presently, we use a genetic algorithm and our developed bioassay to optimize the medium for *Chlorella vulgaris* SAG 211-11b to obtain highest specific growth rate of this alga by changing twelve nutrients simultaneously. Figure 7 shows that this technique is indeed potentially very powerful and that nutrients are converging to optimal concentrations. In the third generation, already five media were found in which the algae grew 75% faster than in M8-A medium (unpublished results).

Improved species

Wild-type microalgae kept in culture collections are merely used in commercial production systems. However, these strains often show suboptimal characteristics. To achieve much higher productivities and obtain a robust cultivation process, algal screening programs are needed that select on desired criteria by natural strain selection or genetically improved strains should be developed with these desired characteristics (Table 3). Characteristics of new developed or newly discovered strains could be tested in bioassays or by other selective screening methods.

A robust production process is needed when cultivating microalgae commercially, because the costs of cleaning, sterilizing and starting up a photobioreactor are relatively high. Therefore, contamination by unwanted species should be prevented. A pure algal culture can be established via plating and/or using antibiotics [33, 34]. This culture can be used as inoculum for a closed photobioreactor in which contamination can be prevented. Also a hybrid system can be used in which algae precultured in closed systems are used to inoculate open systems in which secondary product formation is evoked by stressing the microalgae [35]. Another way to prevent contamination is to select algae that outcompete other organisms (extremophiles) or select species that excrete antibacterial substances. Several microalgae (including *Chlorella, Desmococcus* and *Scenedesmus*) are known to excrete such compounds [36].

Hyper-producing mutants can be selected by quantitative flow cytometry and cell sorting (FCCS). This method allows processing of many cells (up to thousands cells s^{-1}) and therefore is ideal for high-throughput screening purposes. The cells are analyzed on the fluorescence of desired products and hyper-producing cells can be isolated. However, most products have a low fluorescence and FCCS cannot be used. This can be solved by fluorescent dyes that bind specifically to the desired product like Nile Red (9-diethylamina-5Hbenzo[a]phenoxarine-5-one) that stains intracellular lipids. This seems therefore a promising approach to select hyper producing species [37-39].

While genetic modification is widely applied on bacteria and yeast, the field of transgenic algae is still in its infancy [40]. However, with complete genome sequences of algae being identified [41] and the successful transformation of several microalgae, algal research groups and biotechnology companies are now beginning to apply genetic engineering technology to modify key metabolic pathways. As with bacteria and yeast, genomic databases should be constructed that can be combined with biochemical and physiological information to construct genome-scale flux models for the primary metabolism of microalgae. This genome-scale models can be used to get better understanding of cellular metabolism, to develop metabolic engineering strategies and to design media and processes [40].

Table 3. Examples of desired characteristics of a commercial algal strain

Desired characteristic	Advantage	Selection criterion
High specific growth rate	High algal productivity	Biomass formation in time
High photosynthetic yield	Higher efficiency	Biomass per amount of light
High product content	More product per algae	Product per dry weight
No wall growth	No internal wall cleaning needed	Wall growth in time
Inducible auto flocculant	Easy down-stream processing	Flocculation and zeta-potential
Extremophile	Species domination	Growth rate under extreme conditions

Table 4. Relative costs, resulting solid concentrate, energy input and the most important disadvantage (for biodiesel production) of different concentration or pre-harvesting methods. Adapted from Benemann and Oswald 1996

Harvesting method	Relative cost	Obtained solid conc. (%)	Energy input	Main disadvantage
Centrifugation	10	>10	high	expensive
Filtration (cross flow)	4-6	2-6	high	optimal for large or colonial algae
Discrete sedimentation	0.5-1.5	4-6	low	species dependent
Ultrasound*	>10	1-3	very high	expensive, small scale only
Chemical flocculation	4-8	8-10	medium	expensive
Polymer induced flocculation	0.5-1	1-3	low	species dependent

* Based on [27]

Table 5. Learning objectives MSc courses on marine biotechnology at Wageningen University

Introduction to marine biotechnology	Advanced course on marine biotechnology
Learn the basics of photosynthesis	Design of a photobioreactor for a algal product
Determine what limits productivity in algal cultivations	Construct a simplified metabolic network for microalgal growth
Describe state-of-the-art photobioreactors and discuss differences	Gain insight in new developments in the field of marine biotechnology
Describe different operating conditions and discuss how these affect algal productivity	Understand how metagenomic sequence analysis can provide novel leads for biotechnological exploitation
Learn how to cultivate algae in photobioreactors	Design production and research strategies for production of bioactive compounds from sponges
Learn how to develop a production strategy for an algal product	Quantify the maximal productivity of microalgae based bioprocesses
Describe commercial applications of microalgae	Identify biomarker components

For the near future, probably known species will be altered to get desired characteristics [40]. When this field is further developed, probably a few algae will be selected as host organisms in which genes can be inserted to produce or accumulate a desired product [42].

Harvesting

A low-cost harvesting process is required to make the commercial production of algal bulk products possible. Harvesting costs are significant because microalgae grown in cultivation systems are relatively diluted (0.5-3 g L^{-1}) compared to other microbial (yeast, bacterial) processes (50-100 g L^{-1}). In addition, unicellular microalgal cells are small, usually smaller than 20 µm and sometimes even under 5 µm in diameter. Harvesting of algae accounts for about 30% of the total costs in case of algal production in open ponds [43, 44], because as harvesting process centrifugation is used. A better approach would be to use a preconcentration step until 1-5% w/w and than concentrate this further to an algal paste (15-20% w/w) with centrifugation, which is required for further biomass processing [45]. As first concentration step, induced aggregation of the cells (leading to larger and heavier particles) seems promising because this leads, according to Stokes' law, to enhanced sedimentation. We investigated if ultrasound, which is based upon this principle, could be used as pre-harvesting process and optimized the harvesting efficiency and concentration factor [27]. However, for commercial algal cultivation processes, ultrasound as primary harvesting process is too costly due to too high energy costs. Also other harvesting processes like flotation, centrifugation, sedimentation and filtration (Table 4) are too costly or inefficient to be used on commercial scale [1, 45-47].

It was reported that flocculation is induced at higher pH and that calcium and orthophosphate should be present in the medium to provoke flocculation of the microalgae. Calcium ions stick to the algal cells, neutralizing the negative charge of these algal cells and thereby increasing the chance that coagulation occurs [48]. Orthophosphate probably is needed to get a double layer around the algae to obtain a less negatively charged layer around the cells, increasing the chance of coagulation. In commercial systems, this flocculation could be induced by ceasing carbon dioxide supply causing the pH to rise. An advantage of this harvesting process is that after sedimentation of the microalgae, the medium might be recycled.

Auto-flocculation, the spontaneous flocculation of microalgae without addition of any chemicals, seems another good alternative. In this process a polymer, produced by the algae themselves leads to flocculation. Formation of this polymer is probably induced by nutrient limitation [49-51]. These polymers can partly or completely bind to the polysaccharides that are present in the outer membrane of the algal cells. When binding occurs, the polymer can also bind to other algal cells and a network of polymers and algae can be formed, called a floc, which then leads to enhanced sedimentation, like in the ultrasonic harvesting process [52]. Induced flocculation and polymer induced flocculation should be studied and tested on commercial scale to see if they can be used as pre-concentration step.

Education

Marine biotechnology is an exciting new area of biotechnology and therefore gained much interest from industry to cultivate microalgae for the production of bulk products like lipids for biodiesel or as feedstock for other industrial chemical processes. Research is still in an early stage and companies in the field of marine biotechnology start to develop. At present, there are a few applications and probably many will follow. Biochemical engineers are able to develop and build industrial plants for biochemical or microbial (bacteria, yeast) processes. However, they lack specific knowledge about microalgae and especially the design of production processes in which light is required. To obtain such specific knowledge, education of biochemical engineers in the field of marine biotechnology is needed.

At the moment, only a few courses at BSc and MSc level are available that can be used for training in the field of microalgal biotechnology. They are mostly at university level and cover the biology and taxonomy of microalgae. Almost no courses are available that teach people how to cultivate microalgae, engineer cultivation systems, and build and manage complete cultivation plants. To push microalgal biotechnology forward, it should become part of the curriculum of universities and business schools.

Since 2003, microalgal technology was addressed in the optional course marine and animal biotechnology for MSc students biotechnology at Wageningen University. In this introductory course students obtained basic knowledge on marine biotechnology (Table 5). A practical was developed for this course [53].

In September 2008 within Wageningen University an MSc programme in biotechnology was initiated with emphasis on specialization in the field of marine biotechnology. This specialization

includes molecular biology-oriented courses on bioinformatics, metabolic modeling and pathway analysis, and courses focusing on marine biotechnology. For this reason an advanced course was developed, with emphasis on cell biology of marine micro-organisms and design of photobioreactors. An overview of the current learning objectives of both courses focusing partly on microalgae is given in Table 5.

For the advanced course, a distance learning module has been developed, covering a case study on photobioreactor design. In this module the students are asked to work out a mathematical model for the design of a photobioreactor for the production of a high-value product from algae. This case study offers students the possibility to elaborate on design principles and constraints [54]. The constructed model is used by the students to identify bottlenecks in an algal production process and to address future leads for algal productivity improvement.

In addition to the topics covered in the existing courses, more attention should be paid to educate students in down-stream processing and to the derivation of the algal biomass into useful products. It would be worthwhile to include in the education of specialists in algal technology the analysis of a complete production process in terms of energy, exergy and costs. This way they learn to identify bottlenecks and get new insights how a production process can be improved.

In addition to education at university level, education in the field of marine biotechnology should be introduced at secondary school. Classroom experiments with microalgae can be used to show the process of photosynthesis and can form a bridge between physiology, ecology and biotechnology [55] making future algal biotechnologists already enthusiastic for this field.

To achieve increased microalgal productivity at full industrial scale we should not invest in further research and development of microalgal technology only, but also invest in adequate education of dedicated people.

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Appendix I. Cost of artificial lighting

Based on document 6322 635 57271 of Philips **GreenPower LED module HF 8727900 908312 00**			
Needed for algae	100	µmol $m^{-2}s^{-1}$	(PAR)
Per LED module	10	µmol $m^{-2}s^{-1}$	(PAR)
So needed per m^2	10	LED modules	
Per LED module so	10	W	energy
	100	W	energy per m^2
Continuous light	24	hr	
Energy needed	2.4	kWhr $m^{-2}d^{-1}$	
Energy costs	0.15	€ $kWhr^{-1}$	(several suppliers in the Netherlands)
Continuous light	0.36	€ $m^{-2}d^{-1}$	energy costs
Yield	1.8	g biomass mol $photons^{-1}$	(Zijffers 2009)
Amount of photons	8.64	mol d^{-1}	
Max. produced	15.6	gram biomass m^{-2} d^{-1}	
Implicating energy cost of	0.023	€ g^{-1}	biomass
	23	€ kg^{-1}	biomass

Appendix II. Calculation of areal productivities

$$P_{year} = \frac{E_{a,year} \cdot PE}{CV_{biomass}}$$ Equation 1

P_{year}	=	productivity of biomass per year per ha	[ton $ha^{-1}year^{-1}$]
$E_{a,year}$	=	total amount of sunlight (PVGIS, solar irradiation data)	[MJ $ha^{-1}y^{-1}$]
PE	=	photosynthetic efficiency of the photobioreactor	[%]
$CV_{biomass}$	=	heat of combustion of biomass, here 22.9 [56]	[kJ g^{-1}]

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