Prospects for the Development of Cyanobacterial Biofilm Bioreactors

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ABSTRACT

Growing energy requirements and sustainability concerns have drawn the attention to bioreactors, systems where microorganisms are exploited for the production of biofuels, therapeutics and commodity chemicals without fossil fuel combustion. Traditional bioreactors require carbohydrate feedstock to support manufacturing processes, limiting their sustainability and energy-efficiency. Therefore, bioreactors composed of photosynthetic organisms, referred to as photobioreactors, are being increasingly investigated for their ability of sunlight-driven reduction of carbon dioxide into added-value organic carbon chemicals. However, low-productivity of traditional suspension-based photobioreactors, insufficient delivery of CO_2 to the cells and poor robustness are limiting factors that currently challenge photobiomanufacturing. This dissertation identifies possible strategies to improve current photobioreactor design at a cellular level and proposes a biofilm-based design that combines continuous wastewater treatment and sunlight-driven direct biomanufacturing.

Introduction

The exponential increase in human population, expected to reach almost 10 billion by 2050 (United Nations Report, 2017), demands more energy-efficient and environment-neutral production systems to sustain this growth. Organic carbon resources have limited natural availability and, in addition, their oxidation to fuel human activity in the last 150 years contributed to a 25% increase of atmospheric CO_2 (Atsumi et al., 2009), liberated as a waste-product in energy transformation processes. This increase in emission ultimately poses concerns with regards to the recent unnatural warming observed around the globe. In order to obviate this issue, biomass-based feedstock has been described as a more sustainable input to limit CO_2 emissions and promote a carbon-neutral economy. Because carbon biomass is ultimately derived from photosynthetic carbon fixation of atmospheric inorganic carbon dioxide, its oxidation does not release any more CO_2 into the environment than the amount sequestered for biomass accumulation. Current industrial bioproduction of biofuels, commodity chemicals and therapeutics is largely dependent on carbohydrate feedstock fixed by autotrophic crop plants. However, the sustainability of carbohydrate feedstocks has been debated (Aro, 2016), mainly because of the competition between the food and chemical commodity markets. A possible solution to overcome this competition is the sunlight-driven direct conversion of inorganic carbon dioxide into added-value organic carbon compounds (Ducat, Way and Silver, 2011). Photosynthetic organisms are biological systems that naturally offer this alternative, by transforming solar into chemical energy. In particular, cyanobacteria have drawn attention for their ability to produce organic molecules from almost infinite and inexpensive resources, such as CO_2 from the atmosphere, wastewater and sunlight.

The Potential of Cyanobacteria

Cyanobacteria are ubiquitous prokaryotes that perform carbon-fixing, oxygenic photosynthesis. The oxygenic activity is a product of water photolysis, i.e. sunlight-driven water splitting, performed by Photosystem II (Cardona et al., 2012). The electrons extracted from water flow through electron transporters in the thylakoid membranes and drive the pumping of protons into the thylakoid lumen. Electrons and protons are thus separated, and this

electrochemical potential drives NADP+ reduction and ATP synthesis. As shown in figure 1, the chemical energy thus generated drive the reduction of carbon dioxide into several valuable organic carbon compounds.

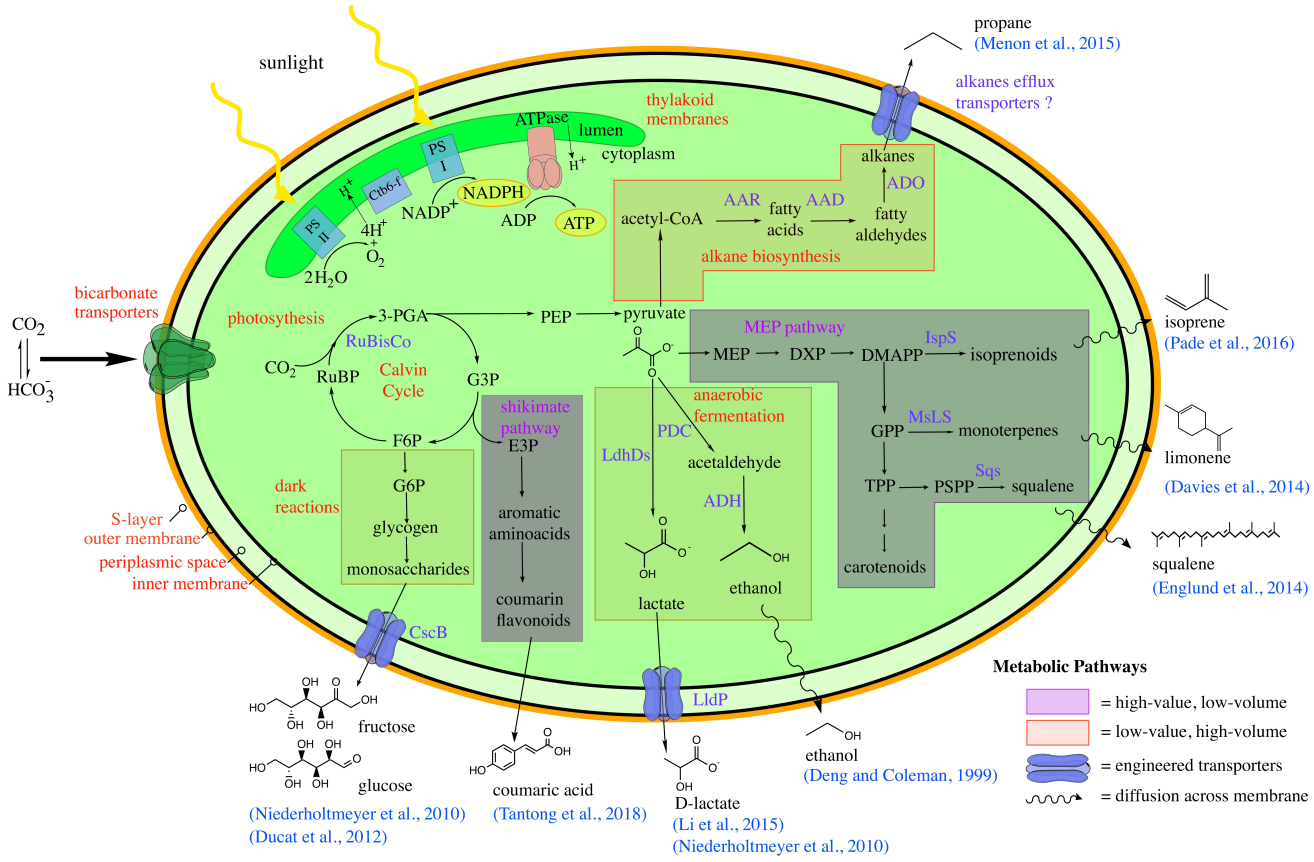

Figure 1. Light energy drives water photolysis by Photosystem II (PSII). Electron shuttling along the thylakoid membranes is facilitated by electron transporters (e.g. Cytochrome b6-f) and drives proton pumping into the thylakoid lumen. This charge separation drives NADP+ reduction by Photosystem I and ATP synthesis by ATP synthase. NADPH and ATP are then used for carbon fixation in the Calvin-Benson-Bassham cycle . Several pathways branch away from this organic carbon roundabout. Emphasis is on the reactions that lead to sugars and lactate export by the introduction of engineered sucrose (CscB) and lactate transporters (LldP). Alkanes have been synthesised in cyanobacteria but no alkane transporters have been identified. A possible solution could be the directed evolution of alkane efflux membrane transporters, as shown in *E. coli* by Foo and Leong (2013). High-value terpenoids and carotenoids (pigment, fragrances, and bioplastics industry) are produced through the Methylerythritol-4-Phosphate (MEP) pathway.
Key enzymes: D-lactate dehydrogenase (LdhDs), pyruvate decarboxylase (PDC), alcohol dehydrogenase (ADH), acyl-acyl carrier protein reductase (AAR), aldehyde decarbonylase (AAD), aldehyde deformylating oxygenase (ADO), isoprene synthase (IspS), M. spicata L-limonene synthase (MsLS), squalene synthase (Sqs)

Cyanobacteria are mostly found inhabiting mat-like structures, referred to as biofilms, characterised by a rich biodiversity. Their autotrophic lifestyle enables them to live in robust biofilm communities found in extreme environments all around the biosphere, from Arctic microbial mats (Roeselers et al., 2007), to Yellowstone Hot Springs (Klatt et al., 2011), on the leaf sheaths of rice plants (Venkatachalam et al., 2016) and on the surfaces of urban monuments (Cuzman et al., 2010). Being the first organisms to evolve Photosystem II, hence the machinery to perform oxygenic photosynthesis, they are thought to be responsible for the great oxygenation of the Earth circa 2.3 billion years ago (Soo et al., 2017). In light of the their carbon-fixing and oxygenic ability, cyanobacteria offer the

possibility to rationally correct the carbon cycle, now compromised by human oxidative activities.
Several features of cyanobacteria render them suitable for photobiomanufacturing. For instance, they can be cultivated in closed bioreactors which can be placed in desert lands and on sea surfaces, which do not compete with other agricultural crops for arable land usage (NASA, 2008). As a result they could minimise the competition between energy and food supply. Moreover, they are more genetically tractable than eukaryotic plants, which have thicker cell walls posing barriers for transformation, and larger genomes that increase the risk of off-target gene editing. Genetic manipulation with CRISPR-Cas9 systems has also been recently optimised for succinate expression in cyanobacteria (Li et al., 2016); and in addition, cheaper, more rapid and precise editing with CRISPR-Cpf1 systems has also been demonstrated (Ungerer and Pakrasi, 2016). Cyanobacteria have also evolved transporters to import nitrates and phosphates and use them as energy sources and building blocks for metabolic activities. This property allows to utilise them for filtering nutrient-rich wastewater in bioremediation processes (Kesaano and Sims, 2014).

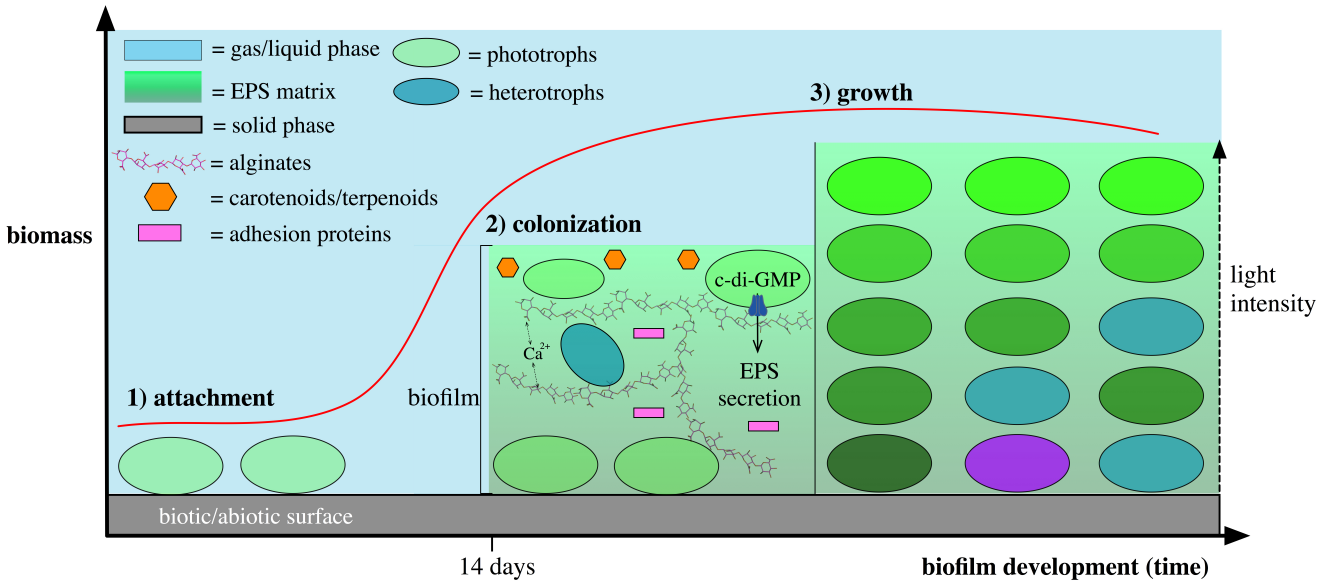

Figure 2. Natural biofilms follow a three step Monod's bacterial exponential growth kinetics (Gonzo et al., 2014). The sigmoidal shape of biomass growth was derived from fluid dynamics models (Clarelli et al., 2000) and chlorophyll absorption data as proxy for cell density. Biofilm development shows an initial limiting attachment phase, that can take up to 14 days (Cole et al., 2014). During the colonization phase new cells join the biofilm seeding and secrete molecular biofilm components. As the biofilm grows, cells organise themselves in stratified layers (Lewandowski and Beyenal, 2013). On the right it is shown the stratification of natural biofilms: on the surface, where light intensity is higher, phototrophic cyanobacteria are clustered, whereas heterotrophic and purple sulphur bacteria (shown in purple) are found at a higher density in the darker bottom layers (Roeselers et al., 2007).

Biofilms are spatially-structured communities of microbes, which are embedded in a self-secreted biomolecular scaffold composed of extracellular polymeric substances (EPS). EPSs are composed mainly of alginate polysaccharides (fig.2), and provide structural support, toxins removal and cell-cell adhesion for the organisms inhabiting the biofilm. Interestingly, EPSs also act as light propagation matrices, facilitating photon transmissions to the organisms at the bottom of phototrophic mats (Flemming and Wingender, 2010). In addition, DNA, RNA and other information-rich molecules found in the extracellular matrix are responsible for communication between the different communities in the biofilm. Protective layers of toxins, UV-light absorbing molecules, reserves of accumulated metal ions, and microchannels are also common features in biofilms. Flemming et al. (2012) reviewed with greater detail the fascinating molecular architectures of biofilm matrices. Naturally occurring biofilms contain many species, including

cyanobacteria, algae, diatoms, and fungi. Indeed, this biological diversity has been shown to be an important factor in maintaining ecosystem stability (Lindemann et al., 2016) and will be discussed as a possible strategy to increase the robustness of bioreactors. Metagenomics analysis using 16 rRNA primers revealed that in Arctic cyanobacterial mats, cyanobacteria species are found clustered at the surface of the biofilm with other oxygen-evolving phototrophs, while heterotrophic bacteria and yeasts thrive in the lower layers (Roeselers et al., 2007), as shown in figure 2. Given their incredible cooperativity and resilience, natural biofilms have inspired the design of synthetic consortia, which could extend biotechnological applications (Jagmann and Philipp, 2014).

The Challenges of Photobioreactors

Photobioreactors are vessels containing photosynthetic microbes performing biochemical reactions. These platforms have been traditionally employed for wastewater treatment or CO_2 conversion into added-value compounds. Photobioreactors for wastewater treatment are widely adopted and are commercially available in mainly four designs: open pond, vertical flat panel, tank reactors and biofilm (Heinemann, 2016). Light-driven carbon conversion platforms usually consist of tanks filled with suspended cells in nutrient-rich media. The need to stir the cultures to deliver feedstock, as well as to aerate and expose all cells to light in such floating-cell reactors, results in energy-inefficient production. In particular, the supply of high concentration of CO_2 to support fermentative pathways is the biggest obstacle for scaling-up (Chisti et al., 2013). The valuable biomass needs to be collected through filtration, centrifugation or flocculation. However, these methods to concentrate biomass require an elevated-energy input and represent up to 20% of the capital cost of bioproduction (Genin et al., 2013). Furthermore, accumulation of overexpressed compounds has been shown to cause cytotoxicity and decreased biomass concentration in suspension-based bioreactor (Anfelt et al., 2013), leading to poor productivity and scalability.

Biofilm Photobioreactors

Biofilm cultivation systems are receiving attention for their potential role as energy-efficient alternatives for traditional suspension-based photobioreactors. For example, cyanobacterial biofilms have been demonstrated as energy-efficient designs to remove contaminants, such as nitrates and phosphates from wastewater (Rai et al., 2016). During the bioremediation process, carbon dioxide is also photosynthetically fixed into cyanobacterial biomass, which can then be harvested to extract valuable compounds, such as lipids and phycobiliproteins. Biomass harvesting in biofilm reactors is easier than in suspension-based designs, as cells can just be scraped off the photobioreactor surface (Gross et al., 2015). Although the biofilm design has been mainly discussed for its application in wastewater treatment (Miranda et al., 2017), direct CO_2 conversion into organic compounds could as well benefit from biofilm designs, compared to suspension-based photobioreactors. As illustrated in figure 3, rather than using cells as feedstock for biomass accumulation, cyanobacteria could be used as catalysts for the direct biosynthesis of high-value products. This can enable to benefit from the advantages of biofilms measured for bioremediation processes, and at the same time overcome the costly step of biomass harvesting currently observed for photobiomanufacturing. Indeed, direct carbon rerouting in artificial biofilms have been described by (Fidler et al., 2007), who embedded a culture of *Haematococcus pluvialis* into a silica-based porous matrix and induced the continuous overexpression of the carotenoid pigment asthaxanthin. This research demonstrated that the immobilised culture and the continuous production strategy resulted in a 17%-fold increase in asthaxanthin extraction. However, the high cost of the silica matrix limits the scaling-up of this system. In addition, necessity to extract asthaxanthin with organic solvents decreases the viability of the microalgae and thus challenges a truly sustainable and continuous production system.

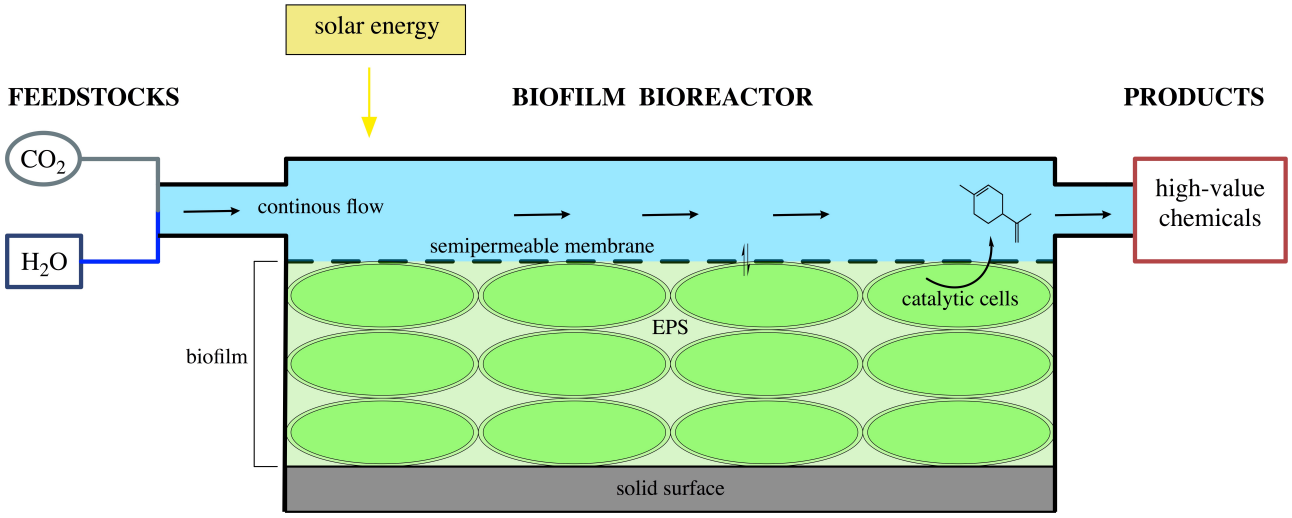

Figure 3. Proposed Biofilm Photobioreactor Design: sunlight, carbon dioxide and water are the sole input required for bioproduction. As Irving and Allen (2011) showed that presence of nutrient-rich wastewater enhances biofilm growth, bioproduction could be coupled to water bioremediation, to increase feedstock sustainability. The biofilm is separated from the nutrient-rich water flow by a semipermeable membrane, which allows metabolite exchange. Products are collected downstream, together with the filtered water if coupled to bioremediation platforms. The biofilm acts as a catalyst rather than biomass accumulation feedstock; here it is shown the photobioproduction of limonene, a monoterpene valuable in the fragrance industry. Its diffusion out of cell membranes facilitates the collection downstream.

Possible strategies to improve biofilm bioreactors

Solid Surface Binding

The pressure of the liquid phase flowing on the surface of the biofilm causes shear forces that can detach cells from the biofilm. The effects of flow rate on cyanobacterial biofilms has been measured in experiments on recently developed biofilm-based biophotovoltaic devices (BPVs) (Saar et al., 2018). These are small photobioreactors that use water as electron source and cyanobacterial electrogenic activity for power generation. The need to drive fluid flow in these systems to enable continuous electron source (water) delivery to the cells caused frictional losses, and hence decreased power generation. Indeed, increasing the flow rate increases mass transport and power generation until a threshold value, when pressure is too high and cells start to detach from the biofilm, and power generation decreases. Analogously, high flow rates are required to promote faster CO_2 delivery in current biofilm bioreactor and unproductive frictional loss is observed when flow rate is too high (Heinemann 2016). Therefore, immobilization of cells on to a solid surface can be adopted as strategy to prevent the shear-force cell loss, without sacrificing continous high flow rates to support nutrient delivery.

Experiments measuring the productivity of algal biofilms for wastewater treatment showed that biofilm growth kinetics depends on the material of the attachment surface. (Christenson and Sims, 2012). Genin et al. (2013) performed measurements to improve material selection for the design of algal biofilm bioreactors and demonstrated that cellulose acetate has the shortest colonization time compared with other low-polar surface energy surfaces. Therefore, the hydrophobic effect between the biofilm and the solid surface, rather than electrostatic interactions, is attributed to stronger attachment of the cells onto surfaces. Hydrophobic surfaces such as cellulose acetate and polycarbonate are thus preferred for the design of the bottom surface of the bioreactor scaffold. Cell immobilisation is a widely researched topic for its applications in biosensors, food technology and vaccine production (Samuelson et al., 2002). Attachment of organisms to solid matrices with antibodies provides the most specific and strong

interactions. However, scaling up with antibody-antigen immobilization strategies interactions is limited by the high cost of antibodies. An alternative developed by Lethiö et al. (2001) uses fungal surface-displayed cellulose-binding-domains to immobilise *Staphylococcus carnosus* cells onto cellulose fibres. This strategy is particularly promising as cellulose-binding-domains are widely available and usually composed of short sequences, hence do not add significant genetic/metabolic burden on the cells. On the other side, cellulose is a great candidate as surface material, which provides strong hydrophobic interactions between cells and solid surfaces, as demonstrated by Genin et al. (2013). In addition, its low-cost and biodegradability further enhance bioreactor scalability and sustainability. Thus, if the cells are engineered to express a surface-displayed cellulose binding protein, this could act as an anchor for cellulose surface immobilization. However, the limitation is that cyanobacteria are unique organisms possessing three membranes: outer, plasma and thylakoid membranes (Pisareva et al., 2011). Consequently, expressed proteins have to cross these phospholipidic barriers to anchor on the outermost surface. In addition, outer cell-wall components such as lipopolysaccharides may physically occlude the surface-displayer proteins.

Ferri et al. (2015) described for the first time an efficient method for the display of recombinant proteins on the outer surface of cyanobacteria. They introduced the autotransporter protein Ag43 (hereafter Ag43) derived from *E. coli*, under a light-inducible promoter in *Synechocystis*. Ag43 is found on the outer surface of *E. coli* and is also involved in biofilm formation and cell-cell interaction. The beta domain of Ag43 encodes all the necessary information for translocation into the outer membrane. They changed the N-terminal translocation sequence, that is specific for *E. coli*, and optimised it for translocation into *Synechocystis sp.* PCC 6803. The new secretion sequon was derived from the highly expressed cyanobacterial outer membrane porin SomA, identified by proteomics studies (Zhang et al., 2009). Ferri et al. (2015) successfully expressed the protein with SomA-derived secretion signal and demonstrated its display on the outer membrane. However, it did not promote biofilm formation as expected from its biofilm-forming functions in *E. coli*, probably due to occlusion of the protein by cell wall extracellular components.

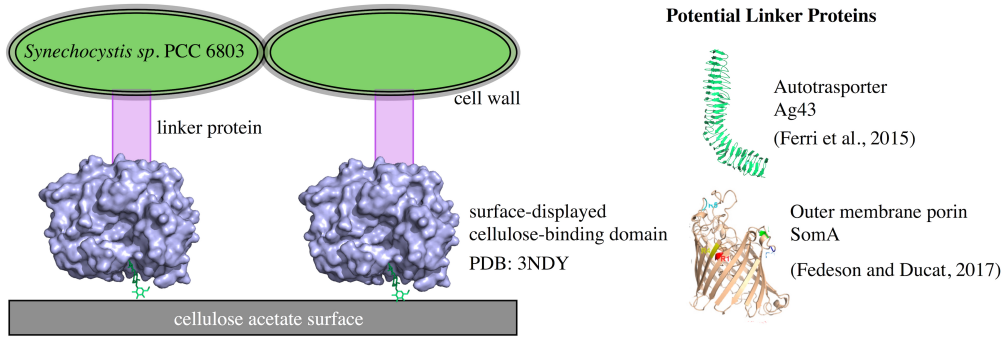

Figure 4. Attachment of biofilm to cellulose acetate can be achieved by surface-display of cellulose-binding domains (CBDs). CBDs are small domains derived from cellulolytic organisms, such as the CBD from *Clostridium cellulovorans* shown here bound to crystalline cellulose (in green). Linker proteins that have successfully demonstrated to display epitopes on the outermost surface of *Synechoscystis sp.* PCC 6803 are shown on the right. Therefore immobilization can be mediated by expression of a CBD-linker fusion protein.

Recently, the same SomA porin has been itself identified as a potential target for surface-display of epitopes of interest (Fedeson and Ducat, 2017). The group expressed a modified version of SomA porin and showed that epitopes exposed by fusion to SomA can mediate physical binding with magnetic beads and abiotic surfaces, useful to concentrate the cells as an alternative to traditional energy-expensive harvesting. In addition, they showed that deletion of the lipopolysaccharides biosynthesis machinery resulted in improved accessibility of the exposed epitopes. This research suggests that for the design of a biofilm bioreactor, the displayed proteins can be functionalised to bind the solid surface rather than magnetic beads, for example by exposing the cellulose-binding domain cited above.

Enhancing Biofilm Growth

While the ecology of phototrophic biofilms is well-characterised, the biochemical processes and genetic basis underlying phototrophic biofilm formation are still not fully illuminated. This understanding is required to induce biofilm development in artificial biofilm bioreactors and increase cell density. Biofilm growth is dependent on several factors, such as nutrient availability, nutrient diffusion, temperature and pH (Kernan et al., 2015).
As expected by the EPS composition, the export of polysaccharides has been demonstrated crucial for aggregation of *Synechocystis sp.* PCC 6803 and adherence to glass tubes (Fisher et al., 2013).
Later, it was found that the planktonic cyanobacterium *Synechococcus* inhibits biofilm formation via a self-suppression mechanism. Planktonic cells secrete signalling molecules that inhibit biofilm formation in the surrounding cells, causing cell detachment and dispersion (Schatz at al. 2013).

Recently, 4 small secreted peptides, Ebf1-4, have been identified as fundamental for biofilm formation in *Synechococcus* (Parnasa et al. 2016). As shown in figure 5, deletion of the genes ebf1-4 completely abolishes biofilm formation. In addition, it has been found through transcriptomics analysis that a cysteine peptidase (PteB) is responsible for secretion and maturation of the biofilm-promoting peptides (Ebf1-4). Therefore, they hypothesised that wild type, planktonic *Synechococcus* uses a secretion system to deposit biofilm inhibitors in the extracellular matrix, which consequently represses transcription of ebf1-4 and pteB. Although the nature of the extracellular biofilm-inhibitor molecule is yet unknown, biofilm-forming cells, useful in biofilm bioreactors, could be evolved via deletion of the self-suppression mechanism and/or overexpression of ebf1-4 and pteB. However, further experiments are needed to test this hypothesis.

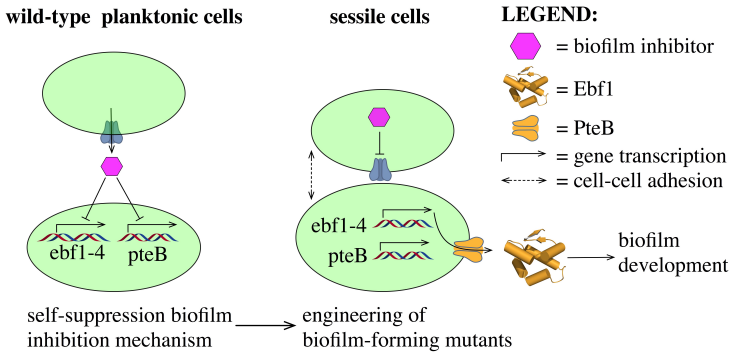

Figure 5. Parnasa et al. (2016) demonstrated the importance of 4 peptides (Ebf1-4) and PteB (peptidase transporter essential for biofilm) in biofilm formation. Wild type *S. elongatus* remains planktonic via transcriptional repression of ebf1-4 and pteB through a biofilm inhibitor signalling molecule (unknown). The structure of Ebf1 has not been solved yet. Shown here is the structure predicted by the *ab initio* protein structure QUARK server from Zhang's lab (Xu and Zhang, 2012) with the Ebf1 sequence (supplementary information, Parnasa et al. 2016).

Agostoni et al. (2016) demonstrated the involvement of the intracellular second messenger cyclic dimeric GMP (hereafter c-di-GMP) in biofilm development. They showed that by transforming the unicellular *Synechocystis sp.* PCC6803 with the c-di-GMP producing enzyme, diguanylate cyclase, cells switch from planktonic to sessile phenotypes. On the contrary, cells transformed with the c-di-GMP degrading phosphodiesterase (PDE) remained unicellular and planktonic in liquid cultures. Although the pathway transducing the signal from c-di-CMP to biofilm outputs are still poorly understood, these results suggest that biofilm-forming phenotypes can be modulated by overexpression of c-di-GMP. Recently the photostable rhaBAD promoter has been optimised for *Synechocystis sp*. PCC 6803 expression, and has been shown to work efficiently and predictably (Kelly et al. 2018). Therefore, biofilm development could be induced by rhamnose addition to *Synechocystis* strains containing diguanylate cyclase constructs downstream of rhaBAD promoters.

Modular and Heterogeneous Consortia

The term biofilm is used to describe a heterogeneous consortium of microorganisms. Indeed, in natural ecosystems, biofilms are characterised by complex microbiomes. Savage et al. (2007) theoretically demonstrated the role of biodiversity in increasing the robustness and efficiency of an ecosystem. Different species in the same ecosystem, such as a biofilm, provide more efficient resource utilisation and more resistance to perturbation through niche differentiation, e.i. the use of different resources by competing species in the same ecosystem. Experimental evidence also suggests that stratification of cyanobacterial strains with different light-harvesting capacities provides more light harvesting distribution in the consortium through niche differentiation (Klatt et al., 2011). Niche partitioning has been shown not only for light harvesting but also for carbon metabolism, as some species are solely autotrophic, other heterotrophic and some can enjoy a photoheterotrophic lifestyle (ibidem).

Therefore, it appears reasonable that mimicking the heterogeneity of cyanobacterial mats found in nature can be a promising strategy in the design of resilient biofilm bioreactors. Indeed, synthetic biology experiments provided evidence for the advantage of heterogeneous cultures. Cyanobacteria have been engineered to secrete hydrophilic molecules, such as sugars and lactic acid, by the insertion of specific transporters in the cell wall (Niederholtmeyer et al., 2010; Ducat et al., 2012). This research paved the way for the use of cyanobacteria as producers of carbohydrate feedstock in mixed heterotrophic-phototrophic cultures (hereafter mixotrophic), that simulate the biodiversity of natural phototrophic mats.

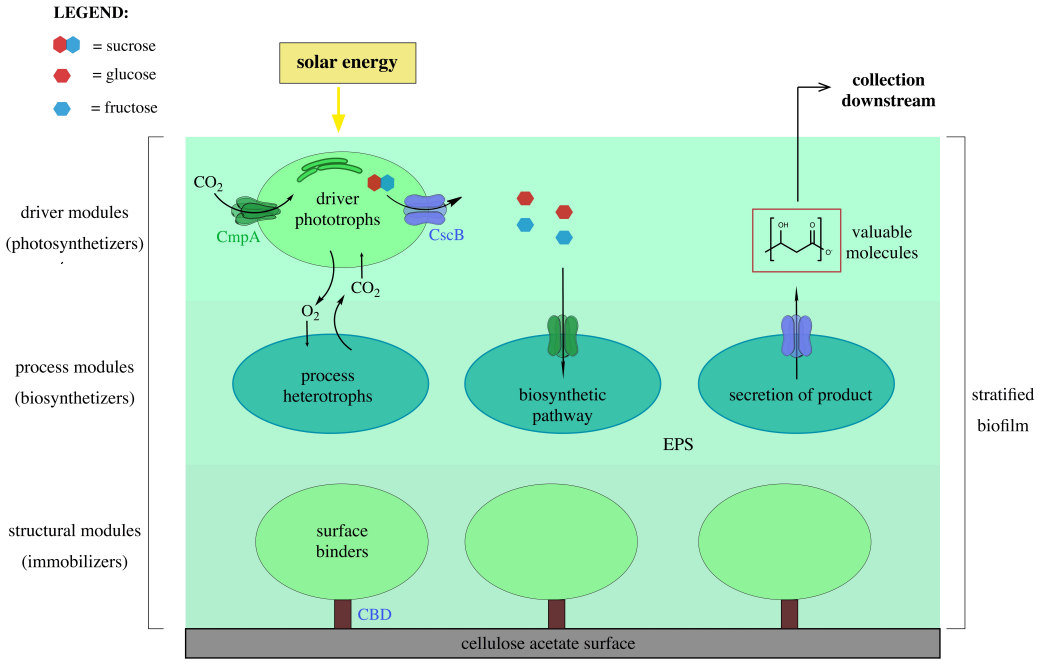

Figure 6. phototrophic organisms (driver modules) import carbon cioxide through bicarbonate transporters (CmpA), perform photosynthesis and secrete sugars (through sucrose transporters, CscB). Heterotrophs (process modules) convert the secreted sugars into added-value compounds. Heterotrophic aerobic respiration liberates CO2 which promotes driver modules photosynthesis, hence more sugar secretion. In exchange, cyanobacterial oxygenic photosynthesis promotes heterotrophic aerobic respiration. Shown on the right is the valuable bioplastic polyhydroxyalkanoate (PHA), as described by Löwe et al. (2016). On the left, it is illustrated the vertical stratification of specialist cells across the biofilm

As illustrated in figure 6, sugars secreted by cyanobacteria can be metabolised as energy source by heterotrophic organisms (i.e. process modules), engineered to express added-value compounds. Since heterotrophs such as *E. coli* and *S. cerevisiae* have been historically exploited as chemical factories, the repertoire of expressible molecules can be significantly expanded , while ultimately the source of energy derives from cyanobacterial photosynthesis.

Li et al., (2017) measured the productivity of a heterogenous biofilm system by co-culturing phototrophic cyanobacterial strains with heterotrophic yeasts. This mixotrophic strategy yielded 60% more biomass than axenic cultures, probably due to the mutualistic relationship between oxygenic cyanobacteria and aerobic heterotrophs (Cole et al., 2014).
Interestingly, cyanobacteria have been recently used for the production of carbohydrate feedstock to support fermentative biomanufacturing processes in "one-pot" reactions (Hays et al., 2017). For instance, it has been showed how *B. subtilis* can express amylase in synthetic light-driven co-cultures with the phototroph *S. elongatus*. A similar strategy has also been adopted by Löwe et al. (2017), who demonstrated heterotrophic production of the valuable biodegradable plastic polyhydroxyalkanoate (PHA), supported by cyanobacterial sucrose secretion.

These results demonstrate that engineered consortia can be programmed to express different bioproducts, by exploiting the interactions between heterotrophic and phototrophic modules in mixotrophic cultures. The same concept can be applied by modularly controlling the microbiome in a biofilm, which provides further stability to the system. The biofilm design could also facilitate the modular construction of the mixotrophic design. The resilience of natural biofilms is thought to be derived from their cooperative and modular nature. The division of metabolic labour by specialist cells in different layers can decrease the extensive burden experienced by single cells (Lindemann et al., 2016). Therefore, different specialist cells, such as sugar-secreting cyanobacteria, product-expressing heterotrophs and surface-binding cyanobacteria can reduce the individual metabolic burden if implemented in the same biofilm, as shown in figure 6. Recently, layers of cyanobacteria have been successfully "printed" using commercial ink-jet printers (Sawa et al., 2017), a similar strategy could be employed to print the different layers of specialist cells, required for more resilient stratified bioreactor designs.

Conclusion and Future Perspectives

In this dissertation, several features have been investigated as advantages for biofilm-based photobioreactors.
Biofilms can be readily immobilized, for example with cellulose-binding modules bound to a cellulose acetate surface. This can provide the stationary conditions required for continuous wastewater and bioproduction processes, and the means to deliver faster feedstock to support burdensome biosynthetic pathways.
Cyanobacterial strain engineering can help in the evolution of biofilm-forming phenotypes and controllable biofilms, for instance by induction of diguanylate cyclase. This has been proposed as a way to increase cyanobacterial density, hence catalytic power required to enhance productivity in photobiomanufacturing platforms.
Finally, it has been discussed how higher photobioreactor resilience can be achieved by integrating the heterotrophic-phototrophic interactions into a synthetic biofilm that mimics naturally occurring cyanobacterial mats.
For the future, several questions still need to be answered in order to catalyse the large-scale adoption of photobioproduction strategies. For example, how can a cyanobacterial bioreactor be designed and controlled so that by the concentration of feedstock the amount of product expressed can be predicted? A deeper system-level understanding of phototrophs and phototrophic biofilms is required to render photobioreactors predictable and reliable, especially when composed of complex multispecies architectures. These efforts could facilitate the transition towards a more sustainable carbon-neutral bioeconomy.

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