Examining Microalgae species as a Biofuel Energy Crop: A Review

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One of the most important issues facing our society right now is the development of CO2 neutral fuels. This truth has come to light during the previous eighteen months thanks to the "Climate Change Economics" [170] in the Stern Report and the "AR4 Synthesis Report" [84] in the National Security Council. The causes and effects of climate change are assessed in greater detail than has been done before in these publications. We conclude that we have crossed the threshold (now 455 ppm CO2-e) 10 years sooner than the prior estimate. Essentially, they characterize atmospheric CO2 levels as over 450 ppm CO2-e (e=equivalent of all greenhouse gases) are currently in the dangerous range. These new discoveries have led the government to set a target (such as the European Union) to reduce CO2, usually in the range of 10-20% by 2020. Germany has recently pledged to cut emissions by 30% by 2020 if other countries do the same.

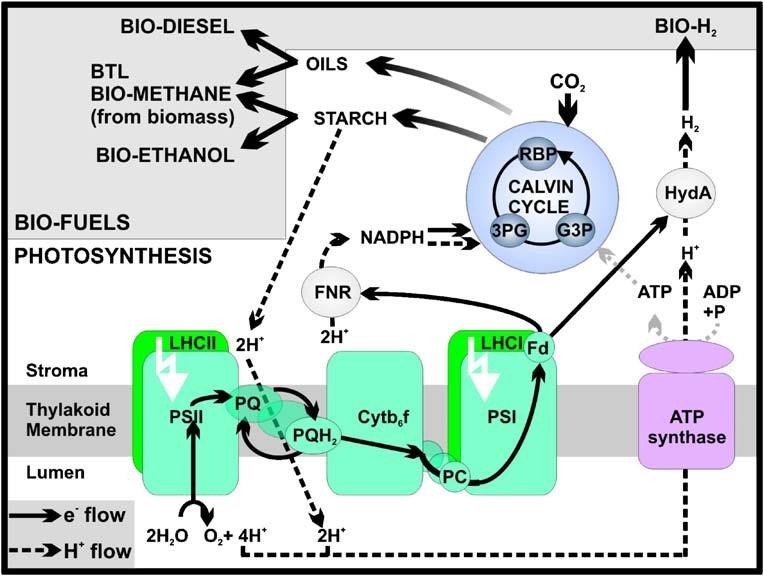
However, these modest reductions are not sufficient to keep CO2 levels within the accepted "optimal" range (i.e. below 450 ppm CO2-e; IPCC [84]). In fact, they can stay stable above 550 ppm. Damage will be greater at this stage [84]. Therefore, although various CO2 reduction scenarios have been developed to offset emissions, the IPCC concluded that emissions should increase by 2015 and that total CO2 emission reductions should be achieved by 50-85% by 2050. More importantly, the IPCC is an understatement because current climate change models lack carbon feedback. As a result, lower emissions reductions (60% by 2020) are seen as overkill, and this concern prompted IPCC Chairman Rajendra Pachauri to say: “What we do in the next 2 to 3 years will determine our future [126]. However, achieving a 60% reduction in CO2 emissions by 2020 or even a 50-85% reduction by 2050 is a major global challenge that requires a lot of improvement in terms of renewable energy. While biodiesel and bioethanol are considered the closest commercial options, consolidation efforts are underway to produce new biofuels. The sectors of biomass-to-liquid (BTL) diesel, biomethane, and biohydrogen are also expanding quickly. With an emphasis on biodiesel, which has the potential to be integrated with the production of renewable, CO2-neutral gas through the power plant chimney and atmospheric CO2 sequestration, this paper gives a brief summary of the history and current advancements in secondary microalgae processes. It specifically seeks to analyze the state of affairs in the biodiesel industry and pinpoint significant prospects for upcoming innovation. The use of biofuels and biodiesel worldwide The oil and power industries make up the two segments of the global energy market. Emissions reductions in both industries are required to fulfill global regulatory targets. About 33% of the world's energy is currently produced by energy, which is changing in a number of ways to produce energy with minimal CO2 emissions (eg nuclear, solar, wind, geothermal, hydroelectric, clean coal technologies).

In contrast, in 2005, oil held a larger market share, accounting for approximately 67% of global electricity consumption, totaling around 15.5 terawatts (489 exajoules per year), as reported by the US Energy Information Administration. Despite the significance of fossil fuels, the establishment of CO2-neutral (such as biodiesel, bioethanol, biomethane, and BTL diesel) and CO2-free fuels for electricity generation has not yet been realized. The availability of biofuels highlights the convenience and potential of the biofuel industry. However, first-generation biofuel systems have been unable to fully harness this potential due to significant economic and environmental constraints (refer to "Challenges in Biofuel Production" and "Economic Viability of Microalgal Biodiesel"). In contrast, second-generation biofuel systems, including lignocellulosic and microalgae biofuel systems, have the capacity to overcome many of these limitations, aiming to reach the ambitious clean energy market target of $500 billion by 2050 or beyond [170]. The majority of biofuels produced to date originate from higher plants that utilize photosynthesis to convert solar energy into chemical energy, stored in various molecules such as lignin, cellulose, starch, and oil (see Figure 1).

Lignocellulose stands as the primary constituent of plant biomass and can serve as a raw material for ethanol production. This conversion can be achieved through processes like gasification or cellulolysis, which encompass chemical or biological enzymatic hydrolysis. Presently, this method is under development for secondary biofuel systems [41, 155] and is commonly referred to as "lignocellulosic processes."

Similarly, starches (e.g., from maize) and sugars (e.g., from sugarcane) are transformed into bioethanol through fermentation [23, 74], while oils (e.g., from sugarcane) are converted into bioethanol. Raw materials like rapeseed, soybean, and palm oil are employed in the production of biodiesel [80, 156]. Microalgae exhibit a remarkable capacity to synthesize cellulose, starch, and oil efficiently [16, 162]. Furthermore, some microalgae and cyanobacteria, which produce more glycogen than starch, can generate biohydrogen under anaerobic conditions [20, 35, 59, 76, 111], and their fermentation processes can be utilized for methane production.

Fig.1 The process of photosynthesis, which converts solar energy into chemical energy, is essential for all biofuel production in plants. (Schenk et al., 2008)



# Flexibility of Biofuel Production Systems:

At the core of all light-driven biofuel production processes lies photosynthesis, which serves as the initial stage in converting light energy into chemical energy. It plays a pivotal role in generating the essential raw materials required for the synthesis of various fuels, including protons and electrons (for biohydrogen), sugars and starches (for bioethanol), fuel (biodiesel), and biomass (for bioethanol).

In higher plants and green algae, light is captured by specialized light-capturing proteins known as LHCI and LHCII (as shown in Figure 1). These proteins are encoded by extensive gene families with significant homology [51], and their expression is influenced by environmental factors, such as light intensity. They bind most of the chlorophyll and carotenoids within plants, playing a crucial role in light collection and dissipating excess energy to regulate the photosynthetic process, particularly in photosystem II (PSII; [81]). The excitation energy, essential for driving the photosynthetic reactions, is transferred to the photosynthetic reaction centers of photosystem I (PSI) and PSII via a highly coordinated network of pigments connected by LHC, PSII, and PSI subunits.

In an initial step, PSII harnesses this energy to initiate the photosynthetic water-splitting reaction, converting water into protons, electrons, and oxygen. Electrons are subsequently transferred to NADPH through the photosynthetic electron transport chain involving plastoquinone (PQ), cytochrome b6f (Cyt b6f), photosystem I (PSI), and ferredoxin (Fd) (as depicted in Figure 1). Simultaneously, protons are released into the thylakoid lumen through the PSII and PQ/PQH2 cycles, creating a proton gradient that drives ATP production via ATP synthesis. Protons and electrons then combine with ferredoxin-NADP + oxidoreductase (FNR) to form NADPH. Both NADPH and ATP are utilized in the Calvin cycle and other biochemical pathways to produce sugars, starches, fats, and various biomolecules, collectively forming biomass. These biomass components serve as the basis for generating bioethanol, biodiesel, biomethane, and BTL biofuels.

Alternatively, in certain photosynthetic organisms, such as the green alga Chlamydomonas reinhardtii, protons and electrons extracted from water (or starch) can be transferred to hydrogenase (HydA) via the electron transport chain (ETC).

The Calvin Cycle represents a pivotal component of the photosynthetic process responsible for carbon dioxide fixation in various organisms, ranging from primitive algae to higher plants. This process relies on ATP and NAD(P)H generated through light-driven mechanisms. In C4 and CAM plants, the Calvin Cycle is integrated with continuous processes leading to CO2 fixation, but the fundamental photosynthetic steps remain unaltered [175].

The Calvin Cycle can be segmented into three key stages, encompassing carboxylation, reduction, and substrate regeneration, with ribulose 1,5-bisphosphate (RuBP) serving as the substrate. The initial phase, involving the conversion of CO2 by reacting it with RuBP, is catalyzed by ribulose 1,5-bisphosphate carboxylase/oxygenase (rubisco). The significance of Rubisco cannot be overstated, as it single-handedly fixes all the carbon monoxide in the world from atmospheric carbon dioxide in a singular process. Furthermore, Rubisco stands as the most abundant protein globally, constituting approximately 30% of the total protein content in most leaves [129]. This abundance is partly attributed to its crucial role in photosynthesis, despite its relatively low catalytic carboxylase activity, processing only 2-3 RuBPs per second [104].

As the name suggests, rubisco performs two catalytic functions: it functions as a carboxylase, integral to the reduction phase of photosynthesis, and under aerobic conditions, it acts as an oxygenase, contributing to photorespiration. O2 and CO2 compete for the same catalytic sites, potentially affecting the efficiency of CO2 fixation in certain aerobic environments.

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For example, although the enzyme is highly specific for CO2 (Tobacco plants) 82 times, *Griffithsia monilis* (red algae)

167 times, Rhodospirillum rubrum (sulphur-free blood bacteria) 12 times [7], O2/CO2 molecular ratio in atmosphere 540:1, 24:125° C Air to water is saturated. In the first step of the Calvin cycle, rubisco catalyzes the formation of two molecules of 3-phosphoglycerate from RuBP, CO2 and H2O. Negative changes in the weak process leads to positive effects. In the second step, in the ATP/NADPH-dependent reduction step, carboxylic acids are reduced by phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase to produce two molecules of glyceraldehyde-3-phosphate. The third step consists of a reaction in which some of the glyceraldehyde-3-phosphate is converted to RuBP, which is necessary to maintain the reduction of photosynthesis [175].

# Addressing problems in biofuel production:

Although the biofuel production process holds significant promise as a means to generate carbon-neutral fuel, the primary production methods are burdened by numerous economic and environmental constraints, making biofuels a prominent subject of debate. While some proponents underscore the advantages of biofuels [157, 167], others raise concerns about their commercialization and diminishing impact [19, 128, 131, 147, 190].

The most common problem with existing biofuel systems is that when there is a large production capacity, they compete with agriculture for arable land used for food production. For example, current biodiesel production from vegetable oil supplemented with small amounts of animal fat and waste cooking oil is estimated to account for only 0.3% of global fuel consumption (about 12 million tonnes in 2007) [24, 125] available and based on future transportation demands. Currently, about 8% of vegetable oil production is used to make biodiesel [125], which causes the price of vegetable oil to increase over the years.

# Area required for biofuel production:

Earth's surface (510,072,000 km2) is estimated to receive an average of ~170 Wm−2 solar energy [31, 196]. This is equivalent to 2,735 YJ of energy per year, equivalent to 5,600 times the world's primary energy consumption in 2005 [196]. Therefore, the solar energy required to produce biofuels is plentiful. But even now oil crops will be planted on all arable land. If 2% of the world is land, 13% is suitable for agriculture, energy conversion efficiency from sunlight to biomass is 1% and oil production is 20%, these will meet half of our energy needs today. Using these figures, biofuel critics often conclude that biofuel production cannot contribute significantly to global oil demand. However, as will be mentioned here, higher photosynthetic efficiency and fuel production have already been achieved, thus paving the way for second generation biofuel technologies with great potential. The increase in arable land has created serious problems and bad practices around the world, giving rise to the term "hill land". For example, rainforests in Brazil and Southeast Asia are being cleared at an unprecedented rate to make room for soybean and palm oil plantations for biodiesel production.

Current biodiesel growth rates in Indonesia, Malaysia and Thailand range from 70% to 250% per year [181]. The increase in the amount of land that can now be used for food production can lead to food insecurity, particularly in countries where more than 800 million people

already face hunger and malnutrition (figures exclude China; [53]). Also, the use of land extensively with fertilizers and pesticides can cause serious environmental problems.

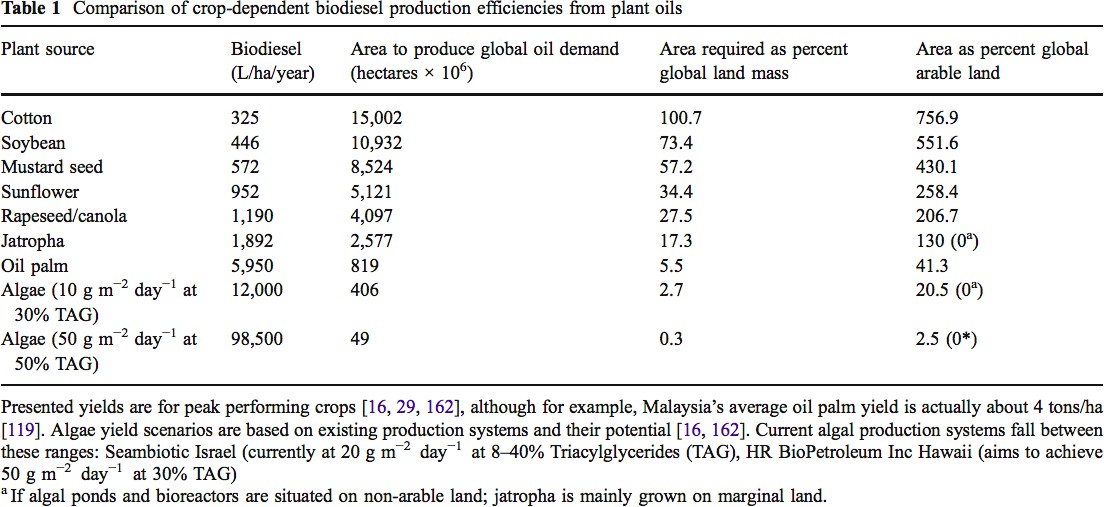
Net energy balance when evaluating the cost and sustainability of the biofuel production process, it is necessary to establish the net energy balance (NEB). Agriculture, harvesting, processing, shipping, etc. Considering the energy required for first-generation biofuels, the NEB is estimated to be about 25% corn ethanol and +∼93% soybean biodiesel [80], but the number depends on the amount of production. detailed Case lifecycle analysis. While this report rejects the claim that the energy cost required to grow crops, machinery and facilities has led to negative NEB values for both biofuels, it has yet to take into account the Intergovernmental Panel on Climate Change's prediction that traditional crops may decline. There will be an increase of up to 50% by 2020 [84]. The carbon balance of the Carbon monoxide calculation process is equally important.

Biofuel crops are generally considered a nearly carbon neutral process because almost all of the carbon in carbon dioxide comes from the carbon dioxide released when burned in situ. But in reality, the overall CO2 balance of biofuel production needs to be evaluated by manufacturer, including energy consumption, plant use and refining, and the process of transporting the fuel from the CO2 that already emits fossil fuels. In addition, oil production from palm oil fields established before the Kyoto emissions target policy is expected to reduce emissions compared to natural gas. Conversely, if the rainforest area has to be cleared first to make room for plantations, the CO2 balance will be negative [14]. CO2 Capture The integration of biofuel production with CO2 separation systems is a significant development that will not only greatly improve the net CO2 balance of the biofuel process, but will also help reduce atmospheric CO2. These usually involve the production of Agrichar via the pyrolysis process.

A number of second-generation biofuel production systems are currently under development that will have more NEBs, use more water and require less arable land. Lignocellulosic technologies and microalgae are of interest ([29,76,96,155]; Table 1). It has been reported that in one region, microalgae produce 15-300 times more biodiesel than crops (Table 1; [29]). In addition, microalgae often have a short cycle (~1-10 days depending on method) compared to traditional crops harvested once or twice a year, allowing them to be grown multiple or sequentially with increased results (Table 1).

Enhanced light capture and conversion rates ultimately lead to reduced fertilizer and food wastage, consequently lowering waste and pollution levels. Utilizing wastewater for algae cultivation represents a viable alternative [75, 103, 120]. Additionally, the cultivation of microalgae for biofuel production on marginal or soilless lands can alleviate land competition concerns and open up new markets in arid, dry, or saline regions. Furthermore, significant freshwater conservation can be achieved when conducting algae cultivation within low evaporation closed bioreactor systems, especially utilizing marine and halophilic microalgae species. The microalgal biomass generated in these bioreactors can also undergo gasification or pyrolysis to yield various biofuels and charcoal, serving as part of CO2 capture strategies. This approach facilitates the disposal of bio-GMO wastes in an environmentally sensitive manner. Carbon-rich biomass pellets can also be stored as part of a carbon sequestration strategy that utilizes CO2 from power plants as a feedstock for biomass production. Another noteworthy aspect of secondary microalgae systems is their suitability for biotechnological processes that can swiftly regenerate algae populations. These attributes hold promise for enhancing "Photosynthetic Efficiency."

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Numerous sectors have seen recent advancements, and it is being identified what constitutes better photosynthetic output and growth tolerance in saltwater or wastewater. The effectiveness of CO2 production and the harvesting process are two areas where secondary microalgae systems have been shown to have certain limits.

# Other Biofuels from Microalgae:

In addition to its use in oil production for biodiesel, microalgae also serves as a valuable feedstock for biofuel generation. The advancement of technologies for algal biodiesel production is closely linked with biomass liquefaction (BTL) methods, encompassing biohydrogen, biogas, bioethanol, and the cultivation of fast-growing algae. Below, we delve into the BTL, biohydrogen, and biomethane processes, as they are particularly pertinent to microalgae systems. The discussion of bioethanol is omitted here, as the process of producing bioethanol from microalgae closely resembles the initial technologies that employed food-derived products from sources like corn and sugar.

Microalgae-based biohydrogen production, which involves the photobiological generation of hydrogen from water, has emerged as a promising and potentially emission-free fuel source. It can be integrated with atmospheric CO2 capture efforts. The concept of biohydrogen production by microalgae has been established for over six decades, with initial discoveries in green algae like Scenedesmus obliques [59] and subsequently in numerous other photosynthetic species, including cyanobacteria [20, 35]. Most investigations into algal hydrogen production have centered around the green alga Chlamydomonas reinhardtii, which serves as a model organism for studying photosynthesis, especially in the context of the aerobic-anaerobic cycle pioneered by Melis and colleagues in 2000 [78, 148]. This species was also prominently featured in studies dating back to 2000 [63, 111].

The biological hydrogen process holds appeal because it harnesses sunlight to convert water into hydrogen and oxygen through a two-step reaction. The initial reaction is common to all aerobic photosynthetic organisms, while the subsequent step involves specific iron-containing chloroplast hydrogenases and is limited to a more selective group of microalgae [57, 77]. Cyanobacteria also have the capability to produce hydrogen from water but employ a distinct biochemical method. Under standard light and aerobic conditions, H+ and e- generated by photosynthetic hydrolysis reactions are utilized to form ATP and NADPH. The second reaction takes place under anaerobic conditions, where the presence of O2 inhibits both ATP production through oxidative phosphorylation and NADH/NADPH formation [67]. In such conditions, certain microalgae, such as Rheinella, redirect the energy stored in carbohydrates like starch toward chloroplast hydrogenases [63, 111]. This redirection facilitates ATP generation via photophosphorylation and prevents an overload in the transport chain [96, 151]. Consequently, hydrogenases, in conjunction with various fermentation processes [93], serve as proton/electron release valves, producing hydrogen gas, which is expelled from the cell by combining protons and electrons and neutralizing ferredoxin [111].

Chlamydomonas reinhardtii is a fundamental organism in solar-powered biohydrogen production from water, and alternative fermentation processes can also be employed. An important advantage of hydrogen production is its rapid release into the gas phase, in contrast to other fermentation byproducts that can accumulate and prove toxic to cells. Recent advancements have led to improved efficiency in algal biohydrogen production. For instance, strains with elevated starch content, reduced cyclic electron flow around PSI (such as Stm6), and increased external storage of glucose (like Stm6glc4) have demonstrated enhancements [42, 95].

To create a profitable commercial algal hydrogen production system, the metabolic flow of hydrogen must be optimized through bioengineering and optimization of related processes in the bioreactor system [76]. Bioengineering includes methods to increase photon conversion from the current ~1% to ~7% commercially available [76]. The "Closed Bioreactor Design" section provides details on actual bioreactor design and ways to reduce costs. Combining hydrogen production with seawater desalination Biohydrogen production from algae can be combined with seawater desalination, even with limited production. Seawater can be converted by marine and halophilic algae into hydrogen (as protons and electrons) and oxygen, which are then burned to create fresh water.

Hence, there is a viable opportunity to integrate electricity generation with desalination by utilizing a fuel that employs hydrogen and oxygen to generate electricity within the nation. Despite the relatively modest freshwater yield, it offers advantages that traditional crops cannot provide. The water yield is directly correlated with the production of H2. With a light-to-hydrogen conversion efficiency of approximately 1% (which aligns with the current state of external light utilization through a photodilution generator), research indicates that 1 million liters of photobioreactor reactor space has the potential to yield up to 610 cubic meters (equivalent to 610,000 liters of hydrogen) of freshwater annually after processing is complete.

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# Algal Biomethane Production:

The significance of biogas production from biomass has grown on a global scale. A recent investigation conducted by the Leipzig Energy and Environment Institute suggests that a substantial portion of Europe's methane requirements can potentially be fulfilled through biogas [18]. Nonetheless, our current comprehension of the biological mechanisms within biogas production facilities remains limited. Consequently, research in this domain is imperative and holds significant relevance for enhancing the biomethane production process. Biomethane has the capacity to be generated from diverse biomass sources and various plant species. An important constraint for the prospective advancement of biomethane-based facilities lies in the accessibility of photosynthetically cultivated biomass.

Presently, a 500 kW biomethane facility necessitates approximately 10,000-12,000 tons of biomass feedstock each year, with corn serving as the primary crop of choice. Typical biomethane yields, ranging from 2,000 to 4,500 m3 per hectare per year, have been documented when utilizing cereals and sunflowers [5, 192]. Corn, characterized by its high yields, exhibits variability dependent on the variety and harvest timing, generating between 5,700 to 12,400 m3 of biomethane per hectare per year [5, 136, 192]. Some grass species, such as ryegrass, have been reported to achieve biomethane production levels of up to 4,000 m3 per hectare per year [192].

Since the production of biomass per hectare by microalgae is five to thirty times that of crops, microalgae are crucial for monitoring [162]. Microalgae have high lipid, starch, and protein contents and low lignin content, which makes them perfect for fermentation-based biomethane production in biogas plants. Lipids are significant because, like biodiesel, they can convert into biomethane at a higher rate (790 L biogas, 72% CH4, 28% CO2) per kg of organic dry matter) than proteins (800 L biogas, 60% CH4, 40% CO2) and carbohydrates (746 L biogas, 50% CH4, 50% CO2) per kg of organic dry matter) [184]. About half a century ago, one of the earliest investigations into the viability of producing biomethane from microalgae was published, with the conclusion that the method is feasible and may be enhanced in the future [65]. Nowadays, it is possible to cultivate microalgae in huge volumes (150–300 tonnes per hectare annually) in indoor algae bioreactors that are helpful for producing biomethane and biomass feedstock. 29 and 138]

Methane production from this quantity of biomass can reach 200,000–400,000 m3 per hectare annually. It should be highlighted, nevertheless, that because biomass is expensive to produce (the commercial value of green algae in Germany, where most biogas producers are located), biomethane produced by microalgae cannot yet compete with biomethane produced from corn or other crops. For instance, Chlorella vulgaris is 100 times more common than maize, but at the moment, its productivity is insufficient to meet the demands of biogas plants. These days, methane from biogas facilities is combined with carbon dioxide (typically between 50 and 75 percent methane), which makes it unusable due to its high impurity level. Since biogas is now mostly burned in combined heat and power plants, fuel storage is no longer popular. Nonetheless, the creation of the first pilot plants for biomethane separation was made possible by the development of effective purification systems, which began in Austria in 2005 (patent no. AT411332B (2003)) and Germany in 2006 (patent no. 304 26 097- BCM-method).

# Microalgae Cultivation:

Traditional outdoor pond algae production and even some indoor algae bioreactors have been commercially successful in producing valuable products such as astaxanthin and nutraceuticals. Biofuel production systems are less economically viable as they have a lower market price. Therefore, optimized biomass production forms the basis of commercial biofuel production [73,76,100,145,166], which requires careful optimization of crop growing systems.

# Optimizing Culture Conditions:

Limitations may arise from the complexity and wide range of elements involved in optimizing culture conditions particular to a strain. These include water quality, pH, salinity [1,30,140,141], mineral and carbon regulation/bioavailability, cell fragility [70], cell density and growth inhibition [16], temperature [30], mixing [12], hydrodynamic and hydrodynamic stress [13], bubble size and distribution [14, 135], gas exchange [52], mass transfer [68]. While controlled agitation of the culture can provide important preliminary information, appropriate models must be properly constructed in laboratory bioreactors [150] to ensure mixing and mass transfer. Traditionally, most research on the relationship between algae growth and nutrition has been devoted to algae in the natural environment and the role of algae growth in ecosystems.

In contrast, algae production systems require maximal biomass growth to attain high cell densities. A more profound understanding of the underlying physical principles and advancements in bioreactor design [66] have resulted in increased achievable cell densities. The formulation of an optimal growth medium also assumes crucial significance as it ensures the provision of sufficient and consistent nutrients essential for maximizing and expediting cell growth, thereby enhancing overall biofuel production efficiency [36, 37]. Algae production processes are versatile and can involve multiple steps, allowing for considerable flexibility in each stage. For instance, techniques such as nitrogen limitation for oil production [165] or sulfur limitation for hydrogen production [111] can be employed. Employing fed-batch feeding for heterotrophic algae culture [100] and enriching photoautotrophic algae culture with CO2 can augment biomass yield, while fine-tuning mineral nutrient levels can enhance the productivity of insect cultures. Nitrogen and phosphorus typically emerge as the primary targets for improving the mineral environment [28, 86, 165, 203], although other minerals also play critical roles in supporting cellular processes and metabolic biochemistry. Mineral ions additionally exert significant influence in areas like osmoregulation, osmotic adaptation [89, 90], and the molecular configuration of photosynthetic complexes [112].

Certain algae varieties exhibit a twofold increase in growth rate and cell count when cultivated in the presence of yeast extract. Evidently, proliferating bacteria can effectively incorporate dissolved organic compounds, suggesting that wastewater can be viewed as a potential resource in specific scenarios. The scarcity of freshwater resources in numerous countries underscores the importance of wastewater recycling, with ongoing research exploring various wastewater utilization methods [10, 153, 154, 163, 201]. Notably, Zaslavskaia et al. [202] illustrated the transformation of Phaeodactylum tricornutum, a functional photoautotroph, into a heterotroph through genetic modification targeting the same gene responsible for encoding the glucose transporter.

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The discovery, characterization, and manipulation of carriers hold substantial promise for future applications in algae cultivation, particularly in the context of biofuel production. Maintaining precise pH levels throughout the culture is of paramount importance, as it exerts a profound influence on all aspects of the medium's biochemistry. The ion uptake within the medium and the metabolic biochemistry of the cells can exert significant stress on pH levels, and in cultures with high activity, their impact can override the potential absence of exogenous buffers. Presently, the best practices and strategies for pH control encompass techniques such as microinjection of potent acids and bases, stabilization in heterotrophic cultures, and the management of CO2 dissolution in both photoautotrophic and heterotrophic cultures [52, 173].

# Outdoor Pond Systems:

Most of the microalgae grown today are grown in open ponds. The construction and operation of open ponds is very efficient and therefore more efficient as long as the culture is preserved [193]. Outdoor pools can come in many shapes and sizes, but the most common design is a racetrack pool. Create a grid of rectangles in the region, with an elliptical channel in each. The impeller keeps the water flowing through the circuit continuously. They typically operate in water between 15 and 20 cm deep because productivity can reach 60–100 mg L-1 day-1 (i.e., 10–25 g m−2 days−1) and biomass concentration can reach 1 g dry weight per litre at this depth [137]. On the other hand, this efficiency is not consistently accessible and cannot be maintained annually. Reservoirs in Asia and the Ukraine also have comparable architecture [15]. Wastewater treatment plants can construct their algae pools to best fit the site; these are typically gravity-flow-guided pools rather than mixed pools. The 11,000-hectare Werribee wastewater treatment plant in Melbourne is one of the biggest of its kind.

The foundation of an algal wastewater treatment pond is made out of excavated trenches or retaining walls. Because more mechanisms (vanes) are needed to ensure the stability of the lake, building a dam costs more. Higher flow rates also necessitate the use of more sturdy structures. However, since transparent materials are not needed to create an open-air pool, various materials can be used for construction. Outdoor pools are also easier to maintain because they have wide open channels that remove surface biofilm. The biggest disadvantage of the open system is that they lose water by evaporation at the same rate as the products in the soil when they are open to the air, and they are also susceptible to harmful diseases. Newly opened ponds are often inoculated with the desired algae culture to encourage algae growth and preserve the pond's flora. However, undesirable species will occasionally appear and can significantly reduce yields even beyond inoculated species. When a big competitor enters the pool, it is very difficult to get rid of it. Of the more than 3,000 photosynthetic organisms collected by the Aquatic Species Program, none were found to dominate the open ponds and have the necessary biofuel and high lipid content [162]. In practice, it has been reported that open ponds are usually of two to six types and have many advantages: rapid growth, protection against animals, tolerance to high oxygen levels, etc. In open ponds, consistent and dependable reproduction of the same species Breeding extremophiles, which avoid and outperform other species in certain settings (e.g., high/low pH or salinity), might, nevertheless, complement system development efforts. For instance, spirulina is frequently the dominating species in soda lakes because it can grow and live at high pH levels (9 to 11.5) [17]. Because of its spiral design, collecting it is also simple. The world's largest producer of Dunaliella is Australia. Because of its high intracellular glycerol concentration, which guards against osmotic stress, this unicellular species of green algae develops effectively in high salinity water. In shallow waters, Dunaliella salina is the best source of protective carotenoids that shield it from intense light [21].

# Closed Bioreactor Design:

Enclosed bioreactors offer numerous advantages beyond conserving water, energy, and chemicals, and they are increasingly becoming the preferred choice for biofuel production as costs decline (see Fig. 3). A pivotal aspect of these systems is their ability to support production volumes up to five times larger in terms of reactor capacity, resulting in a reduced production "footprint" [13]. A key consideration is efficiency, aiming to harness the maximum amount of solar energy from a given land area. Recent studies have indicated that the increased costs associated with bioreactors can be offset by enhanced production efficiency [29]. Most enclosed photobioreactors are designed in the form of tube reactors, plate reactors, or bubble column reactors ([137, 193]; refer to Figures 3 and 4). Additionally, less intricate structures, such as semi-hollow spheres, have demonstrated effectiveness in certain cases [152].

However, there is still a gap between the design of high-end reactors that meet all the needs of algae cells on the one hand, and the design of inexpensive reactors, on the other hand, to improve process economy [193]. Based on current electricity prices and production costs, the cost per square meter of the reactor should not exceed $15. The following paragraphs discuss some aspects of reactor design and current development from a business perspective. Most microalgae exhibit growth/photodynamics where light saturation occurs at medium light intensities. Therefore, they have low activity, photoinhibition, and even photobleaching under direct sunlight for *Chlamydomonas*; [110, 134]. To increase the efficiency of the process, photobioreactors must be designed to scatter light over a large area to provide moderate light intensity to cells. This is usually achieved by arranging tubular reactors in a fence-like structure (Figs. 3) The fence faces north/south to avoid blindness from the field. This elegance is way sunlight is diluted both horizontally and vertically. Dry weight up to 47 g m-2 day-1 can be obtained using this system [27].

The distribution of light around the circle of the tube is very interesting considering the reflections. However, compared to other biochemical engineering processes, this is often not achieved by precise measurements and kinetic methods. The bioreactor surface might be 10 times greater than the matching covering area to increase lighting. Photobioreactors that have bubble columns or plates positioned at an angle to the sun can also be explained in similar ways. Both kinds of structures promote better cell growth, but they need materials that are more transparent, like plastic or glass. However, "make the ratio of surface area to volume as large as possible" is the design philosophy. Since these structures are constructed along a short optical path, they may support larger biomass concentrations. The 400 m2/m3 value is state of the art. Therefore, small culture volume and less mixing power are required for a given biomass yield. Stirring is necessary in all photobioreactors because it inhibits cell aggregation and promotes CO2 and O2 distribution [117]. The partial pressure for CO2 is at least 0. To avoid limitation of kinetic CO2 uptake, 15 kPa must be maintained and meet the stoichiometric requirement of 1.7 g of CO2 per gram of biomass. This highlights the practicality of supplying CO2 that has been purified from external flue gas, such as that from a power plant [45]. While the mixture does not affect light attenuation in the reactor, there exists a relationship between the culture mixture and light attenuation, as each algal cell undergoes varying levels of interaction between darkness and light within the reactor [13]. Dark areas emerge due to mutual cell shadowing on the side of the reactor farthest from the light source. Consequently, it becomes essential to reevaluate algae physiology within the context of the "flashing light effect" [69]. Although high light intensity stimulates the photosystem, it can also lead to photoinhibition. Therefore, microalgae have developed photoprotection mechanisms to dissipate excess energy through fluorescence and heat. This energy wastage is circumvented when algae cells are subjected to low/high light cycles, as low light enables the transfer of energy within the photosystem to metabolic processes. These cycles should occur at a frequency of 10 Hz or faster, with the dark cycle being ten times longer than the light cycle [85]. Under such conditions, algal cells exhibit behavior similar to that under constant light [199]. Photobioreactors can effectively capture the flashing light effect through intelligent mixing to optimize the transition of cells between darkness and light. This concept has already been put into practice, as depicted in Figure 4 [109]. The reactor takes the form of a plastic plate air-lift reactor equipped with integrated baffles, inducing a consistent horizontal liquid vortex. However, the drawback lies in the energy consumption associated with the mixing technology. Nevertheless, such reactors have proven successful. The first CO2 separation demonstration plant was scheduled for construction in Hamburg, Germany, in 2008. In various reactor types, combined energy consumption is estimated to account for 10-30% of the light energy problem. This level is evidently excessive, surpassing acceptable energy consumption thresholds.

Plastic bags made of polyethylene are utilized either as circular reactors (to prevent dark areas within the cylinder) or as plate reactors, which are available for purchase [146, 179]. It has been determined that a simple horizontal plastic pipe laid on the ground lacks the required strength for this purpose. One of the latest advancements in this field is the triangular reactor (as illustrated in Fig. 5). This innovation combines the bubble column principle with mixing through integrated static mixers within an external 'downcomer'. As per MIT's press release and external evaluation [139], even under optimal lighting conditions, this "3DMS-Reactor" achieves a dry air mass of 98 g m-2 per day over a 19-day period. Consequently, this stands as one of the most productive cultures ever developed, with a theoretical maximum average of approximately 100 g m−2 per day. Future improvements should focus on employing thin layers with a high internal area to enhance light distribution, thus increasing biomass concentration while reducing mixing energy requirements [150]. Moreover, gas transport should solely rely on conduction to avoid the formation of negative energy foam.

A different idea for a bioreactor gathers light using plastic Fresnel lenses and uses optical fibers to direct it into a lump reactor. By separating the sun's infrared rays, overheating issues during periods of high solar irradiance can be minimized and the expelled radiation can be utilized to produce energy. This can be used to mix cultures in the currently most energy- intensive bioreactors. This principle eliminates many of the problems associated with large outdoor reactors, but it is very expensive to use and the technology needs to be further developed to reduce its cost and ensure it is successful. Figure 6 presents a schematic of four commonly used bioreactor designs.

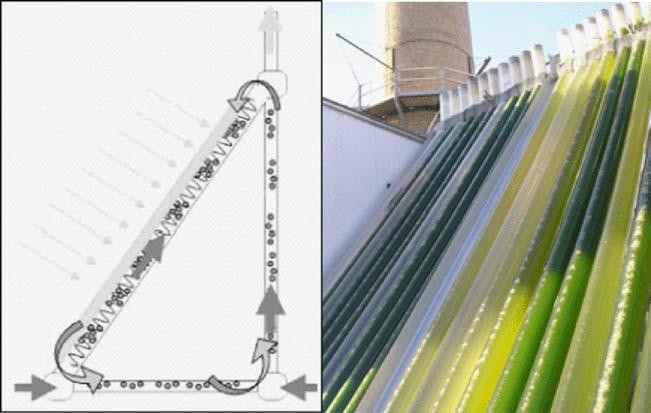
In summary, the design of photobioreactors, particularly those used to produce biofuel, is a fast-growing topic that is critical to the quick development of second-generation microalgal biofuel systems. The best and most profitable outcomes can only be achieved by combining innovative designs with theory and understanding of microalgae dynamics and growth efficiency.



Fig. 3 A high-end closed bioreactor system. The world’s largest closed photo-bioreactor in Klötze, near Wolfsburg, Germany (Bioprodukte Prof. Steinberg; www.algomed.de); the 700 m3 are distributed in 500 km of tubes and produce up to 100 t algae biomass per year



Fig. 4 Flat panel airlift from Subitec [38, 102] with usage of the flashing light effect, here for astaxanthin production



Triangle Airlift Reactor, or 3D Matrix Algae Growth Engineering Scale Unit, GreenFuel, Fig. 5. The patent drawing (US 20050260553) is on the left, while the demonstration plant at Red Hawk Power Plant in Arizona, USA, is on the right. 50]

# Hybrid Systems:

Outdoor ponds represent a cost-effective method for cultivating algae; however, they are susceptible to rapid contamination. In contrast, photobioreactors are highly effective at maintaining sterile cultures but are accompanied by setup costs that are often ten times higher than those of outdoor ponds. Combining these two systems emerges as the most efficient approach for cultivating crops valuable in the production of biofuels. In the practice of algae cultivation, inoculation has long played a crucial role. Open ponds are typically inoculated with the desired strain, which has invariably been cultivated in a bioreactor, ranging from something as simple as a plastic bag to a cutting-edge fiber optic bioreactor. It is worth noting that the size of the inoculum must be sufficiently large to allow the desired species to establish itself in the open system before any unwanted species take hold. However, contamination inevitably becomes predominant over time, necessitating system cleaning and re-inoculation.

Top of Form

Therefore, cleaning or washing the pools should be a part of the aquaculture process to reduce the problem. Aquasearch (Hawaii, USA) demonstrated this process using *Haematococcus pluvialis* to produce astaxanthin. Half of the Aquasearch area is dedicated to photobioreactors and the other half to open ponds. *To promote the formation of astaxanthin, Haematococcus pluvialis* was continually maintained in a nutrient-rich photobioreactor and then part of the culture was moved to an outdoor pond with limited nutrient availability. When the astaxanthin level peaks after three days, the open ponds are harvested, cleaned, and then re-inoculated with sufficient nutrients transferred with the inoculum [83]. This method works particularly well for producing biofuel because, in low-nutrient environments, algae quickly begin to transform solar energy into chemical energy that is stored as lipids for survival (see the section on "Lipid production by microalgae in nature"). There would need to be a succession of photobioreactors of escalating sizes, from beginning culture to the ultimate inoculum, for the large-scale generation of microalgae biofuel. To keep the cost per square meter to a minimum, the complexity level should decrease as the bioreactors get larger. Smaller bioreactors must be maintained under strict axenic conditions; however, as the bioreactor size increases, the level of containment can be loosened if inoculum is continuously supplied to flush each stage of the scale-up, as long as procedures are in place to remove contamination should it arise early in the chain. It is crucial to use an algae species that is both quickly growing during the inoculum scale-up stage and extremely productive during the final open pond stage for such a strategy to be successful. Through this approach, fresh inoculum can be continuously added to open ponds with low nutrient levels that are good for the environment, preventing the domination of invasive species and promoting the ongoing production of algae biofuels.

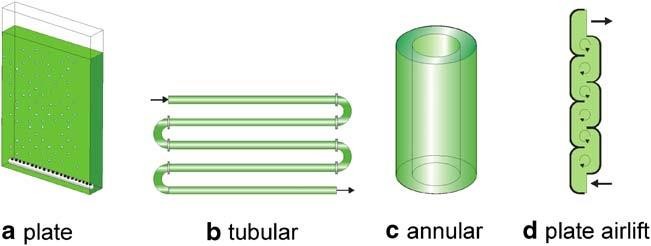


Fig. 6: Various closed photobioreactor designs that are frequently used to produce important compounds The design of a plate reactor follows a classical approach. A tubular reactor is the largest closed photobioreactor. An annular reactor functions as a bubble column and has an empty inner cylinder to increase surface/volume ratio and prevent dark areas. A plate airlift reactor with baffles supports the flashing light effect through controlled fluid barrels. While all of these designs are thought to be generally appropriate, their cost makes them unsuitable for the generation of biodiesel.

# Downstream Processing Harvesting Methods:

Because of their high water content, algae must be removed for harvesting and processing later on. Harvesting microalgae and lowering their water content cannot be done using a single optimal technique. The three most used harvesting methods in algal aquaculture nowadays are centrifugation, flocculation, and microscreening. The development of more inexpensive and energy-efficient harvesting techniques is required to increase the efficiency of the biofuel manufacturing process. Since certain species are far easier to harvest than others, strain selection is crucial in this regard. For instance, the long spiral form of the cyanobacterium Spirulina naturally lends itself to the comparatively energy- and cost-efficient microscreen harvesting technique [11, 16]. Filtration that is both economical and efficient can only be used to filamentous or big colonial microalgae. Although filtration is frequently used in laboratories, it has drawbacks in large-scale settings, including membrane clogging, the development of compressible filter cakes, and, most significantly, high maintenance costs. Stokes' law, which states that the sedimentation velocity is proportional to the square of the cell radius (the Stokes radius) and the difference in density between the cell and medium, can be used to explain both sedimentation and centrifugation. Gravitational force-based techniques are not easily applicable to bacteria, but they are possible for yeast and microalgae with diameters more than 5 μm and rather thick cell walls. Some algae farms use pure sedimentation, but it takes a lot of time and space, therefore it's not a good option for producing biodiesel. Decanting centrifuges have also been effectively used, and separation is improved by commercial centrifuges accelerating to at least 10,000 × g (e.g. [195]). Centrifugation is now thought to be too expensive and energy-intensive for the first microalgae harvesting process. An estimate of 3,000 kWh/ton has been made for the energy intake alone [16]. Nonetheless, centrifugation is a very helpful secondary harvesting technique that can be combined with oil extraction to concentrate an initial slurry (10–20 g/L) into an algal paste (100–200 g/L). Another widely used primary harvesting technique for concentrating algae is flocculation, which is the aggregation and sedimentation (or flotation) of algal biomass [118]. Adjacent settling ponds are utilized for flocculation, settling, and harvesting in raceway or mixing ponds. Although they are very powerful flocculants, inorganic compounds like lime, alum, and ferric chloride are thought to be too costly for large-scale operations. As they are more necessary and the algae can be utilized in downstream applications like animal feed or anaerobic digestion, organic cationic polyelectrolyte flocculants, such as the cationic polymer chitosan, are typically chosen [118]. Because flocculation enlarges the particle size, sedimentation proceeds more quickly (see Stokes' law) or, more effectively, interacts with floating bubbles. However, adding flocculants isn't the preferred approach for low-cost, environmentally friendly manufacturing at the moment. Current advancements entail promoting the self-flocculation of the

cells, which may happen in the event of apH change or carbon constraint. Moreover, polyelectrolytes can be utilized to promote bioflocculation, or spontaneous flocculation. Most likely, the least expensive harvesting method is bioflocculation. While some species flocculate spontaneously, others do so in response to environmental cues, nitrogen stress, pH, and dissolved oxygen levels [16]. However, the unreliability of the time it takes for spontaneous flocculation to occur is an issue. Co-bio- flocculation is an intriguing variant of this that Ami Ben-Amotz recently introduced [3]. In this instance, flocs containing high lipid Nannochloropsis variants were formed using the naturally flocculating alga Skeletonema. Feeding the fish tilapia (O. mosambicus) algae is another intriguing technique that Mike Massingill recently shared. A conveyor belt with 10–14% solids is then used to collect the algal biomass from the sedimented droppings, and it is then air-dried ([3]; Kent Seatech: United States Patent 6447681). Transesterification and Lipid Extraction Nowadays, most biodiesel is made from plant or animal oils by transesterification, which can occur after oil extraction and may or may not disturb cells. As an alternative, methyl esterification combined with the application of immobilized lipases can speed up the process [100]. Basically, a typical extraction procedure is mechanically crushing and then squeezing. For the extraction of oils and other microalgal products, chemical solvents can be chosen in one- or two-step extraction approaches. While high-pressure homogenization ("French press") can be used to disrupt cells, a more modern method is electroporation, where a high electrical field is applied to the biomass leading to perforation of the cell wall and to better extraction. This can even be used directly on living algae [79] or in conjunction with transesterification [58], which yields glycerol and biodiesel when methanol and a catalyst like sodium methoxide are used.

# CO2 Sequestration:

The CO2 levels in the atmosphere are now considered to be "dangerously high" since they have surpassed 450 parts per million CO2-e [84, 170]. Therefore, while the development of CO2-neutral biofuel production systems is vital, the main purpose of their production is to stabilize atmospheric CO2 levels at a level that is considered "dangerously high," rather than bringing it down to a level that is acceptable. Since separating CO2 from other atmospheric gases is a technically tough task, physical sequestration of atmospheric CO2 is sometimes seen as challenging. But over millions of years, photosynthetic organisms have perfected this process, and as a result, they are naturally suited to absorbing CO2 and storing it as biomass. The generation of CO2-neutral biofuels, such as biodiesel, and atmospheric CO2 sequestration would be greatly enhanced if the captured CO2 could be transformed into a more stable form for long-term storage (about 100 years or more). Large pond-style systems for capturing carbon dioxide were examined by Weissman and Tillett [194]. It has been demonstrated that the capture efficiency can reach 99% when operating in ideal circumstances [194, 204]. One gram of glucose requires 1.57 g of CO2 to create, according to the following equation: Light · chlorophyll ¼ C6H12O6 ÷ 6 O2 ÷ 6H2O ÷ 6CO2 Kurano et al. [97] found a ratio of 1.6 to 1 for the fixation of 4 g CO2 L−1 day−1 at growth rates of 2.5 g algae L−1 day-1. Considering that, under some circumstances, glucose can be converted into other substances like lipids or starch, the consumption of CO2 can reach up to 2 g CO2 for every 1 g of algae. One hectare of algal ponds can sequester up to one ton of CO2 per day, assuming a growth rate of 50 g m-2 day−1. Following the extraction of oil (about 30% of the dry weight), 70% of the biomass can be fed into the biodiesel process.

mechanisms for sequestering carbon downstream. Particularly, the "slow burning" method of pyrolysis can transform the sequestered carbon into hard C-chips (Agri-char) [25]. This also has the added benefit that Agrichar, as its name implies, can be sold to the agriculture industry because it significantly raises the soil's fertility and carbon content. Through an impact on soil and crop gas exchange, this can further mitigate the effects of climate change [98, 105]. Additionally, pyrolysis serves as a sterilizing process for biomass waste, offering a waste disposal method that is sensitive to the environment and raising public acceptance of the use of genetically modified microalgae for the production of biofuel.

# Molecular Improvements of Microalgae for Increased Biodiesel Yields:

The following can be kept in mind so as to obtain the best performing microalgae strains for maximal biofuel production:

1. screening a wide range of natural isolates,
2. improve them by metabolic (genetic) engineering or
3. improve them by selection and adaptation.

Thousands of distinct algal strains are available for access in algae collections across the globe. For instance, the Algi-Net Database [4] provides information on the cultures of algae strains that are now cultivated in Europe. Notably, 3000 algal strains were gathered by the US Aquatic Species Program, which evaluated them for their ability to produce biofuel [162]. The Aquatic Species Program's global algae collections and species, along with current developments in genetic engineering and material sciences, offer a solid foundation for the development of microalgal biodiesel production systems. In the future, techniques such as lipidomics, genomics, proteomics, and metabolomics may be employed to identify and cultivate novel strains exhibiting robust growth, elevated lipid biosynthesis rates, extensive resistance to diverse environmental conditions, and the production of valuable byproducts. Approaches from Systems Biology and Metabolic Engineering Transcriptomics, proteomics, and metabolomics techniques can be used to identify bottlenecks in the cell that affect the generation of algal biodiesel. When these techniques are combined, as opposed to when they are used in isolation, biological processes can be better understood. The measurement of metabolic flow can be used to confirm the identification of genes, proteins, or metabolites that exhibit variable expression. These signals point to the rate-limiting mechanisms within the cell. Through genetic or metabolic engineering, algal traits can be precisely tuned thanks to this systems biology method.

The goal of metabolomics is to identify metabolic profiles that characterize an organism's metabolome [56], in this example, lipid-rich algae. To determine the variations in metabolite levels brought on by either genetic or environmental alterations, a statistical technique is employed. Common methods for evaluating this include chemometric analysis after NMR spectroscopy or mass spectrometry (in conjunction with different chromatography techniques) [101, 123, 124]. The buildup may be caused by an enzyme that is downregulated upstream in the metabolic pathway or an enzyme that is upregulated downstream, making the interpretation of these data alone difficult. Consequently, the results of transcriptomics and proteomics should be considered when interpreting this data [121]. The metabolic flux can be investigated using a variety of methods to resolve the metabolic dynamics of microalgae, such as isotope labeling of important metabolite precursors or intermediates and time-dependent monitoring of these isotopes [55] or the monitoring of consumption and production of key compounds [44, 197].

The metabolic flux of an isotope from a given starting compound to a metabolic end product can be visualized time- and sometimes spatially-resolved using radioactive isotopes [34, 158, 198] or stable isotopes for NMR spectroscopy [159, 169] and mass spectrometry [158, 161, 185, 198]. The added benefit of using transcriptomics and proteomics is the ability to find differentially expressed genes and proteins that are either cooperatively regulated or directly engaged in the biosynthesis and breakdown of lipids. For instance, the over- or under-expression of certain regulatory genes and associated proteins in transgenic cells, including as kinases, phosphatases, and transcription factors, can effectively change entire physiological pathways [6, 43, 107]. By employing transgenes encoding for various enzymatic stages in fatty acid biosynthesis/modification pathways, metabolic engineering has been used to modify fatty acid production and composition in a number of plants, most notably canola [39, 49].

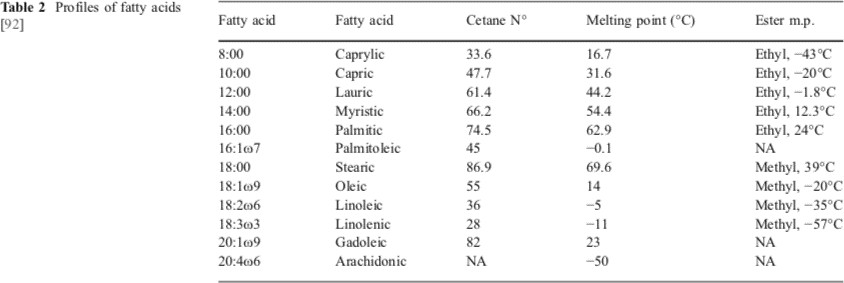
Genetic engineering may be able to increase algal production after the important enzymes and pathways are identified. Only a few chosen algal model species, such as C. reinhardtii, are currently routinely transformed; nevertheless, the area of transgenic microalgae is expanding and has a lot of potential [99, 189]. The "biolistic" procedure is the most widely used of the various transformation methods available for delivering DNA into the algal genome [189]. This method, which involves bombarding algae with microprojectiles coated in DNA, has been successfully used to a range of algae, including diatoms and green algae [8, 94]. It is also the preferred technique for transforming the genomes of mitochondria or chloroplasts [143]. Other techniques for producing transgenic algae include electroporation [164, 174], agitation using silicon-carbide whiskers [46, 47], and cell agitation in the presence of glass beads and DNA [88]. More recently, vectors with nuclear matrix attachment regions (MARs) have been developed to boost transgenic overexpression by raising the degree of foreign gene expression. Dunaliella salina, a halotolerant algae, has been used for this [191]. Doebbe et al. [42] successfully inserted the HUP1 (hexose uptake protein) hexose symporter from Chlorella kessleri into the mutant strain C. reinhardtii Stm6 using Kindle's glass bead approach [88]. As a result, a modified mutant was created that can produce hydrogen using glucose that is given externally. Engineering the photosynthetic light capture apparatus to increase solar energy to biomass conversion is another way to begin the metabolic and genetic engineering of microalgae [122]. Recently, the genome of C. reinhardtii was sequenced, revealing hitherto unidentified genes linked to photosynthetic processes among other things [113]. By genetically altering specific PQ pathway components to reduce photodamage and boost photosynthetic efficiency in high-light environments, electron pathway optimization can also be explored as a means of raising PQ levels and, consequently, the rate of biomass production [122]. Acetyl-CoA is the precursor needed for the production of algal lipids. To increase oil production, acetyl CoA carboxylase and other lipid biosynthesis pathway enzymes have been targeted [142, 162]. In the context of optimization for the generation of biodiesel, lipid metabolism and the manufacture of fatty acids, glycerolipids, sterols, hydrocarbons, and ether lipids in eukaryotic algae have recently been reviewed [72, 115]. Even though C. reinhardtii is used as a model organism to research lipid biosynthesis in green algae [205], certain odd hydrocarbons and ether lipids from Botryococcus braunii have been reported, such as n-

Alkadienes, trienes, methylated squalenes, tetraterpenoids, triterpenoid botryococcenes, lycopadiene; [2, 115]. The quality of the fuel product is significantly influenced by the type of oil used as a feedstock for biodiesel. A possible avenue for increasing lipid quantity and quality (chain length and saturation grade) is the genetic engineering of important enzymes involved in particular fatty acid manufacturing pathways within lipid biosynthesis. Since the alkyl ester content determines the fuel's durability and performance, lipid quality is crucial to the manufacture of biodiesel and ultimately plays a significant role in achieving international fuel requirements. Biodiesel's volumetric energy density is approximately 33 MJ/L, making it roughly 92% equivalent to petro-diesel. On the other hand, biodiesel burns hotter and burns longer than regular diesel fuel because its average hydrocarbon length is longerThe gasoline burns more thoroughly as a result. Considering this, the overall efficiency of biodiesel is almost 97% that of petro-diesel [92]. Nowadays, a variety of oilseed crops are used to make biodiesel, the most popular ones being soybean, rapeseed, and palm oil. Because a given biofuel crop's triacyl glyceride (TAG) profile is largely constant, the characteristics of the biodiesel made from that crop are predictable. When creating a new source of TAGs for biodiesel, several things need to be taken into account. Because they are mostly polyunsaturated, microalgal lipids are more vulnerable to oxidation. There is a significant problem with biodiesel while it is being stored. However, this disadvantage can be overcome by partially hydrogenating the oil by catalysis [29]. According to Gunstone and Hilditch's [71] measurements, the methyl esters of oleic (18:1), linoleic (18:2), and linolenic (18:3) acids had a relative rate of oxidation of 1:12:25. Thus, it is better to keep the amount of polyunsaturated fatty acids in biodiesel to a minimum. Higher polyunsaturated fat content, on the other hand, lowers the cold filter plugging point (CFPP), which is the temperature at which engine fuel begins to crystallize or solidify and clog the filters. The melting points of the main fatty acids are shown in Table 2. It has been noted that the more unsaturated an oil is, the lower its melting point. Therefore, for fuel to function at low temperatures in colder climes, a larger unsaturated lipid concentration is needed. Another metric for characterizing the quality of diesel fuel combustion during compression ignition is the cetane number (Table 2). Higher cetane fuels have been found to have shorter igniting delay times than lower cetane fuels in some diesel engines. As a result, it's critical to confirm that biodiesel's cetane number matches the engine's cetane rating [91]. In light of these factors, the "ideal mix" of fatty acids has been proposed to be 16:1, 18:1, and 14:0 in a 5:4:1 ratio. This kind of biodiesel has extremely low oxidative potential while maintaining a high cetane number and CFPP rating. Algae possess remarkable potential for genetically modifying their lipid pathways, such as through up- or down-regulating fatty acid production or β-oxidation. It should be able to significantly raise the fraction of monounsaturated lipids by deleting or altering the enzymes that the cell uses to synthesize polyunsaturated lipids. Furthermore, it's likely that the homeostasis mechanism of the algal cells would need to change the lipid ratio's amounts of saturated lipids in order for it to stay fluid at low temperatures.

As long as an algae species is cultivated in same conditions, its lipid profile will not change. Every algal species, however, will have a unique lipid profile, so it's critical to use species whose lipid profiles are appropriate for producing biodiesel.

# Photosynthetic Efficiency:

Two other examples that may be helpful for large-scale cultivation, aside from metabolic engineering methods to boost lipid production, are briefly discussed below. These include improving photosynthetic efficiency and choosing and enhancing strains for the best growth, survival, and oil production using wastewater and saltwater resources. Any improvement in photosynthetic efficiency will boost the production of biofuels later on. The initial step in the creation of all biofuels is driven by photosynthesis (Fig. 1). In particular, it absorbs solar radiation and transforms it into chemical energy (such as starch and oil). As a result, improving light capture efficiency is a crucial innovation in the creation of all systems for producing second-generation biofuels. In order to build massive light-harvesting antenna complexes that absorb sunlight and transport the resultant energy to PSI and PSII to power the photosynthetic processes, the majority of wild-type microalgae have evolved genetic methods (Fig. 1). This approach has an advantage in nature since it maximizes light collection in low light. The drawback is that higher plants and algae have to adopt photo-protective systems since too much light harms the photosynthetic apparatus [82, 130]. These usually release most of the captured energy as heat and fluorescence, or "waste" in the context of producing biofuels [134]. The majority of this energy dissipation occurs in the PSII-related light harvesting complexes (LHCII in Figure 1). Reducing the amount of chlorophyll-binding LHC proteins in each cell has been shown to significantly increase the total light conversion efficiency of bioreactors [122, 134]. With this approach, the light capture efficiency of the antenna systems designed specifically for oil production may be carefully adjusted and optimized. The culture is dark green because wild-type algae have extensive LHCII systems that bind chlorophyll. At the same cell density, cultures produced by cell lines with tiny LHCII antenna systems have a much lighter green color (Fig. 7a). When exposed to high light levels, algal cells on the bioreactor's illuminated surface in the wild-type scenario absorb the majority of the light but can squander up to 90% of it as heat and fluorescence [122, 134]. The amount of light that the wild-type cells are exposed to decreases with distance from the lit surface. It is impossible for these darkened cells to absorb enough sun energy to support effective photosynthesis. This leads to a significant reduction in the culture's overall efficiency. Alternatively, the advantage of using smaller antenna cell lines with lower LHCII levels is that they allow more light to enter the bioreactor (Fig. 7a) and better match the light's energy needs for each photosynthesizing cell. Therefore, only the light required by the "small antenna" cells at the bioreactor surface is absorbed, thereby removing superfluous energy fluorescence. Consequently, this permits an increased amount of light—that is, the light lost in the wild-type as heat and fluorescence—to enter the bioreactor, ensuring that even the cells located further inside the culture receive almost ideal light exposure [122]. Small antenna cultures have a better overall photosynthetic efficiency as a result (Fig. 7b). Finally, the following are the main benefits of small antenna mutants for the generation of biofuel: (1) Lower heat losses as determined by fluorescence and LHCII Enhanced light penetration characteristics, less photodamage, increased yield, and enhanced bioreactor efficiency are the other four factors. These efficiency advantages could be further increased by combining enhanced mixing techniques with genetically modified strains.



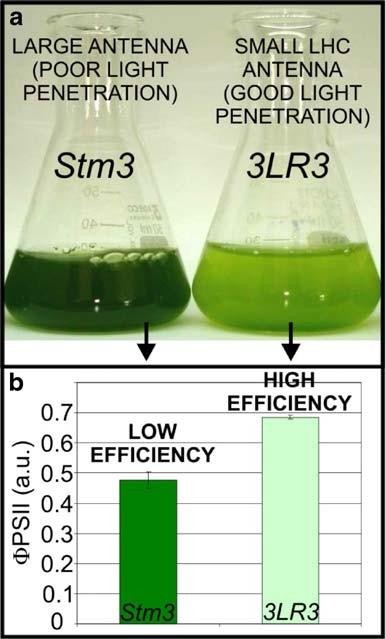


Fig.7. (a) Comparison of cultures of *Chlamydomonas reinhardtii* with parent strain (Stm3) and reduced antenna size (3LR3) at equal cell densities. a Cultures at densities of 6 × 106 cells/mL;

(b) Photosynthetic quantum yield (8PSII); adapted from Mussgnug et al. [122]

# Using Wastewater and Seawater Resources:

Using wastewater and seawater has obvious benefits over using more freshwater resources, which is becoming more and more of a concern due to population expansion and climate change-related water scarcity. But the water quality of both can differ, with wastewater having significant regional variations as well as long-term variations. Important nutrients like phosphorus and nitrogen can be found in wastewater [10, 163], but it can also contain

pollutants, including heavy metals and excessive trace metals, are all quite concerning, especially when it comes to the manufacturing of biodiesel. Cadmium, for example, has been shown to inhibit lipid biosynthesis among many other cellular functions [64]. The same pollutants can also be found in seawater, though not always in the same amounts. Additionally, while using less expensive fertilizers of agricultural grade is preferable from an economic standpoint, it also adds another potential source of heavy metal pollution that may hinder sensitive types of algae.

Although it is preferable to conserve freshwater supplies, strains that have the ability to withstand elevated concentrations of specific toxins can be advantageous in open-pond settings and could mitigate worries about contamination by other opportunistic organisms. The use of algae in phytoremediation and toxicological investigations has led to significant advancements in the fight against chemical contaminations, however its usage has also generated significant controversy [15, 207]. Many contamination problems connected to metals are thought to have their root cause in the induction of oxidative stress [133, 171]. In addition to heavy metals [62, 186–188, 197], various non-metal organo- compounds [54] and trace metals like copper, which are often needed for adequate nutrition, can also be fatal or inhibitive at high concentrations [22, 180]. Resistance mechanisms differ not just on the type of algae species but also on strains within certain species and, of course, on the specific type of toxin. Although the resistance mechanisms, such as decreased accumulation, sequestration, and precipitation, were not fully explained by early studies [32, 33, 108], more recent research has made significant strides in identifying putative protein targets [64] and gene targets [40] associated with heavy metal resistance in algae. There is room for more research on structural aspects such cell wall composition [61] and concurrent studies on resistance mechanisms in plant models [116].

Some algae strains, such Botryococcus braunii, are freshwater strains, however many of the strains designated for oil production are marine strains. Thus, osmotic issues beyond those covered above may arise from using seawater for algae cultivation. Numerous reviews have been written about osmoadaptation in microorganisms [60, 90]. Despite the fact that salt stress has been linked to the production of oil [140, 176, 182, 183], freshwater species can experience a stress response that can be fatal or very inhibitory at osmolarities far lower than those of seawater. Recent developments in this area have also been made possible by genes in microalgae that show anti-salt action [177]. These genes are frequently general anti-stress genes that most likely work by reducing oxidative stress. One such protein is a glutathione peroxidase-like protein from Chlamydomonas, which, when cloned into tobacco, enhanced stress tolerance, particularly salt tolerance (Yoshimura et al., 200). Additionally, glutathione peroxidase has been linked in the past to stress reactions brought on by heavy metal exposure [133]. It is obvious that there is a lot of opportunity here for strain characterisation and selection as well as for breeding, designing, and adapting strains with desired phenotypes that permit the utilization of water resources with varying water quality.

# Recent Advances in Microalgal Production:

Recent developments were showcased at the 1st International Algae Biomass Summit in San Francisco in November [3]. This gathering brought together leaders of newly-established microalgae biofuel companies, existing aquaculture firms, prominent researchers from the Aquatic Species Program, and microalgae scientists worldwide [3]. The event served as a platform to encourage collaboration among world-renowned experts and to explore substantial funding opportunities in 2008 and beyond. The primary objective was to create a highly efficient, cost-effective system for algal oil production and optimize its conversion to JP-8 fuel. The economic benchmarks were set with algae hydrocarbon costs at US $0.48 per liter ($2 per gallon), with a minimum order volume of 210 million liters (50 million gallons) to ensure viability. To achieve this price point, consideration of algae by-products in the economic equation was imperative. Distinguished speakers at the event shared insights from historical experiences, current technologies, and innovative concepts. Key findings and conclusions can be summarized as follows:

Joseph C. Weissman calculated the theoretical maximum yield for algal production to be 100 g m−2 day−1 or 365 tons of dry biomass per hectare per year. Existing limitations include the cost of obtaining CO2 at the required high concentrations for optimal algal growth and the challenge posed by algal grazers, a significant but often overlooked issue.

Cost-effective harvesting methods have been and continue to be a major limiting factor in the industry.

Biofuel production demands biomass at a cost of less than $300 US per ton of dry weight.

Ami Ben-Amotz presented findings of open pond yields averaging 20 g m−2 day−1 and suggested that overall production costs of $0.34 US per kilogram are feasible when the lipid content is sufficiently high. Effective co-bioflocculation using Skeletonema to enhance lipid-rich Nannochloropsis was highlighted as a cost-effective approach.

Tryg Lundquist pointed out the immense production potential of wastewater treatment ponds due to their high nutrient content and existing infrastructure. Limiting factors in this context include CO2 utilization and harvesting methods.

Mike Massingill demonstrated the cost-effectiveness of algae harvesting with the assistance of fish.

Mark Huntley presented hybrid systems achieving growth rates of Tetraselmis suecica at 62 g m−2 day−1 with 30% lipid content, although this was not maintained as a yearly average.

Bryan Willson introduced low-cost bioreactor designs utilizing disposable plastic materials, while Ben Cloud presented setups with standard farm-style configurations at an approximate cost of $15 US per square meter.

These insights and innovations collectively contribute to advancing the field of algal biofuel production.

# References:

* 1. Abu-Rezq T, Al-Musallam L, Al-Shimmari J (1999) Optimum Production Conditions for Different High-Quality Marine Algae. Hydrobiologia 403:97–107
  2. Achitouv E, Metzger P, Rager M-N (2004) C31–C34 methylated squalene from a Bolivian strain of Botryococcus braunii. Phytochem 65:3159–3165
  3. Algae Biomass Summit [(2007) http://www.wsgr.com/news/emailer/](http://www.wsgr.com/news/emailer/) PDFs/algae\_agenda07.pdf. Cited 30 Nov 2007
  4. Alginet (2007[) http://217.114.171.142/alginet/dblinks.html.](http://217.114.171.142/alginet/dblinks.html) Cited 5. December 2007
  5. Amon T, Amon B, Kryvoruchko V (2007) Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. Bioresource Technol 98:3204–3212
  6. Anderson JP, Badruzsaufari E, Schenk PM et al (2004) Antago- nistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance. Plant Cell 16:3460–3479
  7. Andrews TJ, Whitney SM (2003) Manipulating ribulose bisphosphate carboxylase/oxygenase in the chloroplasts of higher plants. Arch Biochem Biophys 414:159–169
  8. Apt KE, Kroth-Pancic PG, Grossman AR (1996) Stable nuclear transformation of the diatom Phaeodactylum tricornutum. Mol Gen Genet 252:572–579
  9. Arthur M, Dean W, Pratt L (1988) Geochemical and climatic effects of increased marine organic carbon burial at the Cenomanian/Turonian boundary. Nature 335:714– 717
  10. Aslan S, Kapdan I (2006) Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. Ecol Eng 28:64–70
  11. Atlantech (2[007) http://www.atlantech.ca/public/products/filters.](http://www.atlantech.ca/public/products/filters) html. Cited 5 Dec 2007
  12. Barbosa B, Jansen M, Ham N et al (2003a) Microalgae cultivation in air-lift reactors: modeling biomass yield and growth rate as a function of mixing frequency. Biotechnol Bioeng 82:170–179
  13. Barbosa B, Albrecht M, Wijffels R (2003b) Hydrodynamic stress and lethal events in sparged microalgae cultures. Biotechnol Bioeng 83:112–120
  14. Barbosa B, Hadiyanto M, Wijffels R (2004) Overcoming shear stress of microalgae cultures in sparged photobioreactors. Biotechnol Bioeng 85:78–85
  15. Becker EW (ed) (1994) Microalgae. Cambridge University Press, Cambridge
  16. Benemann J, Oswald W (1996) Systems and Economic Analysis of Microalgae Ponds for Conversion of CO2 to Biomass, Final Report to the US Department of Energy. Pittsburgh Energy Technology Center
  17. Belkin S, Boussiba S (1991) Resistance of Spirulina platensis to ammonia at high pH values. Plant Cell Physiol 32:953–958
  18. Biogas predictions (2007[) http://www.energetik-leipzig.de/Biogas/Biogas.htm.](http://www.energetik-leipzig.de/Biogas/Biogas.htm) Cited 2 Dec 2007
  19. Blanco-Canqui H, Lal R (2007) Soil and crop response to harvesting corn residues for biofuel production. Geoderma 141:355–362
  20. Boichenko VA, Hoffmann P (1994) Photosynthetic hydrogen production in prokaryotes and eukaryotes—occurrence, mechanism and functions. Photosynthetica 30:527–552.
  21. Borowitzka MA, Hallegraeff GM (2007) Economic importance of algae. In: McCarthy PM, Orchard AE (eds) Algae of Australia: introduction. ABRS, Canberra, pp 594–622.
  22. Boswell C, Sharma N, Sahi S (2002) Copper tolerance and accumulation potential of Chlamydomonas reinhardtii. Bull Environ Contam Toxicol 69:546–553
  23. Bourne JK (2007) Green dreams. Natl Geographic 212(4):38–59.
  24. BP statist[ics (2007) http://www.bp.com/statisticalreview.](http://www.bp.com/statisticalreview) Cited 30 Nov 2007
  25. Bridgwater A, Maniatis K (2004) The production of biofuels by thermal chemical processing of biomass. In: Archer M, Barber J (eds) Molecular to global photosynthesis. Imperial College Press, London.
  26. Brown A, Knights B, Conway E (1969) Hydrocarbon content and its relationship to physiological state in the green alga *Botryococcus braunii*. Phytochem 8:543–547.
  27. Carlozzi P (2003) Dilution of solar radiation through “culture lamination” in photobioreactor rows facing south north: a way to improve the efficiency of light utilization. Biotechnol Bioeng 81:305–315
  28. Casadevall E, Dif D, Largeau C et al (1985) Studies on batch and continuous cultures of *Botryococcus braunii*: hydrocarbon production in relation to physiological state, cell ultrastructure, and phosphate nutrition. Biotechnol Bioeng 27:286–295.
  29. Chisti Y (2007) Biodiesel from microalgae. Biotech Advances 25:294–306.
  30. Cho S, Ji SC, Hur S, Bae J, Park IS, Song YC (2007) Optimum temperature and salinity conditions for growth of green algae *Chlorella ellipsoidea* and *Nannochloris oculata*. Fish Sci 73:1050–1056
  31. CIA World Factbook (2007) https:/[/www.cia.gov/librar](http://www.cia.gov/library/)y[/](http://www.cia.gov/library/) publications/the-world- factbook/index.html. Cited 6 Dec 2007
  32. Collard J-M, Matagne R (1990) Isolation and genetic analysis of *Chlamydomonas reinhardtii* strains resistant to cadmium. Appl Environ Microbiol 56:2051–2055
  33. Collard J-M, Matagne R (1994) Cd2+ Resistance in wild-type and mutant strains of

*Chlamydomonas reinhardtii*. Environ Exp Bot 34:235–244.

* 1. Conklin PL, Pallanca JE, Last RL et al (1997) L-ascorbic acid metabolism in the ascorbate-deficient Arabidopsis mutant vtc1. Plant Physiol 115:1277–1285
  2. Das D, Veziroglu TN (2001) Hydrogen production by biological processes: a survey of literature. Intern J Hydrogen Energy 26:13–28
  3. 36. Dayananda C, Sarada R, Bhattachary S et al (2005) Effect of media and culture conditions on growth and hydrocarbon production by *Botryococcus braunii*. Process Biochem 40:3 125–3131
  4. Dayananda C, Sarada R, Usha Rani M (2007) Autotrophic cultivation of

*Botryococcus braunii* for the production of hydrocarbons and exopolysaccharides in various media. Biomass Bioenergy 31:87–93

* 1. Degen J, Uebele A, Retze A et al (2001) A novel airlift photobioreactor with baffles for improved light utilization through the flashing light effect. J Biotechnol 92:89–94
  2. Dehesh K, Jones A, Knutzon DS et al (1996) Production of high levels of 8:0 and 10:0 fatty acids in transgenic canola by overexpression of Ch FatB2, a thioesterase cDNA from Cuphea hookeriana. Plant J 9:167–172
  3. Deloménie C, Foti E, Floch E et al (2007) A new homolog of FocA transporters identified in cadmium-resistant Euglena gracilis. Biochemical and Biophysical Research Communica- tions 358:455–461
  4. Demain AL, Newcomb M, Wu JH (2005) Cellulase, clostridia, and ethanol. Microbiol Mol Biol Rev 69:124–154
  5. Doebbe A, Rupprecht J, Beckmann J et al (2007) Functional integration of the HUP1 hexose symporter gene into the genome of *C. reinhardtii*: impacts on biological H2 production. J Biotechnol 131:27–33
  6. Dombrecht B, Xue GP, Edgar CI et al (2007) AtMYC2 modulates diverse jasmonate- dependent functions by coordina- tion of a transcriptional cascade. Plant Cell 19:2225–2245
  7. Dong QL, Zhao XM, Ma HW et al (2006) Metabolic flux analysis of the two astaxanthin-producing microorganisms Haematococcus pluvialis and Phaffia rhodozyma in the pure and mixed cultures. Biotechnol J 1:1283–1292
  8. Doucha J, Straka F, Livansky K (2005) Utilization of flue gas for cultivation of microalgae (Chlorella sp.) in an outdoor open thin- layer photobioreactor. J Appl Phycol 17:403–412
  9. Dunahay TG (1993) Transformation of Chlamydomonas rein- hardtii with silicon carbide whiskers. Biotechniques 15:452–460
  10. Dunahay TG, Adler SA, Jarvik JW (1997) Transformation of microalgae using silicon carbide whiskers. Methods Mol Biol

62:503–509

* 1. Dumitrescu M, Brassell S (2005) Biogeochemical assessment of

sources of organic matter and paleoproductivity during the early Aptian oceanic anoxic event at Shatsky Rise, ODP Leg 198. Org Geochem 36:1002–1022

* 1. Eccleston VS, Ohlrogge JB (1998) Expression of lauroyl-acyl carrier protein thioesterase in Brassica napus seeds induces path- ways for both fatty acid oxidation and biosynthesis and implies a set point for triacylglycerol accumulation. Plant Cell 10:613–622
  2. Ecoworld (2[007) http://www.ecoworld.com/home/articles2.cfm?](http://www.ecoworld.com/home/articles2.cfm) tid=405. Cited 10 December 2007
  3. Elrad D, Grossman AR (2004) A genome’s-eye view of the light-harvesting polypeptides of *Chlamydomonas reinhardtii*. Curr Genetics 45:61–75
  4. Eriksen N, Riisgård F, Gunther W et al (2007) On-line estimation of O2 production, CO2 uptake, and growth kinetics of microalgal cultures in a gas-tight photobioreactor. J Appl Phycol 19: 161–174
  5. FAO (2007) Food and Agriculture Organization of the United Nations. [www.fao.org.](http://www.fao.org/) Cited 6 Dec 2007
  6. Fargasová A (1996) Inhibitive effect of organotin compounds on the chlorophyll content of the green freshwater alga Scenedes- mus quadricauda. Bull Environ Contam Toxicol 57:99–106
  7. Fernie AR, Geigenberger P, Stitt M (2005) Flux an important, but neglected, component of functional genomics. Curr Opin Plant Biol 8:174–182
  8. Fiehn O (2002) Metabolomics—the link between genotypes and phenotypes. Plant Mol Biol 48:155–171
  9. Forestier M, King P, Zhang LP et al (2003) Expression of two [Fe]-hydrogenases in *Chlamydomonas reinhardtii* under anaero- bic conditions. Europ J Biochem 270:2750–2758
  10. Fukuda H, Kondo A, Noda H (2001) Biodiesel fuel production by transesterification of oils. J Biosci Bioeng 92:405–416
  11. Gaffron H, Rubin J (1942) Fermentative and photochemical production of hydrogen in algae. J Gen Physiol 26:219–240
  12. Galinski E (1995) Osmoadaptation in bacteria. Adv Microbiol Physiol 37:273–328
  13. García-Ríos V, Freile-Pelegrín Y, Robledo D et al (2007) Cell wall composition affects cd2+ accumulation and intracellular thiol peptides in marine red algae. Aquatic Toxicol 81:65–72
  14. Gekeler W, Grill E, Winnacker E et al (1988) Algae sequester heavy metals via synthesis of phytochelatin complexes. Arch Microbiol 150:197–202
  15. Ghirardi ML, Zhang JP, Lee JW et al (2000) Microalgae: a green source of renewable H2. Trends Biotechnol 18:506–511
  16. Gillet S, Decottignies P, Chardonnet S et al (2006) Cadmium response and redoxin targets in *Chlamydomonas reinhardtii*: a proteomic approach. Photosynthesis Res 89:201–211
  17. Gollueke CG, Oswald WJ (1959) Biological conversion of light energy to the chemical energy of methane. Appl Microbiol 7: 219–227
  18. Gordon M, Polle J (2007) Ultrahigh productivity from algae. Appl Microbiol Biotechnol 76:969–975
  19. Graves DA, Tevault CV, Greenbaum E (1989) Control of photosynthetic reductant— the role of light and temperature on sustained hydrogen photoevolution by Chlamydomonas sp. in an anoxic, carbon dioxide-containing atmosphere. Photochem Pho- tobiol 50:571–576
  20. Grima E, Fernández F, Camacho F et al (1999) Photobioreactors: light regime, mass transfer, and scaleup. J Biotechnol 70:231–247
  21. Grobbelaar JU, Nedbal L, Tichy V (1996) Influence of high frequency light/dark fluctuations on photosynthetic character- istics of microalgae photoacclimated to different light intensi- ties and implications for mass algal cultivation. J Appl Phycol 8:335–343
  22. Gudin C, Chaumont D (1991) Cell fragility—the key problem of microalgae mass production in closed photobioreactors. Bioresour Technol 38:145–151
  23. Gunstone FD, Hilditch TP (1945) The union of gaseous oxygen with methyl oleate, linoleate and linolenate. J Chem Soc 105:836–841
  24. 72. Guschina IA, Harwood JL (2006) Lipids and lipid metabolism in eukaryotic algae. Progress Lipid Res 45:160–186
  25. Haag A (2007) Algae bloom again. Nature 447:520–521
  26. Hahn-Hägerdal B, Galbe M, Gorwa-Grauslund MF et al (2006) Bio-ethanol—the fuel of tomorrow from the residues of today. Trends Biotechnol 24:549–556
  27. Hammouda O, Gaber A, Abdel-Raouf N (1995) Microalgae and wastewater treatment. Ecotoxicol Environ Safety 31:205–210
  28. Hankamer B, Lehr F, Rupprecht J et al (2007) Photosynthetic biomass and H2 production: From bioengineering to bioreactor scale up. Physiol Plantarum 131:10–21
  29. Happe T, Naber JD (1993) Isolation, characterization and N-terminal amino-acid sequence of hydrogenase from the green-alga Chlamydomonas reinhardtii. Eur Biochem 214:475–481
  30. Harris EH (1989) The Chlamydomonas sourcebook: a compre- hensive guide to biology and laboratory use. Academic Press, San Diego
  31. Hejazi MA, Wijffels RH (2004) Milking of microalgae. Trends Biotechnol 22:189– 194
  32. Hill J, Nelson E, Tilman D et al (2006) Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. Poc Natl Acad Sci USA 103:11206– 11210
  33. Horton P, Ruban A (2005) Molecular design of the photosystem II light-harvesting antenna: photosynthesis and photoprotection. J Exp Bot 56:365–373
  34. Horton P, Ruban AV, Walters RG (1996) Regulation of light harvesting in green plants. Annu Rev Plant Physiol Plant Mol Biol 47:655–684
  35. Huntley ME, Redalje DG (2006) CO2 mitigation and renewable oil from photosynthetic microbes: a new appraisal. Mitigation and Adaption Strategies for Global Change 12:573–608
  36. IPCC (2007) Intergovernmental Panel on Climate Change ‘AR4 Synthesis report’. [www.ipcc.ch.](http://www.ipcc.ch/) Cited 30 Nov 2007
  37. Janssen M, Slenders P, Tramper J et al (2001) Photosynthetic efficiency of *Dunaliella tertiolecta* under short light/dark cycles. Enzyme Microb Tech 29:298–305
  38. Jo J, Lee D, Park J (2006) Modeling and optimization of photosynthetic hydrogen gas production by green alga Chlamydomonas reinhardtii in sulfur-deprived circumstance. Biotechnol Progress 22:431–437
  39. Kim J, Kang C, Park T et al (2006) Enhanced hydrogen production by controlling light intensity in sulfur-deprived *Chlamydomonas reinhardtii* culture. Intern J Hydrogen Energy 31:1585–1590
  40. Kindle KL (1990) High-frequency nuclear transformation of *Chlamydomonas reinhardtii*. Proc Natl Acad Sci USA 87:1228– 1232
  41. Kirst G (1977) Ion composition of unicellular marine and freshwater algae, with special reference to *Platymonas subcordiformis* cultivated in media with different osmotic strengths. Oecologia 28:177–189
  42. Kirst G (1990) Salinity tolerance of eukaryotic marine-algae. Ann Rev Plant Physiol Plant Mol Biol 41:21–53
  43. Knothe G (2005) Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. Fuel Processing Technol 86:1059–1070
  44. Knothe G, Gerpen JV, Krahl J (2005) The biodiesel handbook. AOCS Press, Urbana, IL, pp 277–280
  45. Kosourov S, Patrusheva E, Ghirardi ML et al (2007) A comparison of hydrogen photoproduction by sulfur-deprived *Chlamydomonas reinhardtii* under different growth conditions. J Biotechnol 128:776–787
  46. Kroth PG (2007) Genetic transformation: a tool to study protein targeting in diatoms. Methods Mol Biol 390:257–268
  47. Kruse O, Rupprecht J, Bader K-P et al (2005a) Improved photobiological H2 production in engineered green algal cells. J Biol Chem 280:34170–34177
  48. Kruse O, Rupprecht J, Mussgnug JR et al (2005b) Photosynthe- sis: a blueprint for solar energy capture and biohydrogen production technologies. Photochem Photobiol Sci 4:957–970
  49. Kurano N, Ikemoto H, Miyashita H et al (1995) Fixation and utilization of carbon dioxide by microalgal photosynthesis. Energy Convers Manag 36:689–692
  50. Lehmann J (2007) A Handful of carbon. Nature 447:143–144
  51. León-Bañares R, González-Ballester D, Galván A et al (2004) Transgenic microalgae as green cell-factories. Trends Biotechnol

22:45–52

* 1. Li X, Xu H, Wu Q (2007) Large-scale biodiesel production from microalga

*Chlorella protothecoides* through heterotrophic cultivation in bioreactors. Biotechnol Bioeng 98(4):764–771

* 1. Lindon JC, Holmes E, Nicholson JK (2004) Metabonomics and its role in drug development and disease diagnosis. Expert Rev Mol Diagn 4:189–199
  2. LO[E (2007) http://www.loe.org/shows/segments.htm?programID=](http://www.loe.org/shows/segments.htm?programID) 06-P13-00002&segmented=3. Cited 30 November 2007
  3. Mallick N (2002) Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. Biometals 15:377–390
  4. Mann CC (1999) Genetic engineers aim to soup up crop photosynthesis. Science 283:314–316
  5. Marris E (2006) Black is the new green. Nature 442:624–626
  6. McElwain J, Wade-Murphy J, Hesselbo S (2005) Changes in carbon dioxide during an oceanic anoxic event linked to intrusion into gondwana coals. Nature 435:479–482
  7. McGrath KC, Dombrecht B, Manners JM et al (2005) Repressor- and activator-type ethylene response factors functioning in jasmonate signaling and disease resistance identified via a genomewide screen of Arabidopsis transcription factor gene expression. Plant Physiol 139:949–959
  8. McHugh J, Spanier J (1994) Isolation of cadmium sensitive mutants in *Chlamydomonas reinhardtii* by transformation/insertional mutagenesis. FEMS Microbiol Lett 124:239–244
  9. Meiser A, Schmid-Staiger U, Trosch W (2004) Optimization of eicosapentaenoic acid production by *Phaeodactylum tricornutum* in the flat panel airlift (FPA) reactor. J Appl Phycol 16:215–225
  10. Melis A (1999) Photosystem-II damage and repair cycle in chloroplasts: what modulates the rate of photodamage? Trends Plant Sci 4:130–135
  11. Melis A, Zhang LP, Forestier M et al (2000) Sustained photobiological hydrogen gas production upon reversible inactivation of oxygen evolution in the green alga *Chlamydomonas reinhardtii*. Plant Physiol 122:127–136
  12. Merchant S, Allen M, Kropat J (2006) Between a rock and a hard place: trace element nutrition in *Chlamydomonas*. Biochim Biophys Acta 1763:578–594
  13. Merchant SS, Prochnik SE, Vallon O et al (2007) The *Chlamydomonas*

genome reveals the evolution of key animal and plant functions. Science 318:245–250

* 1. Metting F (1996) Biodiversity and application of microalgae. J Industrial Microbiol Biotechnol 17:477–489
  2. Metzger P, Largeau C (2005) *Botryococcus braunii*: a rich source for hydrocarbons and related ether lipids. Appl Microbiol Biotechnol 66:486–496
  3. Minglin L, Yuxiu Z, Tuanyao C (2005) Identification of genes up-regulated in response to Cd exposure in *Brassica juncea* L. Gene 363:151–158
  4. Molina Grima EM, Fernandez FGA et al (1999) Photobioreactors: light regime, mass transfer, and scaleup. J Biotechnol 70:231–247
  5. Molina Grima EM, Belarbi E-H, Acién Fernández FG et al (2002) Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol Adv 20:491–515
  6. MPOC (2007) Malaysian Palm Oil Counsel. In: Fact Sheets Malaysian Palm Oil. 6
  7. Muñoz R, Guieysse B (2006) Algal–bacterial processes for the treatment of hazardous contaminants: a review. Water Res 40:2799–2815
  8. Mus F, Dubini A, Seibert M et al (2007) Anaerobic acclimation in

*Chlamydomonas reinhardtii*: anoxic gene expression, hydrogenase induction, and metabolic pathways. J Biol Chem 282:25475–25486

* 1. Mussgnug J, Thomas-Hall S, Rupprecht J et al (2007) Engineer- ing photosynthetic light capture: Impacts on improved solar energy to biomass conversion. Plant Biotech J 5:802–814
  2. Nicholson JK, Lindon JC, Holmes E (1999) Metabonomics: understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica 29:1181–1189
  3. Nicholson JK, Connelly J, Lindon JC et al (2002) Metabonom- ics: a platform for studying drug toxicity and gene function. Nat Rev Drug Discov 1:153–161
  4. Oilworld (20[07) http://www.oilworld.biz.](http://www.oilworld.biz/) Cited 30 Nov 2007
  5. 126. Pachauri R (2007) <http://www.iht.com/articles/2007/11/20/opinion/> edscientists.php?WT.mc\_id=rssopinion. Cited 30 Nov 2007
  6. Paerl HW, Fulton RS 3rd, Moisander PH et al (2001) Harmful freshwater algal blooms, with an emphasis on cyanobacteria. Scientific World Journal 1:76–113 Apr 4
  7. Palmer MW, Pimentel D, Lal R et al (2007) Biofuels and the environment. Science 317:897
  8. Parry MAJ, Andralojc PJ, Mitchell RAC et al (2003) Manipulation of Rubisco: the amount, activity, function and regulation. J Exp Bot 54:1321–1333
  9. Pascal AA, Liu Z, Broess K et al (2007) Molecular basis of photoprotection and control of photosynthetic light harvesting. Nature 436:134–137
  10. Patzek T (2004) Thermodynamics of the corn-ethanol biofuel cycle. Crit Rev Plant Sci 23:519–567
  11. Perner-Nochta I, Posten C (2007) Simulations of light intensity variation in photobioreactors. J Biotechnol 131:276–285
  12. Pinto E, Siguad-Kutner T, Leitão M et al (2003) Heavy metal- induced oxidative stress in algae. J Phycol 39:1008–1018
  13. Polle JEW, Kanakagiri S, Jin E, Masuda T et al (2002) Truncated chlorophyll antenna size of the photosystems—a practical method to improve microalgal productivity and hydrogen production in mass culture. Int J Hydrogen Energy 27:1257–1264
  14. Poulsen B, Iversen J (1999) Membrane sparger in bubble column, airlift, and combined membrane–ring sparger bioreactors. Biotechnol Bioeng 64:452–458
  15. Prochnow A, Heiermann M, Drenckhan A et al (2005) Seasonal Pattern of Biomethanisation of Grass from Landscape Manage- ment. Agricultural Engineering International: the CIGR Ejournal. Manuscript EE 05 011. Vol. VII
  16. Pulz O (2001) Photobioreactors: production systems for photo- trophic microorganisms. Appl Microbiol Biotechnol 57:287–293
  17. Pulz O, Gross W (2004) Valuable products from biotechnology of microalgae. Appl Microbiol Biotechnol 65:635–648
  18. Pulz O (2007) Performance Summary Report: Evaluation of GreenFuel’s 3D matrix Algae Growth Engineering Scale Unit
  19. Ranga Rao A, Dayananda C, Sarada R et al (2007a) Effect of salinity on growth of green alga *Botryococcus braunii* and its constituents. Bioresource Technol 98:560–564
  20. Ranga Rao A, Sarada R, Ravishankar G (2007b) Influence of CO2 on growth and hydrocarbon production in *Botryococcus braunii*. J Microbiol Biotechnol 17:414– 419
  21. Ratledge C (2004) Fatty acid biosynthesis in microorganisms being used for single cell oil production. Biochimie 86:807–815
  22. Remacle C, Cardol P, Coosemans N et al (2006) High-efficiency biolistic transformation of *Chlamydomonas* mitochondria can be used to insert mutations in complex I genes. Proc Natl Acad Sci USA 103:4771–4776
  23. Reynolds CS (1984) The ecology of freshwater phytoplankton. Cambridge University Press, Cambridge, UK
  24. Richmond A (1986) Outdoor mass cultures of microalgae. In: Richmond A (ed) Handbook of microalgal mass culture 1986. CRC, Boca Raton, pp 285–330
  25. Richmond A (2004) Principles for attaining maximal microalgal productivity in photobioreactors: an overview. Hydrobiologia 512:33–37
  26. Righelato R, Spracklen D (2007) Carbon mitigation by biofuels or by saving and restoring forests. Science 317:902
  27. Rochaix J-D, Goldschmidt-Clermont M, Merchant S (eds) (1998) The molecular biology of chloroplasts and mitochondria in Chlamydomonas. Dordrecht. Kluwer Academic Publishers
  28. Rodhe W (1969) Crystallization of eutrophication concepts in northern Europe. In Eutrophication: causes, consequences, correctives. Washington, DC, National Academy of Sciences, pp 50–64
  29. Rosello Sastre R, Csögör Z, Perner-Nochta I et al (2007) Scale- down of microalgae cultivations in tubular photo-bioreactors—a conceptual approach. J Biotechnol 132:127–133
  30. Rupprecht J, Hankamer B, Mussgnug JH et al (2006) Perspec- tives and advances of biological H2 production in microorganisms. Appl Microbiol Biotech 72:442–449
  31. Sato T, Usui S, Tsuchiya Y et al (2006) Invention of outdoor closet type photobioreactor for microalgae. Energy Convers Manag 47:791–799
  32. Sawayama S, Inoue S, Yokoyama S (1994) Continuous-culture of

hydrocarbon-rich microalga *Botryococcus braunii* in secondarily treated sewage. Appl Microbiol Biotechnol 41:729–731

* 1. Sawayama S, Minowa T, Dote Y et al (1992) Growth of the hydrocarbon-rich microalga *Botryococcus braunii* in secondarily treated sewage. Appl Microbiol Biotechnol 38:135–138
  2. Schaub G, Kolb T, Steiger W (2007) Biofuels. Second- generation biofuels. Chemie Ingenieurtechnik 79:1325–1326
  3. Schmidt CW (2007) Biodiesel: cultivating alternative fuels. Environ Health Perspect. 115(2):A86–A91
  4. Schubert C (2006) Can biofuels finally take center stage? Nature Biotechnol 24:777–784
  5. Schwender J, Goffman F, Ohlrogge JB et al (2004) Rubisco without the Calvin cycle improves the carbon efficiency of developing green seeds. Nature 432:779–782
  6. Schwender J, Seemann M, Lichtenthaler HK et al (1996) Biosynthesis of isoprenoids (carotenoids, sterols, prenyl side- chains of chlorophylls and plastoquinone) via a novel pyruvate/ glyceraldehyde 3-phosphate non-mevalonate pathway in the green alga Scenedesmus obliquus. Biochem J 316:73–80
  7. Sellner KG, Doucette GJ, Kirkpatrick GJ (2003) Harmful algal blooms: causes, impacts and detection. J Ind Microbiol Bio- technol 30:383–406
  8. Shastri AA, Morgan JA (2007) A transient isotopic labeling methodology for 13C metabolic flux analysis of photoautotro- phic microorganisms. Phytochem 68:2302–2312
  9. Sheehan J, Dunahay T, Benemann J et al (1998) A look back at the U.S. Department of Energy’s Aquatic Species Program- biodiesel from algae. National Renewable Energy Laboratory
  10. Shi J, Podola B, Melkonian M (2007) Removal of nitrogen and phosphorus from wastewater using microalgae immobilized on twin layers: an experimental study. J Appl Phycol 19:417–423
  11. Shimogawara K, Fujiwara S, Grossman A et al (1998) High- efficiency transformation of *Chlamydomonas reinhardtii* by electroporation. Genetics 148:1821– 1828
  12. Singh Y, Kumar H (1992) Lipid and hydrocarbon production by Botryococcus spp. Under nitrogen limitation and anaerobiosis. World J Microbiol Biotechnol 8:121– 124
  13. Soeder C (1980) Massive cultivation of microalgae: results and prospects. Hydrobiologia 72:197–209
  14. Somerville C (2006) The billion-ton biofuels vision. Science 312:1277
  15. Spolaore P, Joannis-Cassan C, Duran E et al (2006) Commercial applications of microalgae. J Biosci Bioeng 101:87–96
  16. Sriram G, Fulton DB, Iyer VV et al (2004) Quantification of compartmented metabolic fluxes in developing soybean embryos by employing biosynthetically directed fractional (13)C labeling, two- dimensional [(13)C, (1)H] nuclear magnetic resonance, and com- prehensive isotopomer balancing. Plant Physiol 136:3043–3057
  17. Stern N (2006) The Economics of Climate Change. HM Treasury, London
  18. Stohs S, Bagchi D (1995) Oxidative mechanisms in the toxicity of metal ions. Free Radical Biol Med 18:321–336
  19. Studier FW (2005) Protein production by auto-induction in high- density shaking cultures. Protein Expr Purif 41:207–234
  20. Stumm S, Morgan J (1995) Aquatic Chemistry. New York, Wiley
  21. Sun Y, Yang Z, Gao X et al (2005) Expression of foreign genes in *Dunaliella*

by electroporation. Mol Biotechnol 30:185–192

* 1. Taiz L, Zeiger E (2002) Plant Physiology. Sinauer Associates Inc., Sunderland, USA
  2. Takagi M, Karseno, Yoshida T (2006) Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae Dunaliella cells. J Biosci Bioeng 101:223–226
  3. Tanaka S, Suda Y, Ikeda K et al (2007) A novel gene with antisalt and anticadmium stress activities from a halotolerant marine green alga Chlamydomonas sp. W80. FEMS Microbiol Lett 271:48–52
  4. The Economist (2006) Steady as she goes. in The Economist 379:65–67 (London)
  5. Tredici MR (2003) Mass Production of Microalgae Photo-bioreactors. In: Richmond A (ed) Handbook of microalgal culture. Blackwell, USA, pp 178–214
  6. Tripathi B, Gaur J (2004) Relationship between copper- and zinc-induced oxidative stress and proline accumulation in Scenedesmus sp. Planta 219:397–404
  7. USDA (2007) US Department of Agriculture. [www.usda.gov.](http://www.usda.gov/) Cited 6 Dec 2007
  8. Vázquez-Duhalt R, Arredondo-Vega B (1991a) Haloadaptation of the green alga Botryococcus brauni. Phytochem 30:2919–2925
  9. Vázquez-Duhalt R, Arredondo-Vega B (1991b) oil production from microalgae under saline stress. biomass for energy and industry. 5th E.C Conference. Vol.1: Policy, Environment, Production and Harvesting.Soeder, C., Massive cultivation of microalgae: results and prospects. Hydrobiologia, 1980. 72:pp. 197–209
  10. VDI (2004) Vergärung organischer Stoffe. In:Verein Deutscher Ingenieure Guidelines 2004, Guideline VDI 4630, Düsseldorf, Germany
  11. Villas-Bôas SG, Kesson M, Nielsen J (2005) Biosynthesis of glyoxylate from glycine in Saccharomyces cerevisiae. FEMS Yeast Res 5:703–709
  12. Vymazal J (1987) Toxicity and accumulation of cadmium with respect to algae and cyanobacteria: a review. Toxic Assess 2:387–415
  13. Vymazal J (1990a) Toxicity and accumulation of lead with respect to algae and cyanobacteria: a review. Acta Hydrochim Hydrobiol 18:513–535
  14. Vymazal J (1990b) Uptake of lead, chromium, cadmium and cobalt by Cladophora glomerata. Bull Environ Contam Toxicol 44:468–472
  15. Walker TL, Purton S, Becker DK, Collet C (2005) Microalgae as bioreactors. Plant Cell Rep 24:629–641
  16. Waltz E (2007) Will the current biofuels boom go bust? Nat Biotechnol 25:491–492
  17. Wang T, Xue L, Hou W et al (2007) Increased expression of transgene in stably transformed cells of *Dunaliella salina* by matrix attachment regions. Appl Microbiol Biotechnol. 76: 651–657
  18. Weiland P (2003) Production and energetic use of biogas from energy crops and wastes in Germany. Appl Biochem Biotechnol 109:263–274
  19. Weissman J, Goebel RP, Benemann JR (1988) Photobioreactor design: mixing, carbon utilization, and oxygen accumulation. Biotechnol Bioeng 31:336–344
  20. Weissman JC, Tillett DM (1992) Aquatic Species Project Report; NREL/MP- 232-4174. In: Brown LM, Sprague S (eds) National Renewable Energy Laboratory pp 41–58
  21. Westfalia (2[007) http://www.westfalia-separator.com/en/about/](http://www.westfalia-separator.com/en/about/) aboutnews/newspressedetail.php?ID=1021. Cited 6 Dec 2007
  22. World Energy Council (2007) 2007 Survey of Energy Resources, executive summary[. http://www.worldenergy.org/documents/](http://www.worldenergy.org/documents/) ser2007\_executive\_summary.pdf. Cited 6 Dec 2007
  23. Yang C, Hua Q, Shimizu K (2000) Energetics and carbon metabolism during growth of microalgal cells under photoautotrophic, mixotrophic and cyclic light- autotrophic/dark-heterotro-phic conditions. Biochem Eng J 6:87–102
  24. Yang C, Hua Q, Shimizu K (2002) Metabolic flux analysis in *Synechocystis*

using isotope distribution from 13C-labeled glu- cose. Metabolic Eng 4:202–216

* 1. Yoshimoto N, Sato T, Kondo Y (2005) Dynamic discrete model of flashing light effect in photosynthesis of microalgae. J Appl Phycol 17:207–214
  2. Yoshimura K, Miyao K, Gaber A et al (2004) Enhancement of stress tolerance in transgenic tobacco plants overexpressing *Chlamydomonas* Glutathione peroxidase in chloroplasts or cytosol. Plant J 37:21–33
  3. Yun Y, Lee S, Park J et al (1997) Carbon dioxide fixation by algal cultivation using wastewater nutrients. J Chem Technol Biotechnol 69:451–455
  4. Zaslavskaia L, Lippmeier J, Shih C et al (2001) Trophic conversion of an obligate photoautotrophic organism through metabolic engineering. Science 292:2073–2075
  5. Zhila N, Kalacheva G, Volova T (2005) Effect of nitrogen limitation on the growth and lipid composition of the green alga *Botryococcus braunii* Kütz IPPAS H-

252. Russian J Plant Physiol 52(3):31–319

* 1. Zeiler KG, Heacox DA, Toon ST et al (1995) The use of microalgae for assimilation and utilization of carbon dioxide from fossil fuel-fired power plant flue gas. Energy Convers Manag 36:707–712
  2. Riekhof WR, Sears BB, Benning C (2005) Annotation of genes involved in glycerolipid biosynthesis in *Chlamydomonas reinhardtii*: discovery of the betaine lipid synthase BTA1Cr. Eukaryot Cell 4:242–252
  3. Dwivedi S, Tripathi RD, Rai UN, Srivastava S, Mishra S, Shukla MK, Gupta AK, Sinha S, Baghel VS, Gupta DK (2006) Dominance of algae in Ganga water polluted through fly-ash leaching: metal bioaccumulation potential of selected algal species. Bull Environ Contam Toxicol. 77:427–436
  4. Sandau E, Sandau P, Pulz O (1996) Heavy metal sorption by microalgae. Acta Biotechnol. 16:227–235