A systems-wide understanding of photosynthetic acclimation in algae and higher plants

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Abstract

The ability of phototrophs to colonise different environments relied on the robust protection against oxidative stress in phototrophs, a critical requirement for the successful evolutionary transition from water to land. Photosynthetic organisms have developed numerous strategies to adapt their photosynthetic apparatus to changing light conditions in order to optimise their photosynthetic yield, crucial for life to exist on Earth. Photosynthetic acclimation is an excellent example of the complexity of biological systems, in which highly diverse processes, ranging from electron excitation over protein protonation to enzymatic processes coupling ion gradients with biosynthetic activity interact on drastically different timescales, ranging from picoseconds to hours. An efficient functioning of the photosynthetic apparatus and its protection is paramount for efficient downstream processes including metabolism and growth. Modern experimental techniques can be successfully integrated with theoretical and mathematical models to promote our understanding of underlying mechanisms and principles. This Review aims to provide a retrospective analysis of multidisciplinary photosynthetic acclimation research carried out by members of the Marie Curie Initial Training Project “AcclimPhot”, placing the results in a wider context. The Review also highlights the applicability of photosynthetic organisms for industry, particularly with regards to the cultivation of microalgae. It aims to demonstrate how theoretical concepts can successfully complement experimental studies broadening our knowledge of common principles in acclimation processes in photosynthetic organisms, as well as in the field of applied microalgal biotechnology.

Key words:

biodiversity, European Training Network, mathematical modelling, non-photochemical quenching, photosynthetic optimisation, PhD training, acclimation, interdisciplinary training, microalgal cultivation

1. Introduction

Most life on Earth depends on oxygenic photosynthesis. Photosynthetic organisms such as algae, plants and mosses have the ability to convert solar energy and carbon dioxide (CO_2) into biomass and oxygen. Photosynthetic organisms can be found in highly fluctuating natural environments which exposes them to stressful conditions, particularly regarding light; while light is a necessary source of energy, an excess can cause severe damage (Niyogi and Truong, 2013; Finazzi and Minagawa, 2014). It was therefore essential for plants and algae to develop mechanisms to optimise energy capture, conversion, and dissipation efficiency under different light conditions via specific short- and long-term responses. Long-term responses imply ultrastructural changes in the cell and in most cases *de-novo* synthesis or breakdown of proteins, pigments, and redox cofactors. For instance, under limiting light conditions, photosynthetic cells tend to increase their light-harvesting capacity (Sukenik et al., 1987). This involves biosynthesis of new photosynthetic pigments as well as increasing expression of genes coding for light harvesting protein complexes (LHC in plants). Conversely, plants tend to decrease the number of LHC proteins in high light (Anderson et al., 1995), to avoid absorption of excess light. This leads to a feedback regulation, where the level of irradiance regulates the antenna size of photosystems on the long-term scale of several hours/days (Smith et al., 1990; Melis, 1991; Ballottari et al., 2007).

Short-term responses (timescale of seconds to minutes), which are the focus of this Review, are typically reversible and do not require extensive changes in either gene expression or in the structure of the photosynthetic apparatus. Under high light exposure, excessive photon flux leads to over-excitation of light-harvesting complexes, increasing the accumulation of chlorophyll triplets (Chl*). This triggers the production of potentially damaging reactive oxygen species (ROS; Krieger-Liszkay *et al.*, 2008). To reduce this risk, photosynthetic organisms must increase the thermal dissipation of the excess light. This is typically achieved via a photosynthesis regulation process known as non-photochemical quenching of chlorophyll fluorescence (NPQ), a key rapid-response strategy (Müller *et al.*, 2001).

Photosynthetic acclimation is an excellent example of the complexity of biological systems, where different molecular and submolecular processes interact on different time-scales. Consequently, a diversity of experimental approaches are employed to investigate and understand this process. The acceleration in the development of modern experimental

techniques, coupled with a rapid growth in systems biology approaches, has allowed for our knowledge of photosynthetic acclimation to broaden. In particular, theory and mathematical models are becoming an increasingly useful and utilised approach. Their power lies in providing general theoretical frameworks in which data can be interpreted in a far more sophisticated way than with intuition or purely statistical methods alone. Thus, mathematical models are essentially a simplified representation of the real system. This simplification allows for the identification of common fundamental principles and phenomena and often forms the basis for novel hypotheses. Moreover, they facilitate new predictions and allow for investigations, which are often experimentally challenging, if not impossible. Mathematical models can take many forms, depending on the research aim in question (Pfau *et al.*, 2011). In the context of photosynthesis, the range extends from detailed models of processes occurring within PSII on the timescale of picoseconds to nanoseconds (reviewed in Lazár and Jablonský, 2009) to the biochemically structured models of culture growth in bioreactors (Cornet *et al.*, 1998; Cogne *et al.*, 2011); and to models of photosynthetic evolution (Heckmann *et al.*, 2013).

This Review aims to provide an overview of recent insights on photosynthetic acclimation and consequences on microalgal cultivation achieved by members of the Marie Curie Initial Training Project "AccliPhot" employing a multidisciplinary approach, placing these findings in a wider context of current research activities.

2. Short term stress responses of the photosynthetic apparatus

Oxygen is a strong inhibitor of several stages of photosynthesis, including light harvesting, electron transport and CO_2 fixation. During evolution, phototrophs colonised different environments, with the transition from water to land being particularly challenging. Increased variability in temperature, water availability, light intensities, and UV radiation, made the robust protection against oxidative stress a critical requirement for the success of evolution.

Among these mechanisms, non-photochemical quenching (NPQ) is of particular relevance. NPQ refers to the experimentally observable reduction of fluorescence emitted by photosystem II under light exposure. Based on their different relaxation kinetics (Horton *et al.*, 1996), three main components of NPQ have been proposed. The fastest, energy-dependent component, *qE*, relaxes in approximately one minute. The second, *qT*, which

relaxes within minutes, has been proposed to correspond to state transitions (Joliot and Finazzi, 2010). Finally, the slowest component, *qI*, either represents photoinhibition or a particular form of energy quenching (Dall'Osto *et al.*, 2005). The exact contribution of each component varies between organisms and environmental conditions. As a general rule, *qE* is the major component in moderate to high light, whilst the development of state transitions is supposed to play a role in balancing excitation between the two photosystems, and is therefore prominent under low light, where photosynthesis is limited by absorption. Finally, photoinhibition becomes predominant when incident light exceeds the photosynthetic capacity.

2.1. Energy-dependent quenching, qE

Energy-dependent quenching, *qE*, derives its name from the fact that it directly depends on an excess of absorbed light energy, which leads to a rapid acidification of the luminal space (Horton *et al.*, 1996), immediately activating a signal for the feedback regulation of light harvesting (Niyogi and Truong, 2013). In higher plants, *qE* is the major component of NPQ. For decades, two major research questions have been the subject of investigation: i) what is the exact structural basis for the dissipation of excess absorbed light energy and; ii) what are the precise molecular mechanisms and signalling pathways triggering this? Whilst the focus of this Review is on the second question, it is apparent that both questions are fundamentally interconnected and that an understanding of the structural basis of *qE* forms the basis to understand the underlying mechanisms. Even though the precise location of the quenching sites and the structural and molecular basis for the energy dissipation are still not entirely understood (Holzwarth *et al.*, 2009; Johnson *et al.*, 2009; Zulfugarov *et al.*, 2010; Betterle *et al.*, 2010; Minagawa, 2013), recent advances have been made that clearly identify the xanthophyll pigments and the PsbS protein (subunit S of Photosystem II) as two major factors for *qE* in higher plants (Ruban, 2016; Sacharz *et al.*, 2017). Below, we summarise recent research results regarding the role of these two factors, and illustrate differences and common principles across different photosynthetic organisms.

2.1.1. Xanthophyll cycles

In response to high light, when the lumen pH drops below 6, specialised enzymes are activated and reversibly convert specific pigments (oxygenated carotenoids called xanthophylls) into

their de-epoxidised form in a process known as the xanthophyll cycle. Plant xanthophylls include lutein, neoxanthin, violaxanthin (Vx) and β-carotene. During NPQ, the violaxanthin de-epoxidase (VDE) converts violaxanthin into zeaxanthin (Zx) in two steps, which under low light is reversed by the enzyme zeaxanthin epoxidase (ZEP; Hager, 1967). This conversion occurs on a timescale of minutes and is purported to facilitate a conformational change in the LHCII, switching PSII into a quenched state (Nilkens *et al.*, 2010; Sacharz *et al.*, 2017).

The diatom equivalent of the xanthophyll cycle is known as the diadinoxanthin cycle (Lohr, 2011). It is comprised of diadinoxanthin (Dd) and diatoxanthin (Dt; Olaizola *et al.*, 1994), which, together with fucoxanthin and chlorophyll *a/c* form the main components of the LHC antennae in diatoms (Beer *et al.*, 2006). The diadinoxanthin cycle is a one-step de-epoxidation from Dd to Dt via the enzyme diadinoxanthin de-epoxidase (DDE, active at low pH). It was demonstrated that the photoprotective pigment diatoxanthin is linearly correlated with the extent of *qE* in diatoms (Goss *et al.*, 2006). In low light, the reverse reaction is catalysed by DTE (diatoxanthin epoxidase).

In a comparison of the genes involved in the xanthophyll cycle to those in the diadinoxanthin cycle, more copies of the genes putatively involved in de-epoxidase (VDE, VDL1, VDL2 , VDR) and epoxidase (ZEP1, ZEP2 and ZEP3) reactions have been found in diatom genomes (Coesel *et al.*, 2008). To further our fundamental understanding of qE, the involvement of these components in diatom photoprotection must be understood. This was achieved by the modulation of their expression levels by gene knock-down and gene knock-out approaches in the model organism *Phaeodactylum tricornutum*. Results suggest that not all the VDEs are directly involved in the xanthophyll cycle and that some of them are rather biosynthetic enzymes. Moreover, deregulating the relative content of the diadinoxanthin and violaxanthin pigment pools, indicates that the violaxanthin pool is not involved in the NPQ of diatoms and, furthermore, could be interfering with the photoprotective function of the diadinoxanthin pool (Stella, 2016).

2.1.2. Light-harvesting complex (LHC) protein superfamily and its variants

As demonstrated repeatedly, a key factor in inducing a quenching state in higher plants is the PsbS protein (Crouchman *et al.*, 2006; Sacharz *et al.*, 2017), which is rapidly protonated by a decreased lumenal pH. The precise nature of the proteins involved in quenching induction

that are protonated by a low lumen pH vary greatly between organisms and throughout evolution. However, a common principle appears to hold. In green algae, the light-harvesting complex stress-related (LHCSR) protein is required for quenching (Peers *et al.*, 2009); in the moss *Physcomitrella patens*, descendent from an evolutionary intermediate between algae and higher plants, both LHCSR and PsbS proteins are present and actively contribute to the activation of NPQ (Alboresi *et al.*, 2010); and in diatoms LHCX proteins play a similar role in the activation of *qE* (Bailleul *et al.*, 2010; Zhu and Green, 2010; Lepetit *et al.*, 2013)).

Genetic analysis in the model plant *Arabidopsis thaliana,* has pinpointed PsbS as an essential component of *qE* (Li *et al.*, 2000, 2004). PsbS acts as sensor of lumen pH through protonation of its acidic residues on the lumenal side of the thylakoid. This promotes the rearrangement of the LHCII-PSII supercomplex (Betterle *et al.*, 2009; Goral *et al.*, 2012) leading to *qE* activation. Moreover, PsbS is crucial for survival under fluctuating light conditions (Külheim *et al.*, 2002).

In contrast to PsbS in *A. thaliana*, LHCSR proteins are not constitutively present in the model green alga *Chlamydomonas reinhardtii,* but require high light exposure (Tokutsu and Minagawa, 2013; Petroutsos *et al.*, 2016), active photosynthetic electron flow (Maruyama and Tokutsu, 2014), and a calcium (Ca^{2+}) binding protein (CAS) and Ca^{2+} sensing signals (Petroutsos *et al.*, 2011) to be accumulated in the thylakoids. In *C. reinhardtii*, two LHCSR proteins actively participating in NPQ are encoded in the genome (LHCSR1 and LHCSR3) (Peers *et al.*, 2009; Tokutsu and Minagawa, 2013). The two isoforms possess similar promoter regions followed by an almost identical polypeptide sequence (Maruyama and Tokutsu, 2014). In contrast to PsbS, which has four transmembrane helices and does not bind pigments, LHCSR shares the typical three helix protein motif as well as the pigment binding capacity of LHCII proteins (Bonente *et al.*, 2011; Fan *et al.*, 2015). Moreover, LHCSR3 binds pigments such as chlorophyll *a*/*b*, lutein, violaxanthin, and zeaxanthin (Bonente *et al.*, 2011), which presumably act as a quencher (Tokutsu and Minagawa, 2013). Like PsbS, the protein LHCSR3 also acts as a sensor for luminal acidification, with several residues (aspartate and glutamate) being essential for NPQ induction (Ballottari *et al.*, 2016).

Novel insights into the regulation of photoprotection mediated by both perception of light colour and metabolism in *C. reinhardtii* were recently obtained (Petroutsos *et al.*, 2016) and a molecular link between photoreception, photosynthesis, and photoprotection identified. The

results showed that *C. reinhardtii* is able to detect changes in light wavelength using its photoreceptors, and this also induces photoprotection via the regulation of LHCSR3 (Petroutsos *et al.*, 2016). Moreover, besides light, downstream metabolism can affect the NPQ capacity of *C. reinhardtii* through negative feedback of LHCSR3 accumulation in the thylakoids (Polukhina *et al.*, 2016). These results comprehensively underline how the different processes linked to photosynthesis (light absorption, dissipative electron flow and carbon assimilation for metabolism) are tightly interconnected to allow for the successful acclimation of microalgae to their environment.

LHCSRs are absent in higher plants, but can be found in mosses (*Physcomitrella patens*, LHCSR1/LHCSR2). Organisation of thylakoid membranes is very similar in algae, mosses, and plants, suggesting that LHCSR could possibly be functional if inserted *in planta*. Recent studies show that LHCSR1 from *P. patens* can be over-expressed in *Nicotiana benthamiana* and *Nicotiana tabacum* leading to the accumulation of the protein *in vivo* (Pinnola *et al.*, 2015); however the role of LHCSR in NPQ and which co-factors are required to obtain a fully functional protein in an heterologous expression system remained unclear. By employing a reverse genetic approach using the *npq4* mutant of *A. thaliana*, which lacks PsbS and is thus unable to perform NPQ, as the host for the expression of the full coding sequence of LHCSR1 from *P. patens*, LHCSR1 was successfully expressed as a mature protein in the thylakoid membranes of *A. thaliana npq4*, which could partially overcome the inability of the *npq4* mutant to perform NPQ. When expressed *in planta,* LHCSR1 retains its major structural and functional characteristics such as its ability to bind pigments. Its direct dependence on zeaxanthin (Pinnola *et al.*, 2013) was shown by *in vivo* insertion of LHCSR1 in the *A. thaliana npq1npq4,* a mutant deficient of zeaxanthin and PsbS, generating transgenic plants that stably express LHCSR1 and yet were completely unable to perform NPQ.

Diatoms can reach higher NPQ levels when compared to land plants and green algae (Ruban *et al.*, 2004; Finazzi and Minagawa, 2014; Giovagnetti and Ruban, 2017) which may contribute to their ability to dominate phytoplankton communities in turbulent water environments (Smetacek, 1999). Studies of the molecular mechanisms of light acclimation in the diatom *Phaeodactylum tricornutum* showed that the LHCX1, a member of the light-harvesting protein family, contributes to the dissipation of excess light energy through NPQ (Bailleul *et al.*, 2010). However, LHCX1 is only one member of the expanded LHCX family that diatoms

possess. By performing an *in silico* investigation of the diatom genomes, between 4 and 17 LHCXs in different species were found (Taddei *et al.*, 2016). In order to further dissect their involvement in excess light energy dissipation, an extended characterisation of the *P. tricornutum* LHCX gene family expression and photosynthetic physiology in cells exposed to different light and nutrient stress conditions was performed. It revealed that amongst the four isoforms identified in *P. tricornutum*, only LHCX1 is constitutively expressed. The other isoforms are either induced or repressed by specific treatments, including the LHCX4 which is the only isoform induced in the absence of light. It was also observed that the amount of the *LHCX4* mRNA rapidly decreases following a dark to light transition and that chloroplast-derived signals participate in inhibiting its expression. This poses novel intriguing questions on the role of this isoform in the regulation of chloroplast physiology.

The results reveal a complex regulatory landscape and the existence of multiple stress signalling pathways that tightly control the amount of each LHCX isoform in the cell. We conclude that the observed LHCX gene family expansion reflects a functional diversification of these proteins and may contribute to the regulation of the chloroplast physiology in highly variable ocean environments.

2.2. State transitions, qT

State transitions are another important component of NPQ that refer to the mechanisms of excitation energy redistribution between photosystems (Allen, 1992; Goldschmidt-Clermont and Bassi, 2015; Minagawa and Tokutsu, 2015). In plants and green algae, the physical segregation of PSII and PSI imposes the existence of different antenna systems, which excite the two photosystems independently. Thus, state transitions optimise the relative absorption capacity of PSs via redox regulation by reversible activation of specific proteins.

The reduced state of the plastoquinone (PQ) pool and cytochrome b6/f (cyt b6/f) complex triggers the activation of the protein kinase STN7 (State Transition 7; in algae, Stt7) that phosphorylates subunits of the light-harvesting complex of PSII, some of which can migrate laterally towards PSI (Rochaix *et al.*, 2012). Under conditions in which PSII is more strongly excited than PSI (which may occur due to the different absorption spectra of chl a/b – e.g. wavelengths around 460 nm are absorbed efficiently by chl b but hardly by chl a), antenna migrate from PSII to PSI, a process termed state 1 to state 2 transition (Bellafiore *et al.*, 2005).

This changes the relative cross-sections towards PSI, balancing the light excitation of both photosystems. The reverse reaction is driven by the protein phosphatase PPH1/TAP38 (Protein Phosphatase 1/Thylakoid Associated Phosphatase 38) that dephosphorylates the LHCII associated with PSI and allows for its reallocation to PSII, also referred to as state 2 to state 1 transition (Pribil *et al.*, 2010; Shapiguzov *et al.*, 2010). This mechanism is absent in diatoms (Owens and Wold, 1986), and present at moderate levels in plants (Niyogi, 1999). However, it represents a much larger component in the green algae *C. reinhardtii*, where it can reallocate a large fraction of its antenna between photosystems (Delosme *et al.*, 1996). Whilst state transitions in plants are attributed to optimise light absorption in low light, in *C. reinhardtii* this process also contributes to photoprotection in high light (Allorent *et al.*, 2013) and it is still debated whether it involves a different mechanism than the simple physical displacement of LHCII between the two photosystems (Nagy *et al.*, 2014; Ünlü *et al.*, 2014; Nawrocki *et al.*, 2016).

While the functions of the antagonistic kinases and phosphatases (STN7, STN8, PPH1/TAP38 and PBCP) have been thoroughly investigated in *A. thaliana*, in *C. reinhardtii* information regarding mutants other than *stt7*, which is incapable of phosphorylating antenna and is thus locked in state 1, was still missing (Fleischmann and Rochaix, 1999; Depège *et al.*, 2003). This heightened the need for the investigation of other kinase and phosphatase mutants. Preliminary analysis of an algal mutant deficient in PPH1 indicates that the substrate specificity of the algal phosphatase may be somewhat different from its *A. thaliana* ortholog. Similar studies showed *A. thaliana* to differ from monocots such as barley or maize, where phosphorylation of the minor LHCII antenna CP29 appears to play a role in the regulation of energy-dependent non-photochemical quenching (*qE*) (Betterle *et al.*, 2015).

2.3. Energy spillover as photoprotective mechanism

In red algae and cyanobacteria, the “traditional” mechanisms involved in NPQ are missing and therefore these organisms possess alternate systems to cope with changing environments. The structure of the thylakoid membranes is much simpler than in plants and green algae, and in particular there is no clear spatial segregation of PSI and PSII. Red algae and cyanobacteria possess specific stromal-exposed antenna proteins called phycobilisomes (PBSs). These allow for a direct transfer of absorbed energy from PSII to PSI in a process termed “energy spillover”. In red algae (Yokono *et al.*, 2011; Kowalczyk *et al.*, 2013) and cyanobacteria (Zhang *et al.*, 2007)

it has been shown that this process represents a major contribution to the reduction of chlorophyll fluorescence. Since this mechanism is completely unrelated to PsbS and xanthophyll-related *qE* quenching, and is triggered by a reduced PQ pool rather than by a low pH (Kowalczyk *et al.*, 2013), the molecular mechanisms underlying NPQ in cyanobacteria and red algae appear to differ significantly from plants and green algae. However, recent evidence points towards LHCII complexes in the thylakoid membranes of higher plants, which are neither associated with PSII nor PSI, that may perform a similar role and also facilitate energy spillover in plant chloroplasts (Tikkanen and Aro, 2014) both *in vivo* (Jajoo *et al.*, 2014; Grieco *et al.*, 2015) as well as in reconstituted thylakoids *in vitro* (Akhtar *et al.*, 2016).

In diatoms, both photosystems share similar antennas (FCPs, fucoxanthin chlorophyll a/c binding proteins), and data suggests that the two photosystems may contain specialised antenna pools (Veith *et al.*, 2009). Contrary to what is found in plants, the similarity between FCPs translates into a more homogeneous absorption spectrum of the two photosystems. Despite diatoms not performing state transitions in light (Owens and Wold, 1986), they have succeeded in optimising light utilisation achieving an efficient excitation energy balance at both limiting and saturating light conditions. The peculiar structure of their thylakoids, which is an intermediate between the unstructured one seen in cyanobacteria (and red algae) and the highly structured one observed in plants (and green algae), shows no clear segregation of PSI and PSII. However, the possible existence of energy spillover was never investigated. Using several complementary approaches (spectroscopy, biochemistry, electron microscopy with immunolabelling and 3-Dimensional reconstitution) a comprehensive 3-D map of the photosynthetic membranes and intracellular compartments was generated. This multidisciplinary study reveals how the external membrane systems (the envelope) are organised and operate for the transfer of compounds produced in other intracellular compartments (Flori *et al.*, 2016). It also illustrated how exchanges of ATP/NADPH between plastids and mitochondria and the involvement of mitochondrial respiration contribute to the optimisation of photosynthesis in diatoms (Bailleul *et al.*, 2015).

2.4. Photoinhibition

Photoinhibition as a result of prolonged over-excitation of the photosynthetic machinery contributes to the slowest component of NPQ. Photoinhibition mainly constitutes the degradation and disassembly of the core subunit of photosystem II (PsbA or D1 protein Barber

and Andersson, 1992; Aro *et al.*, 1993). Overall, the extent of photoinhibition is a direct balance between damaged PSII and its repair rate (Murata *et al.*, 2007). Despite the fast turnover of D1 proteins (Sundby *et al.*, 1993; Neidhardt *et al.*, 1998), high amounts of reactive oxygen species (ROS) can enhance D1 degradation (Murata *et al.*, 2007) leading to a decrease in photosynthetic quantum yield (Krause, 1988).

2.5. Identifying common design principles by mathematical modelling of short-term stress responses

The variability of the various mechanisms between different organisms not only illustrates the differences in the molecular characteristics of components involved, but also reveals a commonality of underlying principles. For example, despite all structural and regulatory differences of PsbS (plants) and LHCSR3 (green algae), both function as pH sensors and activate a quenched state. Likewise, the xanthophylls Vx (plants) and Dd (diatoms) are clearly different molecules, but both are enzymatically de-epoxidised to induce energy dissipation.

One of the strengths of mathematical models is that they can provide an abstracted description of a system allowing for the simulation of the dynamics without focusing on the exact molecular details but rather on the fundamental design principles. In the past decade a handful of new kinetic models have been published with the aim of increasing our understanding of underlying principles governing short-term acclimation mechanisms (Ebenhöh *et al.*, 2011; Zaks *et al.*, 2012, 2013; Matuszyńska and Ebenhoeh, 2015). Because all these models aim to explain the dynamics of the acclimation process, a suitable choice for the mathematical description is the use of ordinary differential equations (ODEs). ODEs have a long history of application to biological and physical processes, and have been used to describe a number of general laws of nature (Simmons, 1972) and clear advantages include their universality, the well-established theoretical background, and the highly efficient and widely accessible numerical and computational implementations available.

The ability to monitor regulatory acclimation mechanisms in a minimally invasive way by means of chlorophyll fluorescence measurements, allows for the existing models to simulate the dynamics of the fluorescence signal (Maxwell and Johnson, 2000; Stirbet *et al.*, 2014). Using these models as a reference and guidance, new models that are specifically tailored to support the experimental approaches within the “AccliPhot” project were constructed which

provide a consistent theoretical framework in which new findings can be interpreted and new insight is obtained.

The mathematical model of state transitions in *Chlamydomonas reinhardtii* (Ebenhöh *et al.*, 2014) realistically represents the dynamics induced by transfers from dark to light as well as upon changes from aerobic to anaerobic conditions in the dark. This provides a reliable platform to study short-term acclimation in green alga. To complement the model with the fast component of NPQ, a highly reduced model of NPQ for plants was developed (Matuszyńska *et al.*, 2016). With a set of only six differential equations, not only all the main features of the fluorescence dynamics under low, moderate, and high light intensity were captured, but the model could also be employed to quantify the contribution of *qE* components to short-term light-memory (Murchie *et al.*, 2009; Jahns and Holzwarth, 2012; Ruban *et al.*, 2012). Although the model was constructed for *Arabidopsis thaliana*, it was successfully adapted to the non-model organism *Epipremnum aureum*, demonstrating that a basic mechanism of short-term light memory is preserved across both species. Both models were used to create a modular, unifying framework describing common principles of key photoprotective mechanisms across species in general (Matuszyńska, 2016). The scheme of the model development is illustrated in Figure 1A.

Light signalling pathways are interlinked with other external stimuli such as variations in temperature. To investigate the heat shock response (HSR) in *C. reinhartii*, which is observed upon exposure to large temperature changes (Schroda *et al.*, 2015), a kinetic model based on the mechanisms that sense temperature variations by the accumulation of unfolded proteins was developed (Magni *et al.*, 2016). The HSR activates genes coding for heat shock proteins (HSP), which act as chaperones repairing the heat-induced damage. The system of ODEs describing the signalling network was reconstructed and calibrated from multiple experimental time-resolved data-sets available in the literature (e.g. Schmollinger *et al.*, 2013). We showed that the system can adapt to higher temperatures by shifting to a new steady state. The investigation of the response of *C. reinhartii* to a gradual change in temperature suggests that the number of misfolded proteins is considerably reduced when compared to a drastic temperature change such as those commonly applied in experiments.

3. Metabolism of photosynthetic organisms

3.1. Model predictions on the effect of light stress on metabolism

As mentioned, short-term acclimation processes mainly serve to protect the photosynthetic apparatus from damage of reactive oxygen species resulting from excess light, however, the overall performance is critically dependent on a functional metabolism. The energy-dissipating mechanisms discussed above normally ensure that energy and redox equivalents produced do not exceed the energy that can be consumed by metabolism. However, how can metabolic fluxes be adjusted if this regulation is no longer functional, such as when it is halted experimentally via *e.g.* a sudden drop of CO_2 concentration or in knock-out mutants that lack important mechanisms such as *qE*? This question can be addressed by genome scale metabolic models (GSMs) representing the entire metabolic capabilities of an organism. Such models belong to the class of structural (or stoichiometric) models which, in contrast to kinetic models, are defined in terms of the reaction stoichiometry and thermodynamics, and are designed to describe the topological characteristics of the system rather than its kinetic behaviour (Heinrich and Schuster, 1996). They are built based on all the enzymes encoded in its genome (Fell *et al.*, 2010). Suitable analytic techniques then allow the identification of potential metabolic behaviour under given environmental and genetic conditions (Thiele and Palsson, 2010). Analysis of structural models generally depends on the steady-state assumption, which states that the rate of consumption and production of internal metabolites remains balanced within the time frame under consideration (Heinrich and Schuster, 1996). This assumption leads to an equation, from which statements about the distribution of metabolic fluxes can be made. However, since this equation is underdetermined, a prediction of the fluxes is not possible without additional assumptions.

Many approaches, such as Flux Balance Analysis (FBA) (Varma and Palsson, 1993, 1994), overcome this problem by calculating a flux distribution that optimises a certain objective function under given constraints, which include limitations of individual flux values due to thermodynamic constraints, demand for biomass production, observed growth rates *etc*. The two most common objectives are either the maximisation of growth rate (Varma and Palsson, 1994) or minimisation of total flux (Holzhütter, 2006; Poolman *et al.*, 2009).

Genome-scale models of *A. thaliana*, *C. reinhardtii* and *P. tricornutum* were constructed from their respective BioCyc databases (Caspi *et al.*, 2015), which contain the biochemical reactions of organisms based on their genome sequences, and previously published models (Chang *et al.*, 2011; Cheung *et al.*, 2013; Hunt *et al.*, 2014). They were then manually curated to fill the gaps and to ensure conservation of mass and energy (Gevorgyan *et al.*, 2008; Poolman *et al.*, 2009), resulting in networks containing approximately 500 (*P. tricornutum*) and 2500 (*C. reinhardtii* and *A. thaliana*) reactions. Gap-filling (Satish-Kumar *et al.*, 2007; Christian *et al.*, 2009) is a necessary process, because gene annotation is far from perfect. In each of the resulting networks, around 50 reactions had to be added during the gap filling process. All three models were used to identify possible metabolic cycles acting as energy dissipation modes under supra-optimal light conditions. In all models the results suggested that photorespiratory reactions may play a constructive role, rather than being an unavoidable inefficiency. The results for *P. tricornutum* showed that glycolate can either be excreted or recycled within the system depending on environmental conditions and that there is a potential link between photorespiration and lipid synthesis in this organism (Figure 1B) (Singh *et al.*, 2015).

3.2. Mixotrophic growth

The evolutionary secondary endosymbiotic event between a photoautotrophic eukaryotic cell and a heterotrophic eukaryote (Gibbs, 1981) believed to be the origin of modern diatoms such as *P. tricornutum* has resulted in some unique features in their biochemistry when compared to other photosynthetic eukaryotes, particularly in terms of the subcellular localisation of enzymes and the presence of some enzymes more commonly found in prokaryotes. *P. tricornutum* possesses lipid biosynthesis pathways comparable to those present in higher plants, both of which contain eukaryotic and prokaryotic pathways (Hu *et al.*, 2008). However, how *P. tricornutum* channels fixed carbon towards the production of lipid molecules is still poorly understood. Generally, under optimal conditions, phototrophs use most of the energy derived from carbon fixation for growth and for the biosynthesis of carbohydrates (Melis, 2013). By contrast, under unfavourable growth conditions *P. tricornutum* ceases growth and initiates the accumulation of storage molecules such as lipids (Cheng and He, 2014). To find conditions which simultaneously increase the algal biomass and lipid production in *P. tricornutum*, novel strategies are needed.

Although successful examples of metabolic engineering such as the implementation of genome editing technology that disrupted the UDP-glucose pyrophosphorylase gene leading to a 45-fold increase of triacylglycerol accumulation in *P. tricornutum* (Daboussi *et al.*, 2014), obvious constraints for using genetically modified organisms in an industrial context exist.

In *C. reinhardtii* it is well established that optimal growth can be established by mixotrophic conditions, in which an additional carbon source is applied in the presence of light (Chen and Johns, 1996), which simultaneously increases lipid production (Moon *et al.*, 2013). Lipid production can be further increased if starch synthesis is inhibited (Li *et al.*, 2010). Also mixotrophic cultivation of diatoms including *P. tricornutum* has shown great promise (Cerón-García *et al.*, 2013) but the full potential of this approach has not yet been reached.

During periods of light, microalgae can both respire and perform photosynthesis simultaneously, the basis of which is the poorly understood chloroplast-mitochondria interaction. In diatoms, it was recently shown that the NADPH generated in the plastid is exported to the mitochondria to generate additional ATP. The ATP produced can then be transported to the chloroplast providing the extra energy needed for the carbon fixation (Bailleul *et al.*, 2015), demonstrating the close interaction between the two compartments. By implementing an interdisciplinary approach, the genome-scale model of *P. tricornutum* developed was used to calculate metabolic fluxes and aided in the experimental activities by testing the potential of new culture conditions *in silico* that predicted a simultaneous increase of biomass and lipid production (Singh *et al.*, 2015). In the model, an increase in the light intensity and the addition of sodium bicarbonate led to a significant increase in lipid production. Experiments were designed using these parameters, which resulted in an increase in lipid production and growth rate (Villanova *et al.*, unpublished). The addition of glycerol enhanced biomass production by a factor of two as compared to growth on medium lacking glycerol; approximately 9 million cells/mL when grown in the absence of glycerol to 18 million cells/mL in the presence of glycerol. The combination of theory and experiments allowed for the elucidation of the main pathways involved in mixotrophic growth and the identification of gene targets for possible future metabolic engineering of *P. tricornutum* to optimise the efficiency of mixotrophic cultivation approaches. Other limiting factors such as medium composition, light, pH, aeration/mixing, temperature, *etc.*, have to be taken into account (Merchant and Helmann, 2012) for a successful implementation of mixotrophy for industrial

exploitation. Efforts to optimise the medium composition by an "AccliPhot" industrial partner, Fermentalg (a company producing high-value bioactive compounds), led to the development of a novel medium that optimises growth by the addition of micronutrients that are limited in natural seawater (Villanova, 2016). The optimised growth conditions were tested in laboratory-scale 2L PBRs that possess a better system control (temperature, pH, light, aeration/mixing) comparing to open pond (Sheehan et al., 1998).

4. From bench to bank: scaling up microalgal cultivation for industry

In order to translate our novel understanding of short-term light acclimation and its effect on metabolism to industrial processes, optimised large-scale cultivation techniques are required. Considering the future potential of algal biotechnology, one fundamental research goal of the microalgal biotechnology field is to investigate scale-up approaches by understanding the performance of algal populations in bioreactors, increase lipid production by implementing mixotrophic growth conditions, and assess the extent to which the models developed for controlled laboratory conditions are applicable to outdoor, industry-scale cultivation. Some examples of cultivation scales can be found in Figure 2A-D. A substantial amount of research efforts are placed on *C. reinhardtii* and *P. tricornutum* due to the extensive knowledge on the behaviour, including photosynthetic mechanisms, of *C. reinhardtii* and because of the ability of *P. tricornutum* to synthesise a number of commercially-relevant molecules including lipids such as triacylglycerols (TAG) and polyunsaturated fatty acids (PUFA) (Kates and Volcani, 1966; Siron *et al.*, 1989; Rebolloso-Fuentes *et al.*, 2001; Fajardo *et al.*, 2007).

4.1. Bioreactors and engineering

To gain insight into the performance of algal populations in bioreactors a biochemically-based structured model for the autotrophic growth of *C. reinhardtii* in photobioreactors (PBRs) using knowledge of the detailed underlying metabolic network previously determined (Cogne *et al.*, 2011) was developed. The model is reduced to a minimal set of 7 reactions derived from metabolic investigations of light-limited growing cells in PBRs (Rügen *et al.*, 2012). Structuration of the model including a fully detailed description of cellular energetics leads to the formulation of only three kinetic equations, namely photon uptake rate and light-dependent kinetics for pigment synthesis and maintenance, thus setting the degree-of-freedom of the system to zero. The model involves the introduction of only 3 parameters that

are estimated by experimental data. The experimental approach included a wide range of experimental conditions: batch cultures at 100, 300, 500 and 700 µmol m^{-2} s^{-1} incident photon flux density as well as various steady-states at 200 and 600 µmol m^{-2} s^{-1}. The elaborated model was found to accurately represent the behaviour of *C. reinhardtii* cultures with a good predictability and robustness as illustrated in Figure 3A and 3B. Kinetic model analysis showed that increasing pigment content has a negative effect on population-level growth dynamics. Furthermore, measurements of oxygen uptake rate in the light showed that respiratory activity increases relative to the photosynthetic oxygen production rate. The increasing maintenance flow due to the existence of an increasing dark zone inside the PBR suggests concomitant oxidative and reductive processes.

4.2. Novel approaches to scaling up microalgal cultures

Whilst PBRs are closed systems ideal to keep monocultures (Grima and Fernández, 1999), which is especially desired if the final product is a bioactive molecule for human consumption (Mata *et al.*, 2010), operational costs are high, preventing industrial-scale production of low- or medium-value compounds. Other options include open raceway ponds, simple open-air cultivation systems that have been in use since the 1950s (Chisti, 2007). They are highly susceptible to contamination, and unless the desired species is a halophile or thermophile (Parmar *et al.*, 2011), it is hard to maintain monocultures. Irrespective of the cultivation method, the establishment of unwanted organisms is a serious obstacle for large-scale microalgae cultivation (Day *et al.*, 2012; Wang *et al.*, 2013). Despite intense research on microalgal culture upscaling, very little is known about the identity and characteristics of these invading organisms, responsible for microalgal culture 'crashes' which lead to loss of biomass, and therefore, loss of revenue.

Bacteria, which have co-existed with diatoms for more than 200 million years, form a crucial part of a complex ecosystem and have been shown to enhance the growth of diatoms (Bruckner *et al.*, 2011; Amin *et al.*, 2015). Increased understanding of the interactions could allow for the exploration of 'synthetic ecology' as a novel scaling up technique (Kazamia *et al.*, 2012).

To gain insight into the dynamics of the bacterial communities associated with diatoms, we translated the complexity of a natural system into a reproducible, systematic experimental

approach where the microbiome of batch-grown 5L non-axenic cultures of *P. tricornutum* were investigated using barcoded 16S-V6-Next-Generation-Sequencing. The results identified four major players within the microbiome and a network of putative interactions between *P. tricornutum* and each of the bacterial factions was proposed, thus providing a framework to understanding the dynamics of diatom-associated microbial communities. Species-specific co-culture experiments were carried out, and preliminary results show increased growth rates and maximal cell densities when *P. tricornutum* is co-cultured with representative members of the four identified families (Moejes, 2016; Moejes *et al.*, 2016).

The proposed network of putative interactions was translated into a set of ordinary differential equations which, together, constitute a computational dynamic model. The proposed mathematical model is able to capture the population dynamics and therefore represents a simple yet important proof of concept of the hypothesised community-level interactions. Further experimental measurements of biomass production rates and concentrations of metabolites exchanged within the community will allow the model to develop from qualitative to quantitative, providing a powerful and practical predictive tool for culture monitoring. The interdisciplinary analysis provides a framework to understanding the dynamics of diatom-associated microbial communities and represents a solid starting point for systematic investigation of organism interactions mediated by metabolite exchange (Moejes *et al.*, 2016). While at the current state, the model resembles a classical population dynamics model (Verhulst, 1838; Lotka, 1925; Volterra, 1926), a promising approach to combine FBA and kinetic models is by considering the steady state solution of FBA as input for a set of differential equations defining the evolution of metabolite concentrations. In such dynamic FBA (dFBA) (Mahadevan *et al.*, 2002), constraints on the fluxes change at each time step, based on defined reaction kinetics and on the FBA solution at the previous time step. To advance our understanding of population dynamics of bacterial communities associated with photosynthetic organisms, an integrated modelling framework was developed inspired by the dFBA modelling approach utilised by (Harcombe *et al.*, 2014) coupling the complexity of structural models with the simplicity of ODE. This modelling framework can now be used to consolidate our understanding of the mechanisms regulating symbiosis or produce new hypotheses to be experimentally tested.

5. Perspectives and Outlook

Collectively, the projects undertaken by the members of the "AccliPhot" consortium underline how by increasing our understanding on the different processes linked to photosynthesis (light absorption, dissipation, electron flow and carbon assimilation for metabolism) we are successfully unravelling the mysteries of photosynthetic acclimation. The complementary research on four model species (green alga *Chlamydomonas reinhardtii*, the diatom *Phaeodactylum tricornutum,* moss *Physcomytrella patens* and the higher plant *Arabidopsis thaliana*) opens completely novel perspectives on the evolution and diversification of different adaptation mechanisms in phototrophs. Providing novel support to theoretical studies, this information can feed into encompassing models of photoprotection, shedding light on unsolved evolutionary and functional questions of photosynthetic acclimation.

A unique feature of "AccliPhot" was the successful integration of theoretical approaches with experimental ones. Dynamic models were used to explain dynamic responses of photosynthesis, to confirm that our understanding of the underlying quenching mechanisms is basically correct, and to highlight common principles in evolutionarily distant species. Structural models were employed to fill knowledge gaps, explain physiological properties and to support synthetic biology approaches. Combining these approaches allowed construction of a computational framework, in which bacterial community dynamics associated with large-scale cultures can be investigated, thus paving the way towards the establishment of controlled synthetic communities. All these efforts demonstrate the value of interdisciplinary collaborations, by which biological problems are elucidated from various complementing aspects.

Furthermore, the project improved our knowledge of algal growth in photobioreactors as well as highlighted the need for advancement of scaling up approaches (i.e. mixotrophic growth, co-cultivation with other organisms such as bacteria) essential to optimising industrial-scale cultivation of microalgae. Continued work to understand population dynamics in PBR will aid in PBR design, e.g. to ensure maximal light absorption, a good gas transfer rate, efficient nutrient distribution and avoidance of dark zones. In conjunction with the novel mixotrophic growth conditions developed, this will pave way for optimised industry-scale algal cultivation in PBRs. We also show that applying laboratory and ecological data to create synthetic ecologies, in theory, has the potential to optimise scaling up techniques, particularly for open

raceway pond cultivation, which is a cheap large-scale technique but very susceptible to contamination, allowing for the production of low- or medium-value compounds to become an economically-viable option. Further research is required to explore the full potential of applied microbial ecosystem management for a sustainable bio-economy.

One of the fundamental goals of "AccliPhot" was to illustrate the importance of an interdisciplinary approach to scientific research, and we believe that this Review is a testament to the successful marriage of theoretical and experimental approaches. Although this multidisciplinary approach is not a novel idea, we have never encountered a comparable large-scale project, in which the numbers of theoretical and experimental scientists are as balanced as was the case in "AccliPhot". The working principle that every research question is addressed both by experimental and theoretical methods is reflected in the development of successful mathematical models which have assisted in experimental design, and where experimental data has facilitated the advancement of the models to predictive tools.

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8. Figure Legends

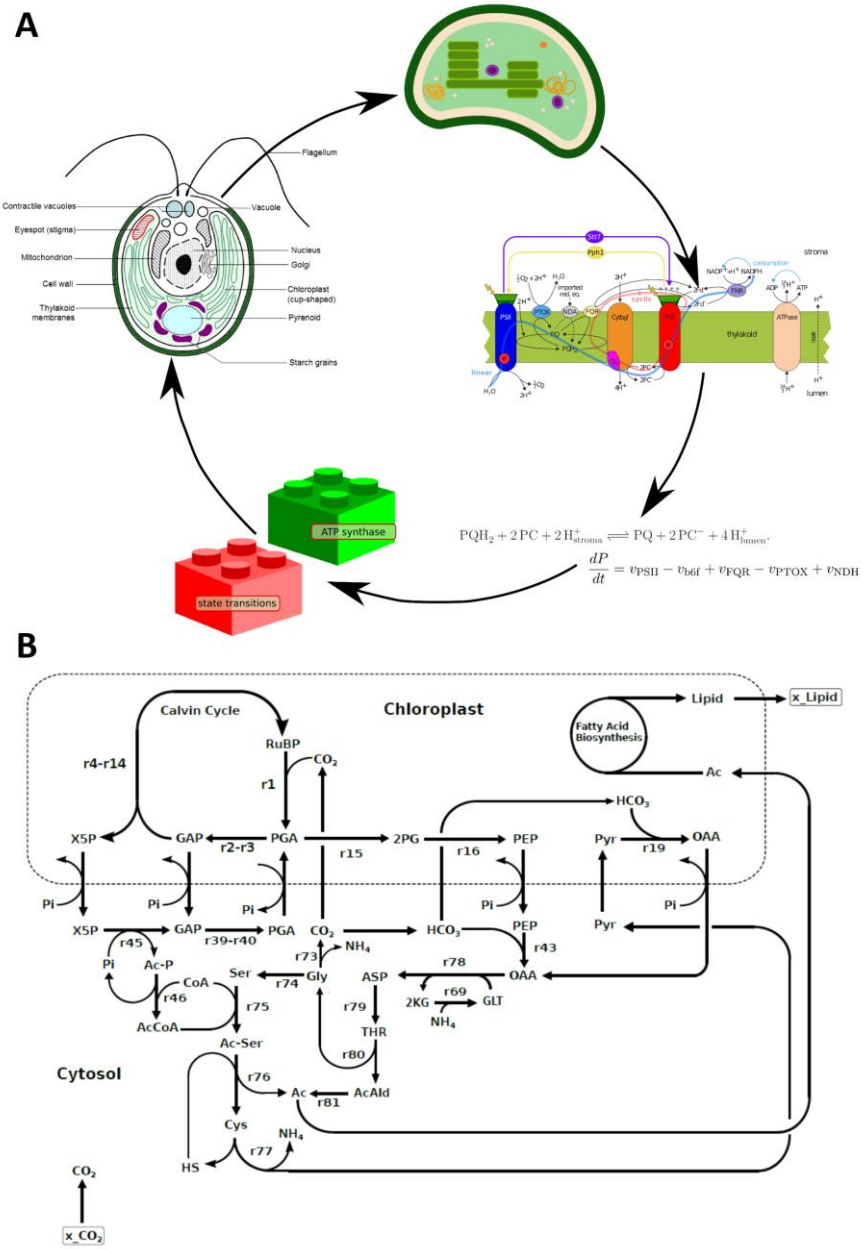


Figure 1: Schemes of two modelling approaches. (A) The scheme of the reduction process applied to developed kinetic models. The complexity of a model organism (here *Chlamydomonas reinhardtii*) was reduced to include only processes taking place in the chloroplast, precisely, the thylakoid membrane. Key biochemical reactions of the photosynthetic electron transport were translated into the mathematical terms, using ODEs. A set of reactions describing a specific process (from ATP formation through various NPQ mechanisms, like state transitions) were clustered together in a form of modules, that can be put together to reconstruct the model organism *in silico*. **(B)** The scheme of a Genome Scale Model reconstructed to perform Flux Balance Analysis in *Phaeodactylum tricornutum*. The

network of reactions exhibit change in flux in response to increase lipid demand. This model was used to identify reactions with co-related change in flux to change in lipid demand in phototrophic condition i.e. source of energy and inorganic carbon was light and CO2 respectively. External metabolites are distinguished from internal metabolites with prefix 'x '.

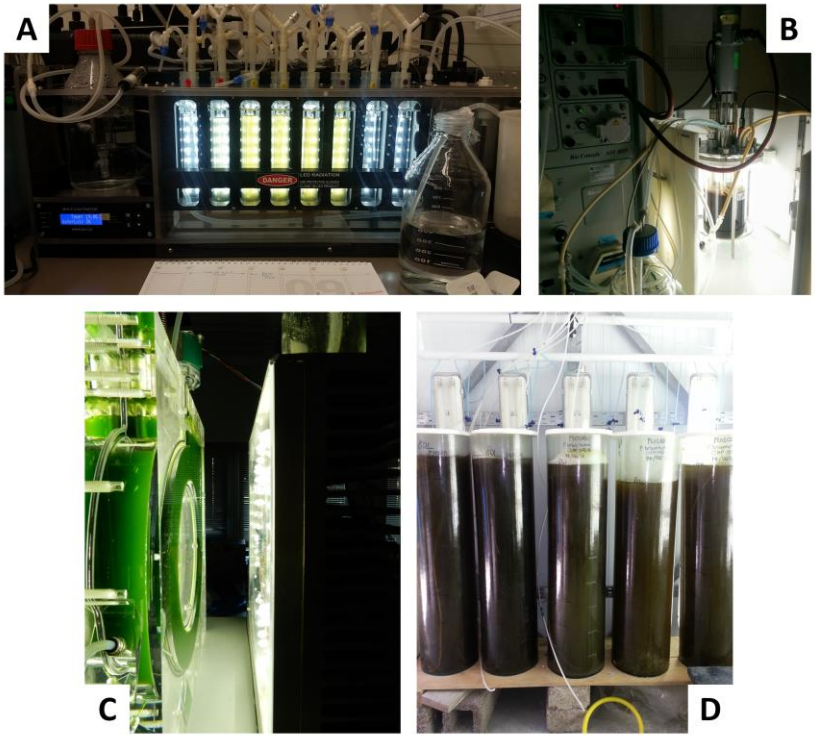
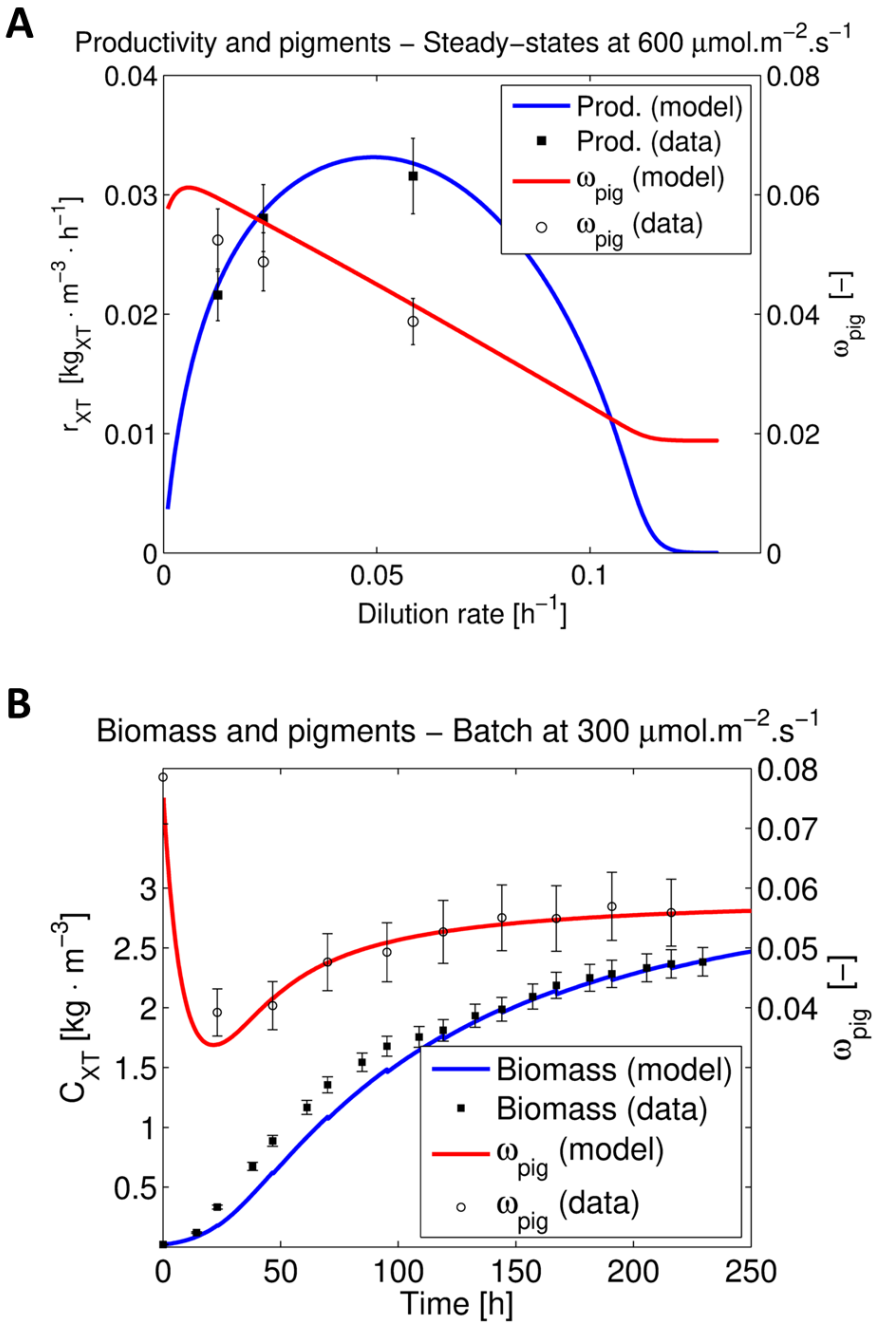


Figure 2: Different algal cultivation scales implemented. (A) A mulicultivator (80 mL) was used to do systematic investigations on the effect of the presence of bacteria on *Phaeodactylum tricornutum* growth (Moejes, 2016). **(B)** A 2L chemostat utilised to study the effect of mixotrophic growth on *P. tricornutum* (Villanova, 2016). **(C)** A 2L Torus photobioreactor implemented for kinetic growth analysis and modelling of *Chlamydomonas reinhardtii* at the population scale. **(D)** 80L vertical columns investigating the population-level response of scaling up *P. tricornutum* cultures (Moejes, 2016).



571 **Figure 3: Graphs illustrating how model accurately represented the behaviour of**
572 ***Chlamydomonas reinhardtii* cultures. (A)** Graph showing the biomass productivity and the
573 pigment mass fraction as a function of the dilution rate for steady-state cultures with an
574 incident photon flux density of 600 µmol m^{-2} s^{-1}. Full lines are model predictions, data points
575 are shown with error bars. **(B)** Graph showing the biomass concentration and the pigment
576 mass fraction as a function of time for a batch culture with an incident photon flux density of
577 300 µmol m^{-2} s^{-1}. Full lines are model predictions, data points are shown with error bars.