Physiological studies to optimize growth of the prototype biosolar cell factory Synechocystis sp. PCC6803

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1   Harvesting sunlight with cyanobacteria and algae for sustainable production in a bio-based economy

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1.1   Introduction: Limits and opportunities in oxygenic photosynthesis

Oxygenic photosynthesis in cyanobacteria, algae and plants provides, directly or indirectly (via fossil reserves), the fast majority of the energy for the living world. Cyanobacteria and microalgae are responsible for about half of this conversion of free energy (28). These organisms convert solar energy into chemical energy by the conversion of CO_2 into organic carbon compounds, evolving oxygen – the essential substrate of respiration in chemo-heterotrophs – in parallel. If all solar energy that reaches the Earth's surface were used with 100 % efficiency, it would take only one hour to satisfy the energy demand of the world's population for a year (29). Even though clearly not all of this radiation can be harvested in oxygenic photosynthesis, it is important to analyze the efficiency of light-energy conversion in (oxygenic) photosynthesis in detail, in order to identify molecular targets for improvement. Over the years, the results of a wide range of measurements and estimates of the "solar-to-biomass" free-energy conversion efficiency have been published. The most-cited values range from 4.6 to 6 % for C_3 and C_4 plants, respectively (29), and 8 to 10 % for cyanobacteria and algae (30, 31). Which of the latter two single-celled (micro) organisms actually is most efficient in converting radiation free energy into the free energy of biomass is difficult to decide on the basis of the available data, though it is clear that the prokaryotic representatives among them have a distinctly lower maintenance energy demand for growth. These efficiency numbers were derived from lab-scale experiments. The highest reported solar-to-biomass conversion yield reported for algal productivity at a larger scale so far is only 3 %, which still is significantly higher than for a plant such as switchgrass (i.e. 0.2 %) (31).

Free-energy losses in photosynthesis occur at various points in the light-energy transduction pathway, such as the limited spectral width of photosynthetically active radiation (PAR, equal to ≈45 % of the incident radiation from the sun), losses due to non-photochemical quenching (NPQ), and those due to futile cycles in downstream metabolism, such as some forms of 'photorespiration'. Hence it is often observed that oxygenic photosynthesis requires significantly more than the theoretical 8 photons (32, 33) per molecule of fixed CO_2. Actual numbers in fact range from 9 to 17 (34-38).

Compared to land plants, cyanobacteria and microalgae have little non-photosynthetic overhead (i.e. roots, stems, etc.). Furthermore, they can grow at high densities and growth rates, and are capable of continued production throughout the year. Together with their high intrinsic photosynthetic efficiency, this makes them promising candidates for becoming the preferred production host for biofuels and fine chemicals in a sustainable bio-based society.

Currently, algal cultivation is mainly done in open ponds, which can be subdivided into natural waters (lakes, lagoons, ponds) and artificial ponds or containers (39, 40). The most commonly used systems include large shallow ponds and raceway ponds, either paddle-stirred or unstirred, with a water depth of 15–30 cm (41). Even though these are relatively simple to build and operate, they are only suited for growth of (consortia of) algae that

can cope with predators and invaders, or with the extreme conditions required to suppress the former, as such open systems will inevitably be infected. In the absence of efficient mixing and hence slow mass transfer of CO_2, and with poor light utilization, this will lead to low productivity. Beyond these open (pond) systems, also many closed photobioreactors (PBRs) have been developed, which are less prone to infection and often more efficient in terms of mass transfer and light utilization (39, 40). However, these closed systems are more difficult to scale up to mass-production capacity without reducing the illumination surface and/or mixing efficiency, and require significant capital investment.
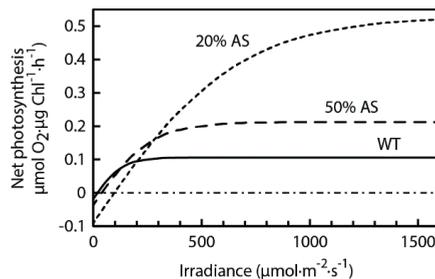

Fig. 1.1. Photosynthesis – Irradiance (PI) curve simulated for WT and antenna size mutants with 20% and 50% of WT antenna levels, respectively. The dash-dotted line at 0 µmol O_2 µg Chl^{-1} h^{-1} indicates the compensation point. Though the compensation point is reached at a higher light intensity than WT, antenna size mutants have a much higher saturation level and allow for better light penetration, leading to overall higher productivity in dense cultures. Reproduced from Formighieri et al. (2012).

Efficient product formation in large-scale culturing facilities for cyanobacteria and microalgae generally requires dense cultures with very efficient light harvesting. These are characteristics for which the organisms are not selected for in their natural environment. As light intensity in Nature varies depending on the time of the day, the weather, the season, and other environmental factors, such organisms are adapted to maximize light harvesting under varying and suboptimal conditions so that they deprive their competitors of light. However, photosynthesis only initially responds linearly to an increase in light intensity: It already saturates at moderate (sunlight) intensities. In fact, on a sunny day at sea level and moderate latitude the photosynthetic capacity in cyanobacteria and microalgae is saturated most of the time (31). This leads to dissipation of excess energy into heat by photo-protective mechanisms, to avoid production of an excess of Reactive Oxygen Species (ROS) in the organisms involved.

The cellular capacity for light harvesting is based on antenna size, which is defined as the number of light-harvesting molecules (chlorophyll *a* and *b*, phycobilisomes (PBS), carotenoids, etc.) that are attached to, and serve, the two photosystems, and the abundance of these photosystems. Green algae contain approximately the same number of each photosystem, whereas in cyanobacteria PSI is ≈5-fold more abundant than PSII (42-44). Both photosystems, but particularly PSI, contain several dozen chlorophyll molecules as intrinsic antenna pigments (45, 46). In addition to those intrinsic antennae,

mobile antennae exist – notably phycobilisomes in cyanobacteria and the Light-Harvesting Complex (LHC) in green algae – that can adjust exciton transfer to the two separate photosystems, via so-called 'state transitions', so that equal excitation rates can be obtained in the two photosystems, which is a prerequisite for balanced phototrophic growth. Both changes in light color and light intensity may necessitate such a redistribution, which usually takes several minutes in LHC-containing organisms (47, 48) and tens of seconds in cyanobacteria (49-51).

In dense (mass) cultures, light penetration is poor, which will lead to a large part of the culture being in light intensities below the compensation point, i.e. the light intensity at which photosynthesis equals respiration. This in turn will lower the overall efficiency of such a production system to less than the theoretical maximum. Additionally, mixing of these cultures causes cells to experience light/dark cycles as they shuttle between the dark deeper layers and high light intensities near the surface of the reactor, which can have positive as well as adverse effects on biomass yield, depending, amongst others, on the incident light intensity and the geometry and mixing characteristics of the reactor (30, 52, 53). The highest productivity per volume is achieved with a biomass concentration and PBR configuration that leaves light intensity above the compensation point at the far end of the PBR, in order to minimize losses from respiration. In addition, incident intensities should not be much higher than the saturation intensity. The former point has experimentally been confirmed with mass cultures of *Arthrospira platensis* (54) and computationally rationalized (28) (**Fig. 1.1**). In outdoor culturing, nevertheless, incident light intensity is primarily dictated by latitude and climate. Further adjustment can only be made through the geometrical design of the PBR (55) and via genetic engineering of the cell's antenna complexes. The latter aspect will be discussed in part 3.

1.2 Large-scale culturing of oxyphototrophs for a bio-based economy

Direct exploitation of the free energy from sunlight, through natural oxygenic photosynthesis, for use in a bio-based economy, requires the use of large shallow photobioreactors. The distributed nature of sunlight implies that it is not of much use to have a light-path in the reactor of more than a few centimeters at moderate cell densities. In its simplest form, this approach aims at producing biomass, which subsequently can be processed, e.g. via anaerobic digestion, into methane, or even into crude oil via pyrolysis or other chemical conversion processes *(56)*. With optimal areal productivity of the algae, this approach has been brought close to economic feasibility and is expected to reach this point within the next 10 to 15 years (57). However, as biomass may contain specific components (e.g. polyunsaturated fatty acids (PUFAs), carotenoids, etc.) with a much higher value than the average (liquid) energy carrier, a bio-refinery approach in which such valuable products are first separated, may significantly enhance the profitability of these culturing systems (58).

The bio-refining approach can be extended to the subcellular fraction that contains the polysaccharides or the triglycerides. These can then be converted into fermentation products such as ethanol, butanol, etc. and into biodiesel, after trans-esterification with methanol, respectively (57, 59-61). For this latter approach (eukaryotic) microalgae may be preferable over cyanobacteria in view of their larger cellular storage capacity. This also holds for the production of products with even higher added value that are stored in the cytoplasmic compartment, like e.g. pharmaceutical proteins (note that some polysaccharides synthesized by cyanobacteria may form an exception as they are stored in the extracellular compartment). Directed engineering to increase the cellular content of the refined product in the algae may further boost this approach, be it that this engineering is complicated by the presence of multiple subcellular compartments, several of which have their own dedicated genetic system. Hence complicated regulatory circuits will need to be optimized, similar to the 'crisscross' regulation in sporulation (62), as e.g. in terpene synthesis (63).

For maximal overall efficiency, however, it is beneficial to engineer a shortcut in phototrophic metabolism, which then allows such engineered organisms to directly channel fixed carbon into a fermentation/fuel product or into a chemical commodity (23, 64, 65). This strategy can be applied to both microalgae (66) and cyanobacteria, but has been much more successful in the latter class of organisms. This may be due to the ease with which the prokaryotic phototrophs can be engineered genetically, but also an increased permeability of their cell envelope may play a role in this. This 'shortcut' strategy – also referred to as the 'Photanol approach', see Hellingwerf and Teixeira de Mattos (2009) – is particularly attractive if the final product of metabolism can leave the cells, either through cloning of a dedicated export protein (as for sucrose (67)) or via passive leakage (as for lactic acid (68) and ethanol (64, 69)). Several large-scale initiatives are underway that are based on a cyanobacterial cell factory for a small molecule. Their economic competitiveness is currently under investigation (70).

1.3   Cyanobacterial cell factories

Use of cyanobacteria for the production of biofuel and chemical commodities has primarily been focused on *Synechocystis* and *Synechococcus*, with smaller efforts directed towards *Anabaena* and *Cyanothece* (a nitrogen-fixing strain). A proper molecular genetic toolbox is available for all four of these species, be it that knowledge about (regulatable) promoters and regulatory translation signal is still limited. Various metabolic pathways have been introduced into these organisms, like for the production of ethanol (23, 64), lactic acid (68, 71), sucrose (67), ethylene (72), 2,3-butanediol (73, 74), isoprene (75), iso-butyraldehyde (76), iso-butanol (77), 1-butanol (65), fatty acids (78) and alkanes (79).

After the expansion of the cyanobacterial metabolism with a heterologous metabolic pathway, and the subsequent optimization of the pathway itself (65), optimization of product formation can be pursued by streamlining cyanobacterial CO_2-fixation and intermediary metabolism. For sucrose production, this was not even necessary: A salt

stress makes the cells channel 90 % of its fixed CO_2 into this sugar and even increases the overall rate of CO_2 fixation (67). However, most of the products listed above are derived from pyruvate, of which the level in wild type cyanobacteria is rather low (80). To increase the rate and level of formation of these products the capacity of the exogenous catabolic/fermentation pathway needs to be optimized (69, 72, 81). Furthermore, competing pathways that catabolize pyruvate need to be eliminated (and pyruvate formation stimulated). This strategy has successfully been applied e.g. in the production of ethanol and lactic acid (82). The choice of enzymes that can contribute to increased pyruvate formation extends all the way to Ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO)(76), which catalyzes the initial reaction of the Calvin cycle, be it that engineering of an 'electron sink' by itself already leads to increased RuBisCO activity, provided the heterologous metabolic pathway has sufficient capacity (67, 81).

   The developing field of synthetic-systems biology clearly has the potential to help resolve all the complicated mechanisms that lead to (sink) regulation in cyanobacterial photosynthesis. Eventually then the point will be reached in which nearly all (i.e. > 95 %) carbon fixed by an engineered cyanobacterium is channeled into product, and only a small fraction into the synthesis of new cells. Such cells then would truly be 'cell factories'. Ideally the remaining growth rate will equal the death rate of these cells in a production environment. Metabolic control theory can then provide the tools to predict the optimal expression level of the heterologous product-forming metabolic pathway, to prevent that this pathway would become a metabolic burden (68).

1.4   Development of micro-algal cell factories

Because of their specific and complex genetic and spatial organization eukaryotic algae are attractive for cell factory applications for which a large intracellular storage capacity is required. As they are able to either directly synthesize (e.g. in the chloroplast) or temporarily store (i.e. in the vacuole) products within cellular sub-compartments, this increases the flexibility and efficiency of design-driven synthetic biology strategies. A further advantage is their ease of harvesting, due to their large cell size (>10 μm).

   Chlamydomonas reinhardtii is generally considered as the best genetically accessible green alga. Both its chloroplast genome (83, 84) and its nuclear genome can be targeted selectively for knockout mutagenesis and gene insertion. Heterologous gene expression, combined with cytosolic protein production is well established in this organism (85), be it that their efficiency could further be improved. Of course, *C. reinhardtii*, in contrast to the cyanobacteria, brings all the advantages of a eukaryotic production system, in terms of presence of systems for post-translational modification. Significantly, also efficient protein secretion has recently been engineered into this organism (86, 87). Neutral lipid metabolism in microalgae is complex, compartmentalized, and not yet fully understood (88), which makes it difficult to define unambiguous targets for molecular engineering (89). Nevertheless, in parallel to the approach of synthetic biology, also new physiological strategies are being developed to enhance lipid production in microalgae (90-93).

Besides *C. reinhardtii*, for only a few additional green algae (e.g. *Nannochloropsis gaditana* and *Ostreococcus tauri*) and one diatom (i.e. *Phaeodactylum tricornutum*) a toolbox for genetic engineering has been developed (94-96). *C. reinhardtii* strains with improved phenotypes of interest have already been constructed (97, 98). The well-established protocols for transformation of *C. reinhardtii* with heterologous genes have also been the driver of the application of this organism in the expression of edible therapeutics in its chloroplasts (99).

Another microalga for which many researchers eagerly await increased accessibility of its genome(s) is *Botryococcus braunii*. This green alga can produce large amounts of the triterpene botryocene, in such a way that the organism is able to deposit a large part of this 'biocrude' product in the extracellular matrix. This has great advantages for downstream processing of this fuel product. Molecular techniques are now rapidly providing a view on the mechanisms and pathway by which this product is made and deposited (100, 101), but the race is on to see whether genetic engineering to enhance its productivity will take place in this green alga itself, or in a relative that now already is genetically accessible, and in which the relevant genes may be heterologously expressed.

1.5   Improving light utilization by truncating antenna size

As discussed above, moderation of the changes in light intensity to which cells that grow in a well-mixed photobioreactor are exposed, may optimize their photosynthetic efficiency. A promising approach to achieve this is to reduce their antenna size in such a way that it keeps the PSII/PSI excitation ratio in balance. This brings the advantages that: (i) Cells will be less prone to quench excitation energy non-productively and (ii) make the contents of the photobioreactor more transparent. The latter aspect will make light gradients in the reactor shallower and therefore put less stringent demands on cellular adaptation mechanisms to changes in light intensity, and may simplify the engineering of photobioreactors in which cells can carry out photosynthesis with high efficiency.

Though initially it turned out to be difficult to generate truly useful mutants with a truncated light-harvesting antenna (Tla), indeed there are now examples of mutants in which a significantly reduced antenna size leads to a higher biomass yield in dense cultures. In green algae, random mutagenesis has led to the identification of various mutants with reduced antenna size (102-104), from which rationally designed mutants were derived (105-107). One particular Tla mutant that has extensively been studied is Tla1 in the green alga *Chlamydomonas reinhardtii*. It was originally identified after random mutagenesis, but has also been used in knockdown and overexpression experiments (108). The *tla1* gene is thought to be a regulator of chlorophyll antenna size as its knockdown significantly reduces the antenna size of both photosystems and chlorophyll *b* synthesis. Furthermore, its overexpression causes a slight increase in antenna size and chlorophyll *b* synthesis. Other, related, mutants like Tla2 and Tla3 have a similar phenotype in which the peripheral antenna complexes are strongly reduced in abundance, as well as the PSI and PSII content (103, 104). The genes *tla2* and *tla3* are postulated to

encode chaperone proteins CpSRP43 and CpFTSY, which assist translocation of light-harvesting complexes to the thylakoid membrane. In their absence, the membrane proteins aggregate and are subsequently degraded, possibly leading to feedback inhibition in light-harvesting protein and chlorophyll synthesis, thereby also indirectly causing the reduction of PSI and PSII content observed in these mutants. All Tla mutants display a reduction of the number of auxiliary chlorophyll-containing proteins, rather than in the number of chlorophyll molecules associated with the core photosystem proteins, which contain only a fraction of the total chlorophyll content of the cell. This is similar to the high-light phenotype (109, 110).
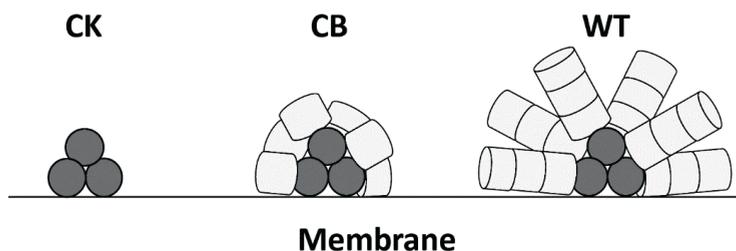

Fig. 1.2. Structure of the phycobilisome (PBS) of *Synechocystis* sp. PCC6803 (WT) and mutants (CK and CB). The PBS consists of a tri-cylindrical core, from which radiate six rods composed of three phycocyanin (PC) hexamers. In the CB mutant, only one layer of PC hexamers remains and no PC remains in the CK mutant. The PAL mutant lacks all components of the PBS. Adapted from Tian *et al*. (2012).

However, these auxiliary complexes have other functions, beyond light harvesting, making a complete knockout undesirable (48, 107). In both green algae and in cyanobacteria, antenna mutants are also shown to have an extensive impact on cell morphology, particularly on the thylakoid membranes (42, 108, 111). A challenge for the future is to find mutants that selectively show a truncation of the fixed antennae, rather than of the mobile ones, and hence a reduction of the number of light-harvesting proteins rather their absence, thereby preserving state transitions and thylakoid morphology. Whereas a change in morphology might not be so harmful under mass culture conditions, there is no escape from (quickly) changing light conditions in sunlight-driven reactors because of the inherent fluid dynamics and weather conditions. Such fluctuations in light intensity may be especially problematic for mutants impaired in light adaptation.

   Though the issue of light attenuation and PBR design clearly leaves much room for further improvement in solar-to-biomass conversion efficiency, electron transport rate is an area in which production in cyanobacteria can be optimized. In cyanobacteria the PSII/PSI ratio is much lower (1 : 5-6) than usually found in algae and plants (where this ratio is around 1). Interestingly, despite the stark difference in ratio, cyclic electron transport only "significantly contributes (80 %) to overall electron transport under mixotrophic growth conditions, compared to photoautotrophic growth (when it is below 10 %) (112)". Cyclic electron transport has been shown to be required for photosynthesis

to adjust the NADPH/ATP ratio, generated for assimilation of CO_2 and other processes in the cell (113), as well as for the detection of excess of light energy, through the acidification of the lumen (114, 115). In order to maximize electron transport towards NADPH, the ratio should be 1 : 1, assuming equal photon capture of both systems. Fortunately, the PSII/PSI ratio is tunable by changes in light quality/quantity and knocking out selected genes, among which there are genes that directly affect the antenna size of the PBS (112, 116, 117).

Similar to truncation mutants in green algae, in cyanobacteria selected truncation mutants do not affect the light-harvesting ability of the two photosystems themselves, but rather that of the external antenna (118, 119). Phycobilisome (PBS) deletion mutants in *Synechocystis* sp. PCC 6803 were shown to also affect the PSII/PSI ratio which is increased to plant levels (42, 112). Contrary to the Tla2 and Tla3 mutants in *C. reinhardtii*, the research of Bernát *et al.* (2009) shows that deletion of the PBS (i.e. the PAL mutant) causes the PSII/PSI ratio to increase significantly due to a reduction of the amount of PSI while simultaneously the amount of PSII increases. Linear electron transport in this mutant was increased up to 5.5-fold compared to wild type. Considering that the rate of linear electron flow in cyanobacteria appears to be low, this is a welcome additional effect, though the growth rate of the PAL mutant was slower than that of the corresponding wild type.

An antenna mutant which leaves the core structure of the PBS largely intact, but lacks phycocyanin (PC), called the "Olive" mutant, also shows an increased rate of linear electron transport, as well as a significantly faster growth rate (>2x) under the selected growth conditions (112). To date, few studies used cyanobacteria with a modified PBS antenna size in a (small scale) mass cultivation system and this yielded mixed results (120-122). The mutants used had either only one layer of PC attached to the core of the PBS (i.e. the CB mutant), just the allophycocyanin (APC) core of the PBS (CK and *Δcpc*) or no PBS at all (PAL). **Fig. 1.2** (adapted from (123)) schematically depicts the core, the rods and the PBS mutants. For additional details, the reader is referred to (124) and elsewhere in this book. All mutants did show higher productivity at high culture density, in agreement with predictions based on green algae research, but grew slower than the wild type. The fact that growth rates during exponential growth were lower compared to wild type, contrary to the "Olive" mutant discussed above (which is similar to CK), indicates that antenna mutants are not easily comparable and that multiple aspects of the selected growth conditions are likely to be important. The results of Collins *et al.* show that in the case of the CB mutant, significant amounts of PC were found in the cytosol, indicating that despite truncation of the PBS, significant overhead is created. Additionally, the morphology of the thylakoid membranes was disturbed in both the CK and PAL mutant, further suggesting that at this stage a clear-cut comparison to wild type is hard to make.

Nevertheless, algal Tla mutants have been shown to have increased productivity on a per Chl basis as a result of less wasteful dissipation of energy and increased light penetration into deeper layers of the culture (102, 107, 109, 111). The apparently

conflicting results point towards stringent requirements for the optimization of these two properties, i.e. antenna size and rate of linear electron transport, for actual improvements. These truncations can therefore only be an improvement for solar energy conversion if a strain complies with the following characteristics (31, 112):

- The absolute amount of PSII per cell or chloroplast is not reduced
- The functional PSII and PSI antenna size is smaller than in wild type
- The quantum yield of photosynthesis, i.e. the number of photons required per CO_2 fixed, is unaffected
- The light-saturated rate of photosynthesis, as measured in O_2 $[Chl]^{-1}$ s^{-1}, should be inversely proportional to the measured antenna size
- The absolute rate of linear electron transport is not decreased
- The phenotype does not create respiratory "drag" by useless synthesis of proteins

Additionally, it has been suggested by Formighieri *et al*. (2012) that respiration increases in low-chlorophyll mutants as they can grow at higher densities and thus the compensation point is reached at a higher light intensity. This would then require the light intensity to be significantly higher before a net gain is observed (**Fig. 1.1**). Because NPQ partly relies on pigments such as carotenoids that are also involved in light-harvesting, care must be taken to not hamper the photoprotective mechanism in an effort to reduce the antenna size (125).

In this chapter, we have summarized multiple ways in which photosynthetic organisms can be genetically adjusted to increase photosynthetic efficiency. In most cases this is akin to irreversible photo-acclimatization which leads to reduced antenna size, either via chlorophyll content or via PBS composition. Regulation of expression and assembly of these systems is still poorly understood, which has led to the identification of several ineffective mutants that superficially appear to have the desired traits, yet don't necessarily improve productivity in mass cultures. Therefore, it is both of practical and of fundamental value to further study these aspects in order to develop strategies to domesticate algae as we have done with crops over thousands of years (126). Fortunately, our tools have improved.

1.6   Large-scale culturing in 2D and 3D photobioreactors

The use of large-scale cyanobacterial photobioreactors for direct product formation has several advantages, not the least of which are: (i) the avoidance of the need to use arable land, (ii) its closed minerals cycle and (iii) its low water requirement (25, 61). This approach may already compete favorably with traditional agriculture as is apparent from the study of Ducat *et al*. (2012) on sucrose production by an engineered cyanobacterium, which led these authors to conclude that "Sucrose production in engineered *S. elongatus* compares favorably with sugarcane and other agricultural crops". An important aspect of this approach, which so far received relatively little attention, is the down-stream

processing for product recovery. Depending on the specific product selected for large-scale production, use can be made of selected physico-chemical properties like volatility and water miscibility (127, 128). As an alternative, two-stage approaches can be selected, like in Günther *et al.* (2012): Glycolate is produced by excessive photorespiration in green algae, which then leaks out into the external medium. In a second reactor/phase this product is subsequently converted into methane, which is then recovered (66).
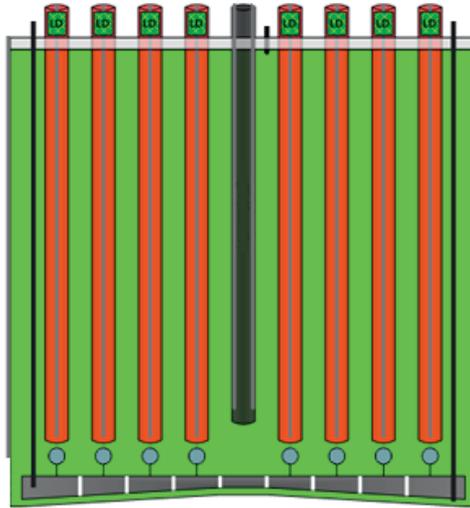

Fig. 1.3. A schematic design of a 3D photobioreactor viewed from the side. Indicated are LED (red) and sensor rods (dark grey) submerged in the culture volume and bubbling vents (light grey). Critical in its design is efficient illumination of the culture volume in order to minimize respiration and photoinhibition as well as efficient gassing for proper mixing, to provide CO_2 and to remove O_2.

Likewise, it would be possible to convert the lactic acid produced by cyanobacteria into an organic solvent through fermentation by lactic acid bacteria. Nevertheless, in a future, sustainable, society that puts efforts into closing the global carbon cycle, additional forms of application of cyanobacterial and algal mass cultures can be foreseen. In that society, a large part of the energy needs will (have to) be covered by the generation of renewable electricity, with the use of solar panels, wind turbines, and tidal and hydro-electric plants. As the daily rate of generation of renewable electricity will not be constant, new innovative ways will have to be developed to use 'peak electricity'. Because a closed carbon cycle also implies that fossil long-chain hydrocarbons will no longer be available, this provides an opportunity for engineered cyanobacteria and algae to show that they can use their natural capacity for CO_2 fixation as an alternative source.

In the slightly more distant future this may even lead to an entirely new form of agriculture: Current best photovoltaic cells have a solar power to electricity conversion efficiency of 44.5 %; one may therefore anticipate that within a decade, panels with 50 % efficiency will be available (129). Likewise, the efficiency of light emission by LEDs shows a trend of improvement that make one predict that in the same period 70 % efficiency may

be achievable (130). Therefore, the free energy of sunlight should be transportable to a 3-D (i.e. volumetric) photobioreactor with 35 % power efficiency. If we let these LEDs emit light with a wavelength of ~ 690 nm, textbook schemes suggest that the energy of this monochromatic light should be convertible to NADPH and ATP, and hence to Calvin cycle intermediates with approximately 30 % free energy conversion efficiency (131). Hence this type of photosynthesis should have an overall efficiency of 0.5 x 0.7 x 0.3 (x 100 %) = 10.5 %. This is considerably higher than any type of plant-photosynthesis and is not hampered by factors such insects, drought, hail, etc., factors that bring the actual crop yield significantly below the theoretical maximum. A schematic design of such a 3D PBR is shown in **Fig. 1.3**.