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# Improving distribution and absorption of light in microalgal photobioreactors through plasmon enhancement, entrapped cultivation and controlled motion

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## Abstract

Microalgae represent a promising source of renewable biomass for the production of biofuels and valuable chemicals. However, the development of high throughput microalgal cultivation methods and energy efficient biomass harvesting technology is necessary to improve the economic viability of large scale microalgal biomass production.

The issue of poor distribution and absorption of light is one of the hurdle that can be addressed to improve the productivity in microalgal cultivation systems. As it happens, microalgal photosynthetic activities have shown great dependence on the irradiance to which the microalgal cells are exposed. Quantitatively, microalgal growth increases with increasing light intensity until a saturation level beyond which the microalgal photosynthetic machinery can be subject to photodamage. Qualitatively, most microalgal species have exhibited a propensity for wavelengths in the blue and red regions of the visible electromagnetic spectrum whereas other wavelengths can induce photoinhibition. Further, a change in the incident light can lead to photoacclimation where the microalgal species activate the preferential synthesis of certain compounds or completely alter their metabolic activity.

Despite such importance of light for microalgal growth and biomass production, only a small fraction of microalgal cells receives an optimal irradiance in current microalgal cultivation systems (open and enclosed ponds). The remaining microalgal cells are found either in the over illuminated zones (e.g. top surface of open culture) of the cultivation systems where they are exposed to photoinhibition or in the poorly illuminated zones where their growth is limited. In this dissertation, this issue is addressed by developing a multi-fold approach to improve the distribution and absorption of light in microalgal photobioreactors.

First, a plasmonic film light filter technology is developed. By virtue of enhancing the irradiation of blue and red lights using silver nanospheres and gold nanorods, this technology can enhance microalgal biomass production by up to 50% and increase photosynthetic pigments production by up to 78%. A short light path capable Tris-Acetate-Phosphate-Pluronic (TAPP) microalgal cultivation and harvesting system is also developed. Adding to the interesting light manipulation features, this energy efficient microalgal cultivation and harvesting system that exploits the thermoreversible sol-gel transition properties of the copolymer pluronic can increase the harvesting rate of microalgal biomass via gravimetric sedimentation by a factor of ten. Further, the adhesion properties of microalgal cells on the surface of photobioreactors are studied as a way to control the impacts of biofouling on light penetration and light absorption in microalgal cultivation systems. Furthermore, the rheological properties of microalga *C. reinhardtii* broths are studied and the interesting properties of such complex fluids are used as tools to control the periodical motion of microalgal cells from dark sections to well illuminated sections of typical photobioreactors for enhanced microalgal growth.

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## DEDICATION

I dedicate this dissertation to my daughter to be born Briyendy Corrine, my wife Wondy-Neecodeliny and her family, my siblings Emerson, Tania, Roosevelt and Glaelle Francesca and to all my relatives and friends.

## IN MEMORIAM

Franckel Estime, Dinah Estime, Marie Ynorese Aristide & Adina Jerome.

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# Chapter I

# Introduction

## 1.1 Microalgae for sustainable biorefinery

The use of renewable energy has been reported since before the Christian Era with the use of vertical axis windmills to grind grain in Persia or the use of wind energy propelled boats in ancient Egypt [1]. However, the adverse impacts of fossil fuels energy on the environment and on human health and the raising awareness for global warming have specifically impelled the development of clean and renewable energy production systems over the past decades [2,3]. The list of alternative energy systems to replace fossil fuels is long and includes solar photovoltaics, wind energy, hydroelectricity, tidal energy, biofuels etc. [4] Nonetheless each of these alternatives fails to meet the increasing energy demands and some suggest that significantly reducing fossil fuels consumption will require the combination of different of the listed sustainable energy alternatives [5].

In search of alternatives to fossil fuels, interests have risen for microalgal biomass. Microalgae comprise of the wide range of microorganisms including cyanobacteria that contain chlorophyll a and a thallus not differentiated into roots, stem and leaves [6]. Like crude petroleum, microalgal biomass has the potential to generate not only different kinds of fuels but also a wide range of commodity products. Hence the expression microalgal biorefinery to reference this similarity between the traditional petroleum based refinery [7]. Through thermal and biochemical conversions, microalgal feedstocks have been processed to generate biodiesel, bioethanol, biohydrogen and other types of biofuels [8]. There has also been a great interest to exploit microalgae as feedstock for the production of expensive and highly valuable pharmaceutical ingredients. For instance, chlorophyll and carotenoid pigments from microalgae have found several applications in food and pharmaceutical industries as food additives, cosmetic agents and antioxidants for medical purposes [9,10]. In addition, most microalgal species are rich in proteins

and therefore have great nutraceutical potential [11]. Algal extract rich in proteins, carbohydrates and free amino-acids have also been characterized as a suitable replacement for yeast extract used to feed microorganisms [12].

Adding to the large range of products that can be obtained from microalgae, other attributes motivate the investment in microalgal biomass production. Contrary to other biofuels feedstocks such as corn or sugarcane, microalgae do not require as much human labors to grow and can adapt to harsh environmental conditions [13]. Moreover, microalgal biomass has a far higher lipid content and use much less land than the competing feedstocks [14]. More interestingly, microalgal biomass production does not have high raw materials demands. Sometimes the nutrients demand is simplified to only flue gas feed and wastewater [15]. Technologies that simultaneously offer effective wastewater treatment and significant microalgal biomass generation have known great advances over the last decade [16]. The use of flue gas as carbon source for microalgal biomass production has also been proven to be a valuable option [17]. All this means that cultivation of microalgae can serve a dual purpose: environmental remediation and valuable biomass production. Therefore, the development of microalgal biomass production technology is of great interest.

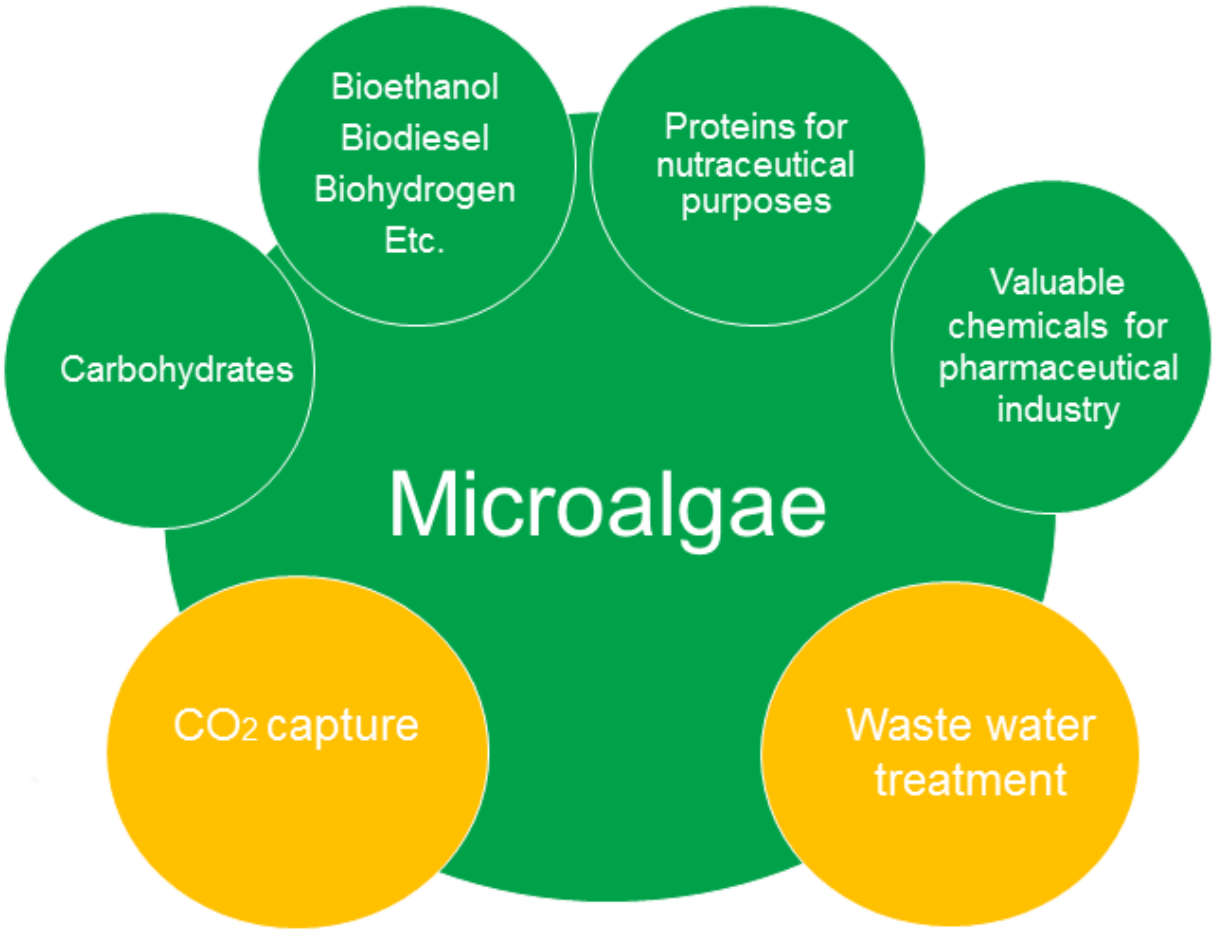


**Figure 1.3: Illustration of microalgal biorefining potential.** Varieties of biofuels and specialty chemicals can be obtained from microalgae, using mainly carbon dioxide from flue gas and wastewater as raw materials.

## 1.2 Production and processing of microalgal biomass

Despite all the benefits mentioned above, commercial utilization of microalgae for the listed purposes is still hardly financially feasible [18,19]. There is indeed a great need to improve the productivity in microalgal cultivation systems in order to make large scale biomass production a viable enterprise. Before discussing the current challenges with the production and processing of microalgal biomass, it is necessary to elucidate the processes involved in a typical microalgal biorefinery.

In nature, microalgal growth relies mainly on the conversion of the solar energy into chemical energy through photosynthesis. Chemically speaking, photosynthesis can be described as a light energy driven reaction where carbohydrates and oxygen are produced using carbon dioxide and water (Equation 1) [6]. Suitable light, carbon dioxide and water are the principal requirement for microalgal photosynthetic activity although other micronutrients will deem necessary for effective microalgal growth and proliferation. Therefore, any attempt to use microalgae as feedstocks for biofuels or specialty chemicals production first requires the ability to satisfy these primary conditions for effective microalgal biomass generation.

$$6CO_2 + \ 6H_2O \xrightarrow{light} C_6H_{12}O_6 + 6O_2 \qquad (1)$$

Human-made structures for microalgal biomass production are often classified into open pond cultivation systems and closed ponds cultivation systems often referred to as photobioreactors [20]. In the handbook of microalgal culture by Richmond, photobioreactors are defined as "culture systems for phototrophs in which a great portion of the light does not impinge directly on the culture surface, but has to pass through the transparent reactor's walls to reach the cultivated cells" [6]. Photobioreactors are often classified based on their shape, their constituting

materials or their mode of operation. The design or adoption of a specific microalgal cultivation system is often motivated by the ability to maximize light-energy conversion, enhance nutrient availability or to optimize the environmental conditions to ensure high productivity in a cost-effective manner.

After generating the necessary microalgal biomass, several downstream processing steps are necessary to obtain, at a certain level of purity, the fine chemicals (lipids, proteins etc.) needed [21]. Usually, the microalgal biomass is first separated from the culture medium through a harvesting process. Common harvesting techniques include centrifugation, sedimentation, filtration, flocculation etc. These harvesting techniques are also divided into subcategories based on their specificity [22]. In most cases, the harvested microalgal biomass still contains a consistent water concentration that additional dewatering and drying are required to avoid spoiling and denaturation. Microalgal biomass drying techniques comprise mainly of sun-drying, spray-drying, drum-drying, freeze-drying, etc. [23]

Further, due to the nature of the cell wall of most microalgal strains, the disruption of the cell wall is usually necessary prior the extraction of the targeted compounds. Current cell wall disruption methods include ultra-sonication, bead-milling, homogenization, osmotic stress, freezing etc. [24] The specific extraction of the compound will then require redulition of the biomass and often the use of several organic solvents to follow the standards bioseparation procedures. The last step of obtaining the lipids or proteins needed often consists of another extraction method that may involve chromatography, adsorption, ultrafiltration electrophoresis and many others [25].

These steps described above illustrate the general path to obtain useful chemicals through microalgal biomass production and biorefining (figure 1.2). However, some of these steps may be omitted or combined due to emerging technologies or due to the intended application for the biomass. For instance, production of bio-oil through thermochemical conversion of microalgal biomass does not require cell wall disruption or solvent extraction. On the other hand, the obtention of certain other useful compounds from the microalgal biomass usually requires other additional steps such as chemical reactions. For example, fatty acids methyl esters constituting biodiesel are obtained through transesterification of microalgal lipids. Transesterification is the reaction where a short alcohol (methanol) chain is used to cleave the glycerol group off a triglyceride (lipid) molecule resulting in three molecules of fatty acid methyl esters and one molecule of glycerol (equation 2) [26]. Adding to the transesterification reaction, other steps to purify the biodiesel are also needed.

$$Triglyceride + 3R'OH \leftrightarrow 3R'COOR + Glyrecol \qquad (2)$$

In summary, the production and processing of microalgal biomass involve a lot of steps and procedures and the optimization of microalgal biomass production requires thorough consideration and significant improvement of each step.

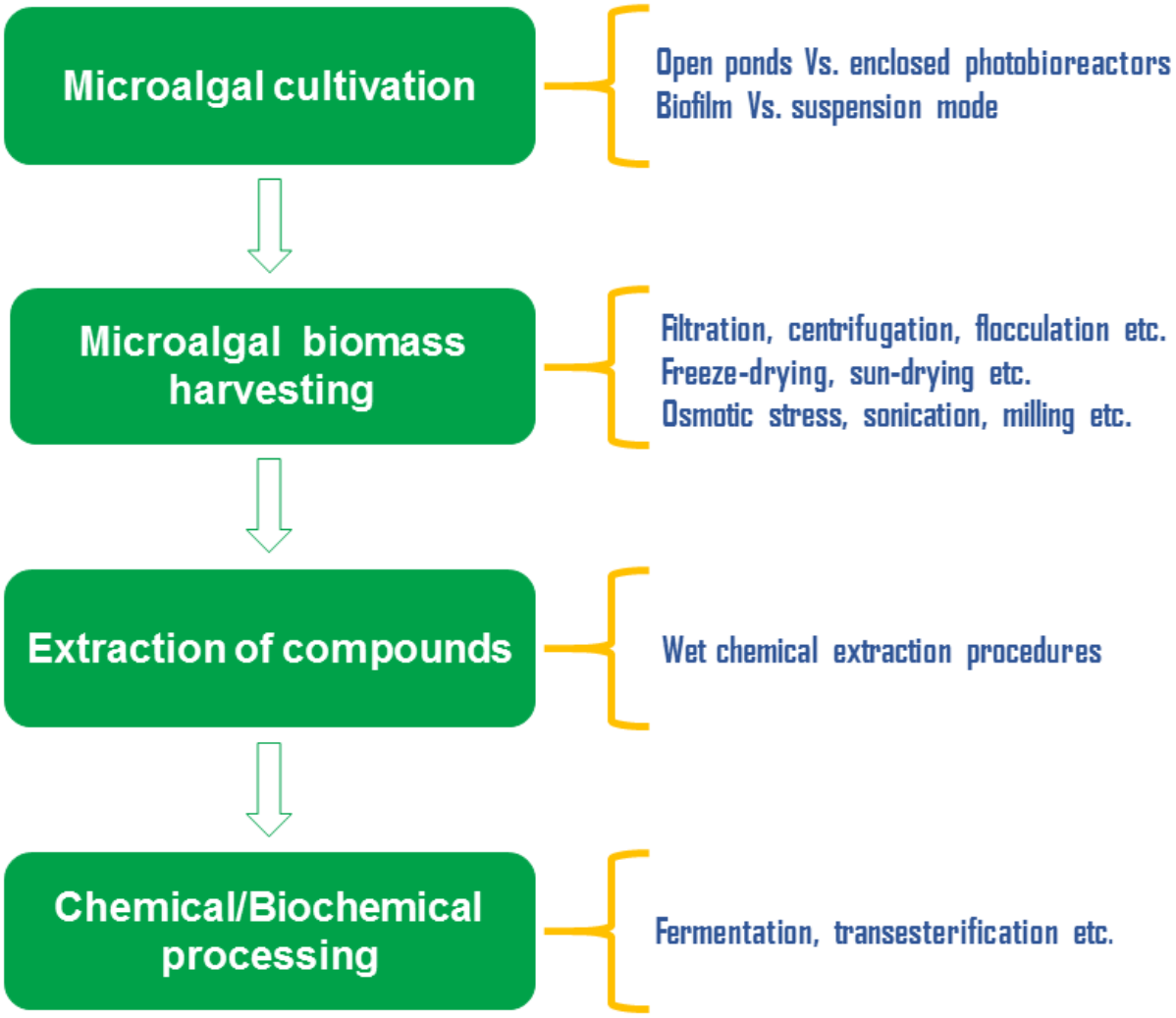


**Figure 1.4: Illustration of microalgal biorefining processes.** Microalgae are first cultured in open or enclosed systems. Then, the microalgal biomass is harvested. This is often followed by the extraction of the valuable compounds and further chemical processing to obtain the target specialty chemicals.

## 1.3 Challenges with microalgal biomass production and microalgal biorefining

The previous section should give an idea about the unit operations that would be needed for large scale microalgal biomass production and microalgal biorefining. In this section, some of the technical issues that hinder cost effective microalgal biomass production and microalgal biorefining at different level and the recent technology improvement are briefly discussed (figure 1.3).

First and foremost, in order to produce microalgal biofuels at a reasonable price compared to the cost of petroleum fuels, there is a need to maximize the productivity in microalgal biomass production systems. To address this issue, two simultaneous approaches have been explored: the identification or engineering of microalgal strains with high growth rate and high lipid content and the optimization of the bioprocess parameters.

Concerning the first approach, there has been active research to identify microalgal species that possess the potential for high throughput and cost effective production of lipids for biofuels or the production of a different compound for other applications. Further, extensive research has been carried out to improve the ability to manipulate the biochemical factory of microalgae through genetic engineering, transcription factor engineering and biochemical engineering [7,27]. Genetic engineering approaches aim mainly at understanding the metabolic pathways of specific compounds (e.g. lipid) for channeling of metabolites to biosynthesis by overexpressing key enzymes in recombinant microalgal strains. Transcription factor engineering targets the enhancement of production of a particular metabolite by means of overexpressing the transcription factors regulating the metabolic pathways involved in the accumulation of the target metabolites. Biochemical engineering strategies involve investigating physiological conditions

such as high salinity and nutrient deprivation stresses to induce accumulation of carbohydrate, lipid or other valuable compounds [7].

As for the optimization of the bioprocess parameters, there is a long list of parameters that need further improvement for an effective control of microalgal cultivation conditions and a significant reduction of the associating capital and production costs [18]. An entire section of this chapter will be devoted to discuss the critical problem of light distribution. Adding to this one, there is a wide range of problems including temperature fluctuations, control of PH and medium composition, carbon dioxide dissolution and distribution, limiting mass transfer and effective mixing that are deemed worthy of exploration.

Although successful microalgal cultivation has been reported as feasible over a wide temperature range (5-35°C), the cultivation temperature is not without effects on the microalgal growth and the biochemical composition [28]. A number of studies have focused on the effects of temperature on microalgal growth rates and the production of lipids, carbohydrates, proteins and photosynthetic pigments [29,30]. However, maintaining an optimal and uniform temperature has been a design challenge, more severe for outdoor culture exposed to solar radiation and ambient conditions [31].

The medium composition and the PH affect the biomass productivity in many ways. This is mainly due to the fact that the metabolic pathways of photosynthetic microorganisms depend not only on light and carbon but also on trace quantities of various of inorganic elements [32]. For instance, iron and copper are known to serve as catalysts for many metabolic pathways. While the deficiencies in these elements paralyze or cause alteration of certain metabolic activities, an excess in the concentration of these trace elements can lead to growth medium contamination [33].

Moreover, nitrogen limitation in microalgal culture has been seen to enhance lipid accumulation and decrease growth and the reverse effects have been seen when the conditions are reversed [34]. Further, phosphorus limitation is known to increase oxygen production in microalgal culture. The presence of oxygen can lead to the worst impacts on microalgal culture by oxidizing several nutrients and enzymes forming oxygenic byproducts that suppress microalgal growth [35,36]. The biggest obstacle is that there are some poorly understood but verified interconnections and/or diverging effects between the concentrations of these elements (Nitrogen, phosphorus, etc.) in microalgal culture. Any imbalance affects the PH which in turn affects the composition of the medium and the metabolic activities. Therefore, finding and maintaining the appropriate concentration of nutrients is a hurdle for commercial microalgal cultivation that necessitates thorough research.

Another difficulty encountered in large scale microalgal cultivation is the need to maintain a sufficient and uniform carbon distribution throughout the cultivation system. The low solubility of carbon dioxide in water makes continuous pumping of carbon dioxide inevitable in most microalgal cultivation systems. This increases significantly the operating cost for microalgal biomass production [37]. Moreover, although inorganic carbon is necessary for microalgal photosynthesis, a high concentration of inorganic carbon can alter the metabolic pathways or lead to contamination of the culture medium due to change in PH [38,39]. For these reasons, a lot of studies have focused on predicting and optimizing the temporal and spatial inorganic carbon profiles in microalgal culture [40].

A deep understanding of nutritional requirements for microalgal growth will facilitate the preparation and implementation of appropriate growth medium, light and temperature conditions for optimal growth of different microalgal species. However, in order to ensure a homogenous

distribution of nutrients and to eliminate or mitigate the effects of temperature and light gradients, proper mixing is required. The importance of mixing in microalgal culture has been largely documented with a variety of experimental results. Low and inadequate mixing has shown to lead to a decrease in the mass transfer rate of necessary nutrients [41]. On the other hand, excessive mixing rate has proven to cause shear-induced damage of microalgal cells [42,43]. There are currently different commercially available systems for mixing of microalgal culture [44]. Nonetheless, due to the time dependent complex fluids nature of microalgal culture, thorough studies on the flow properties are still necessary [45].

Beside the challenges with microalgal cultivation, the harvesting of microalgal biomass has also been a hurdle that greatly impacts the commercial production of microalgal biomass. The nature of the problems varies depending on the harvesting method in use and a brief summary of the major ones are presented in the next paragraphs.

Sedimentation is one of the simplest and less expensive microalgal harvesting technique where the suspended microalgae are separated from the growth medium through gravitational settling. One of the drawback of harvesting through sedimentation is the land area requirement for the settling ponds [46]. The biggest impracticality is the fact that the settling process, depending on the microalgal cell size and density, can be extremely slow and merely feasible [47].

Filtration is another microalgal harvesting method using semi-permeable filters to allow the selective passage of water and nutrients and the retention of microalgal cells. There are currently a lot of filtration technologies using dead-end filtration, tangential flow filtration, rotary vacuum etc. [48] The most reported issues with filtration are the low efficiency with small microalgal cells and the high rate of fouling and clogging [48,49,50].

Harvesting by flocculation entails the addition of polymers in the microalgal broth to aggregate the cells into readily settled clusters. This technique has been extensively investigated with the use of different flocculating agents acting either on the PH of the medium, the cell wall surface charges, the bridging mechanism etc. [51,52] The major drawbacks of this useful technique are the fact that efficacy can vary significantly based on the characteristics of the broth or the microorganisms and also the concerns for toxicity from the chemicals use as flocculants [53].

Centrifugation is a well-known harvesting method where the settling rate of microalgal cells is drastically increased due to enhanced gravitational force. Although harvesting through centrifugation can be rapid and efficient, this process is highly energy intensive and increase the capital and processing costs of microalgal biomass production. Moreover, the high gravitational force sometimes entrains some valuable materials and causes shear-induced damages to microalgal cells [53].

There are other emerging methods being tested for microalgal harvesting. There are also many other issues related to microalgal cultivation and harvesting. Not to mention the challenges with microalgal cell wall disruption and the extraction processes and chemical reactions necessary to obtain the commodity products. This section, far from presenting an extensive list of the challenges related to microalgal biomass production and processing, serves as a useful foundation for the flow of ideas throughout this dissertation.

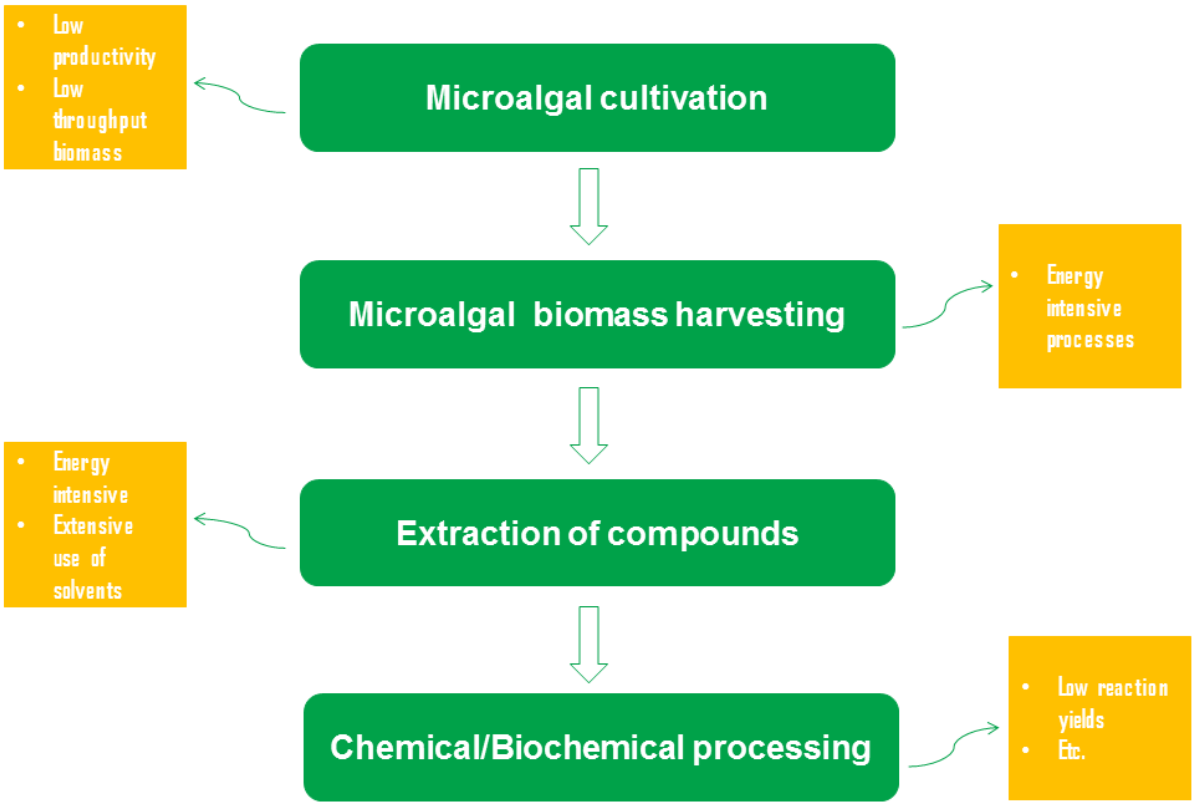


**Figure 1.3: Illustration of current challenges in microalgal biomass production and biomass processing.** The most critical issues include the low productivity and the high energy consumption in each step**.**

## 1.4 Effects of light on microalgal biomass production

Light is a crucial parameter for phototrophic production of microalgae. The impacts of light on microalgal photosynthetic activity depend both on the quantity and the quality of the irradiance. Quantitatively, the intensity of light has been classified into three regimes of irradiance to categorize the effects on microalgal biomass production: light limitation, light saturation and light inhibition. Within the light limitation range, microalgal growth rate increases with increase in light intensity, even though the extent of the effect of changes in the light intensity varies from species to species [54]. At saturating light intensities, the rate of photon capture exceeds the rate of linear photosynthetic electron transfer. As a consequence, a large fraction of the captured light energy is dissipated as heat or fluorescence b non-photochemical quenching. These dissipative energy losses result to inefficiency in the conversion of light into chemical energy through photosynthesis [55]. Lastly, very high irradiances in the light inhibition regime cause photodamage of photosystems which may lead to death of microalgal cells [56].

The quality of light is also important to microalgal growth because microalgal photosynthetic activity is often wavelength specific. For example, cyanobacteria have been known to exhibit a large propensity for light in the blue and the red regions of the electromagnetic spectrum whereas other wavelengths of the electromagnetic spectrum have been reported for increasing the risk for photoinhibition [57].

The quantity and quality of irradiance do not only impact the microalgal growth but also the composition of the resulting biomass. In fact, two expressions are often used to refer to the effects of irradiance on microalgal biomass production: Photoacclimation and chromatic acclimation. The former, more general, refers to phenotypical changes that occur in response to

changes in both the intensity and quality of light. The latter, more specific, are often used to refer to preferential synthesis of light-harvesting pigments or changes within the photosystems to accommodate to changes in incident light quality (wavelength) [58].

It has been reported that photoacclimation can affect the synthesis of major chemicals that are useful for microalgal biorefinery. For instance, the work by Napolitano confirmed that triglyceride production can be increased through light enhancement [59]. Further, in many cases, light enhancement has resulted in alteration of fatty acid synthesis by inducing the production of mono-unsaturated fatty acids and concomitantly disfavoring the formation of poly-unsaturated fatty acids [60]. It has been also proven that the generation of the precursors for the synthesis of carbohydrates like glucose and starch can be stimulated by irradiance. Nonetheless, research is being undertaken with the goal of understanding the photochemical mechanisms that explain the correlation between the irradiance and the accumulation of carbohydrates. Lastly, syntheses of photoactive pigments such as chlorophylls and carotenoids have shown great dependence on the irradiance. Many reported experiments have proven that production of such pigments can be easily increased or decreased through manipulation of the quality and/or the intensity of the incident light [7].

Although the quantity and the quality of light have such importance in microalgal biomass production, light management in microalgal cultivation systems has been a challenge for large scale biomass production. As it happens, only a small fraction of microalgal cells receives an optimal irradiance in current microalgal cultivation systems (open and enclosed ponds). The remaining microalgal cells are found either in the over illuminated zones (e.g. at top surface of open culture) where they are exposed to photo-inhibition or in the poorly illuminated zones where their growth is limited. The recent review by Ooms *et. al.* describes some recent emerging

engineering strategies to optimize the distribution and absorption of light in microalgal cultivation systems [61]. These include the screening of suitable locations with weather patterns and sunlight favorable for the installations and operations of photobioreactors. Some other efforts focus on the development of artificial light systems to meet the irradiance requirements of microalgal culture such as LED lightening and flashing light systems. Recently, a few studies have confirmed successful conversion of undesired incident photosynthetic wavelengths to more benign and desired wavelengths for microalgal photosynthetic activities. Lastly, the emerging field of plasmon enhancement of light along with other new ideas including the development of short light path systems and controlled motion strategy will be discussed throughout this dissertation to help address the issue of poor distribution and absorption of light in microalgal photobioreactors.

## 1.5 Motivation and objectives

The issue of poor distribution and absorption of light in microalgal photobioreactors can only be addressed by taking into account the other challenges pertinent to the cultivation and harvesting of microalgae. This is important because any strategy to improve the distribution and absorption of light in microalgal culture should be assessed in terms of the beneficial impacts on microalgal biomass production but also on how it affects other crucial parameters inherent to large scale microalgal biorefining as well as how it affects the production cost. With this challenge in mind, a multi-fold approach to improve the distribution and absorption of light in microalgal photobioreactors is presented in this dissertation. This consists of exploiting the plasmonic properties of metallic nanoparticles for light enhancement, using surface micropatterning to better control the impacts of biofilm and biofouling on light delivery, developing short light path cultivation systems for efficient light absorption and using flow

manipulation to control the exposure of microalgal cells to ideal light zones and to create an optimal light/darkness balance.

### 1.5.1 Development of plasmonic light filters for enhanced microalgal growth and biomass composition

Plasmon resonance is an optical phenomenon arising from the collective oscillation of conduction band electrons in a metal under the irradiation of light [62]. This phenomenon occurs in the visible region of the electromagnetic spectrum for nanoparticles made of noble metals like gold, copper and silver. Its applications to bio-detection, optical sensing and solar photovoltaics have shown great potential [57]. The wavelength specificity of the microalgal photosynthetic activity motivated the use of plasmonic nanoparticles to enhance the irradiation of blue and red lights desired by photoactive pigments and to minimize the irradiation at other wavelengths that may cause photoinhibition in microalgal culture. In chapter two of this dissertation, we present our efforts and the major results on developing a plasmonic film technology to be used as light filter in microalgal photobioreactors and investigating the impacts of such plasmon enhancement on microalgal growth and biomass composition [63].

### 1.5.2 Development of a short light path energy efficient microalgal cultivation and harvesting system

Short light path microalgal cultivation systems are largely advantageous compared to traditional cultivation of microalgae in relatively deep open ponds. This is because it allows a more efficient control of light delivery, mitigates the effects of shading and also because it can minimize the need for constant energy intensive mixing. In chapter 3, we present a new short light path energy efficient microalgal cultivation and harvesting system. This system with

thermoreversible sol-gel transition properties exhibits some interesting microalgal cultivation and harvesting capabilities that are also discussed in the same chapter [64].

### 1.5.3 Controlling light delivery through manipulation of cells attachment on the surface of photobioreactors

Microalgal cells adhesion to surface along with biofilm formation should be seen at two angles in the context of microalgal cultivation. In suspended culture, microalgal cells adhesion to surface would hinder light penetration inside the photobioreactor and therefore would affect growth. On the other hand, biofilm cultivation of microalgae (attached culture) would be advantageous for shortening considerably the light path and also minimizing the energy requirement for harvesting. The ability to manipulate microalgal cells attachment was thus one of our goals. In chapter 4, we discuss the manipulation of microalgal adhesion through surface micropatterning in light of our experimental results.

### 1.5.4 Rheological characterization and flow manipulation to improve light absorption in microalgal photobioreactors

Efficient flow control within microalgal cultivation systems can be very useful to address the issue of non-homogenous growth due to unbalanced light exposure while optimizing the energy requirement for mixing. Moreover, the ability to create an optimal light/darkness balance can greatly improve microalgal biomass production [65,66]. Doing all this requires a deep understanding of the hydrodynamic properties of microalgal suspensions, complex fluids still poorly studied. In chapter 5, we present our results and discuss this strategy involving the characterization of the rheological properties of such microalgal systems and the use of these properties as building blocks to control the shuttle motion of microalgal cells between the poor and highly illuminated sections of typical photobioreactors.

## 1.6 References


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# CHAPTER 2

## Development of plasmonic light filters for enhanced microalgal growth and biomass composition

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**Abstract:**

In search of strategies to enhance light absorption in microalgal culture, a plasmonic film light filter technology was developed. First, polymer films consisting of spherical silver nanoparticles were fabricated and used as plasmonic filters that selectively enhance blue light absorption in microalgal cultures. For the microalgal species *Chlamydomonas reinhardtii,* after ten days of cultivation the use of these plasmonic filters led to an increase in the microalgal dry biomass by more than 25% and an increase in chlorophyll and carotenoid pigments by more than 35%, compared to the control cultures without using these films. Further, light enhancement by plasmon resonance did not affect lipid and carbohydrate accumulation within individual microalgal cells. However, the higher cell densities obtained with the plasmonic filters resulted in enhanced overall carbohydrate and lipid production. Motivated by these results, the fabrication of plasmonic film filters containing silver nanospheres and/or gold nanorods were tailored to selectively backscatter different wavelengths of the incident light that are favorable for microalgal growth and pigments accumulation. The experimental results proved that microalgal biomass production and photosynthetic pigments production can be increased by up to 50% and 78% respectively through light enhancement induced by the use of plasmonic film filters.

## 2.1 Introduction

In the context of renewable energy production, microalgae represent a promising viable and sustainable feedstock that can replicate the traditional refinery approach, often referred to as the bio-refinery [1]. Through thermal and biochemical conversions, microalgal feedstock can be used to produce biodiesel, bioethanol, biohydrogen and other types of biofuels [2]. Moreover, chlorophyll and carotenoid pigments from microalgae have several applications in food and pharmaceutical industries as food additives, cosmetic agents and antioxidants for medical purposes [3,4]. Furthermore, algal extract is rich in proteins, carbohydrates and free amino-acids and can serve as a replacement for yeast extract used to feed microorganisms [5]. However, commercial utilization of microalgae for such purposes is still far from being financially feasible due to high extraction costs and low productivity in confined environments [6]. As a result, strategies for improving microalgal biomass growth, carbohydrate and lipid production, as well as pigment accumulation have been extensively investigated. For instance, transcription factor engineering and genetic engineering approaches have been applied to enhance lipid overproduction [7]. Physiological stresses such as high salinity and nutrient deprivation are also often used to induce accumulation of carbohydrate, lipid or other valuable compounds [1].

In the first chapter of this thesis, the effects of light intensity on microalgal biomass production was extensively described based on well-known theories as well as largely reported experimental results [8]. We also described how blue and red lights are optimal for the photosynthetic activity of most microalgal species whereas other wavelengths of the electromagnetic spectrum may in some cases cause photo-inhibition [9,10]. Recently, the light intensity dependency and wavelength specificity of microalgal photosynthetic activity have been largely studied and exploited as tools to develop technologies to improve biomass growth in

microalgal cultures. For instance, Wondrackzek *et al*. developed a photoluminescence converter that enables conversion of undesired green light in the incident spectrum to desired red light by photoactive pigments [11]. Organic and inorganic dyes, phosphors and quantum dots have also been tested for the conversion light wavelengths with very little photosynthetic potential into light wavelengths with higher photosynthetic potential [12,13].

The application of localized surface plasmon resonance by metallic nanoparticles to the enhancement of light in microalgal cultivation systems was first introduced by Torkamani *et. al.* [14]. Considering the light backscattering properties of silver and gold nanoparticles, this work developed a mathematical model to show how such localized surface plasmon resonance by metallic nanoparticles can effectively enhance light irradiance in microalgal cultures. The effects of such enhancement of light on microalgal growth was also modeled and tested in a miniature system with two petri-dishes. Specifically, the use of a silver nanoparticle suspension, as a plasmonic filter that backscatters blue light into microalgal culture, was shown to result in an increase in photosynthetic growth by more than 30%, compared to a control without nanoparticle suspension. Following this, experiments conducted by Eroglu *et. al.* with microalgal species *Chlorella vulgaris* showed how nanometallic suspensions can be used to increase photosynthetic pigment accumulation [3]. The work by Ooms *et. al*. also demonstrated how wavelength selective scattering from nanopatterned surfaces was able to enhance the growth rate of cyanobacterium *S. elongatus* in modular bioreactors by 6.5% while improving the power efficiency by 52% as compared to systems that utilize broadband reflectors [15]. These promising results motivated further studies and the development of plasmonics-based light enhancement technologies that could be realistically integrated in the design of large scale enclosed microalgal photo-bioreactors.

It was also important to investigate the concomitant effects of plasmon-enhanced growth on microalgal growth, accumulation of photosynthetic pigments and production of lipids and carbohydrates. Investigating such effects was important because the way an improvement in any given factor affecting microalgal biomass production impacts different biomass production output factors has shown to be highly dependent on the type of enhancement. For instance, stress conditions such as high salt concentration and nitrogen deprivation that increase lipid production have been observed to have a negative effect on microalgal growth [1,16]. Similarly, nutrient starvation that increases lipid production in cyanobacteria has been shown to either increase or have little effect on carbohydrate generation, depending on the bacterial species [17,18]. Further, enhanced microalgal growth by light enhancement could be accompanied either by an increase or a decrease in triglyceride production as suggested by the works by Napolito and the work by Guedes *et. al.* respectively [19,20]. It has been also shown that, in many cases, light enhancement can cause alteration of fatty acid synthesis by inducing the production of mono-unsaturated fatty acids and concomitantly disfavoring the formation of poly-unsaturated fatty acids [16].

Motivated by the above listed reasons, we developed a plasmonics-based light filter technology to enhance light absorption in microalgal culture and improve microalgal biomass production. First, flexible polymeric films consisting of spherical silver nanoparticles were fabricated and used as filters that selectively enhance the irradiation of blue light in microalgal cultures. This technology is much more easily implemented, scalable, durable and safer compared to that based on nanoparticle suspensions. Previous simulation results have demonstrated how plasmon resonance by spherical silver nanoparticles increases the available irradiance in microalgal culture [14]. By virtue of this, the plasmonic film would enhance photosynthetic activity. It was also hypothesized that the additional blue light provided by

plasmon resonance would increase photosynthetic pigment contents through chromatic acclimation [21]. Further, it was important to assess potential effects on lipid and carbohydrate production. The plasmonic film was therefore tested for a *Chlamydomonas reinhardtii* culture and its effects were assessed through analyses of microalgal biomass, photosynthetic pigment, lipid and carbohydrate production. The successful results with the selective backscattering of blue light with silver nanospheres inspired the development of gold nanorods-based and combined gold nanorods and silver nanospheres-based plasmonic films light filters to enhance the absorption of blue and red lights by microalgae.

## 2.2 Materials and methods

### 2.2.1 Synthesis of silver nanospheres

Silver nanoparticles were synthetized by using sodium borohydride ($NaBH_4$) as a reducing agent for silver nitrate [22]. Using a pipette, 20 ml of 1.0 mM silver nitrate solution was gently added to a 60 ml solution of 2.0 mM sodium borohydride. The reaction was performed at 4°C on a magnetic stir plate. Synthetized spherical silver nanoparticles had diameters of 12 ± 2 nm, as confirmed by dynamic light scattering measurements.

### 2.2.2 Synthesis of gold nanorods

Gold nanorods with extinction wavelength peak at 675 nm were synthesized using a modified seeding-mediated growth method similar to that presented by Nikoobakht & El-Sayed [23]. First, a seed solution was prepared by mixing 5 ml of an aqueous solution of 0.5 mM HAuCl4 with 5 ml of cetyl trimethyl ammonium bromide (CTAB) 0.2 M and 0.6 ml of cold ice sodium borohydride (NaBH4) 10 mM. The mixture was then vigorously stirred for 2 minutes and this seed solution was stored at 25°C. The gold nanorods were then synthesized by first adding 0.06 ml of 4 mM AgNO3 to 5 ml of 0.2 M CTAB solution at 25°C. Following this, 5 ml of 1 mM of HAuCl4, 70 µl of 0.0788 M of ascorbic acid and 12 µl of seed solution were successively added to the mixture under gently mixing. This final mixture was then incubated at 30°C for gold nanorods growth. The color of the mixture that changed from yellow to transparent after the addition of ascorbic acid, was observed to gradually exhibit a blue color characterizing the formation of gold nanorods with aspect ratio that leads to extinction of light wavelength around 675 nm. After 30 minutes of incubation at 30°C, the mixture exhibited a dark blue color and was used for the intended plasmonic film fabrication purpose after the spectrophotometric characterization of the gold nanorods.

### 2.2.3 Preparation of plasmonic films consisting of silver nanoparticles

Plasmonic films consisting of silver nanospheres were prepared by adding polyvinyl alcohol (PVA) powder to the silver nanoparticle suspension such that final PVA content in the mixture was 5% by weight. The final mixture was dried in a petri-dish for two days. Then, the film was peeled off and stored at room temperature until use. The resulting plasmonic film was about 1 mm thick when 20 ml of the mixture was put to dry in a 100 mm diameter petri-dish. The thickness may vary slightly if more volume is added or the PVA content is higher. The absorption spectrum of the plasmonic film exhibited a single peak near 400 nm but was broad enough to cover the entire blue region of the electromagnetic spectrum (figure 2.1).

### 2.2.4 Preparation of plasmonic films containing gold nanorods

To prepare the plasmonic films containing gold nanorods, a 5% weight aqueous solution of polyvinyl alcohol was first prepared. Then the gold nanorods solution was centrifuged (8,000 RPM, 10 minutes) and washed twice. Subsequently, the supernatant was disregarded and the dark blue gold nanorods ink was redissolved in the aqueous polyvinyl alcohol solution. Plasmonic films with a single primary extinction peak at 675 nm could be fabricated by pouring this last mixture in a petri-dish and letting it dried for three days at the room temperature (figure 2.2). To prepare plasmonic films with dual extinction peaks, the blueish aqueous polyvinyl alcohol mixture containing gold nanorods were mixed with the yellowish polyvinyl alcohol mixture containing silver nanospheres leading to a greenish mixture containing both silver nanospheres and gold nanorods with extinction peaks at around 400 nm and 675 nm (figure 2.3). This mixture was similarly poured in a petri-dish and dried under the same conditions. After the

three-days drying period, the 1mm thick films were completely dried and were peeled off the petri-dishes and could be used for light filtering in microalgal cultivation systems.

### 2.2.5 Microalgal strain and culture conditions

The wild type microalgae *Chlamydomonas reinhardtii* CC-124 obtained from the Chlamydomonas Resource Center (University of Minnesota, St. Paul, Minnesota) was used in this study. The experimental setting consisted of a 250 ml conical flask with the base wrapped with a plasmonic film. The base of the flask was then covered with black tape (Scotch Super 33+ Vinyl Electrical Tape, 3M, St. Paul, Minnesota) so that incident light could enter only from the top of the culture. Another conical flask wrapped with only black tape (without the film) was used as a control. Microalgal cells were allowed to grow in a 50 ml minimum medium enriched with 20 mM of sodium bicarbonate, with the flasks placed on a rotary shaker (100 rpm) [13]. The space was continuously illuminated by full spectrum compact fluorescent lamps (CFL 60W, Fancierstudio, San Francisco, California). The photosynthetic active radiation at the top surface of the culture was at 100 ± 5 $\mu E/m^2/s$, as measured by quantum meters (MQ, Apogee Instruments, Inc., Logan, Utah). The temperature was controlled at 23 ± 1°C. Triplicate samples were collected every two days to measure the optical density of the culture at 675 nm ($OD_{675}$) [14]. After ten days of cultivation, the microalgal cells were harvested for dry mass determination and compositional analyses.

### 2.2.6 Lipid analyses

Lipid generation was assessed using three techniques: gravimetric quantification, fluorometric scanning and gas chromatography. For gravimetric quantification, a modified Bligh and Dyer method was used [24]. Briefly, each microalgal sample was centrifuged (4,800g, 5 min, 20°C) to remove excess water. Cell lysis for lipid release was assured by sonication (Q500

Sonicator, Qsonica, LLC., Newtown, Connecticut) with a 20/10 seconds ON/OFF cycle for 2 minutes; 5 ml of chloroform, 10 ml of methanol and 5 ml of deionized water were then added to the sonicated samples. To facilitate phase separation, the samples were placed on a rotary shaker (100 rpm) for 6 hours and 5 more ml of chloroform and deionized water were then added prior to centrifugation (4,800g, 5min, 20°C). Following this, the lipid rich chloroform layer was removed and dried for gravimetric measurement.

Prior to trans-esterification, dry lipids were dissolved in 1ml of toluene. Then, 2 ml of a solution of anhydrous sodium methoxide in methanol (0.5 M) was added and the reaction was allowed to proceed for 3 hours in a 65°C water bath. Subsequently, the mixture was neutralized with acetic acid and fatty acid methyl esters (FAME) were extracted by adding 5 ml of hexane to the mixture.

Using ASTM 6584 solution as standard, fatty acid methyl esters were characterized with two gas chromatography systems: a Hewlett-Packard Model HP 5890 series II gas chromatograph with Flame Ionization Detector (FID), and an Agilent 7890 gas chromatograph with Agilent 5975c Mass Spectrometer (MS). For the GC-FID system, the injector temperature was set at 300°C; the oven was initially held at 60°C for 2 minutes and then increased to 150°C at a rate of 6°C /min. The oven temperature was subsequently held at 150°C for 10 min and then elevated from 150 to 350°C at a rate of 10°C/min and held for 1 min. For the GC-MS system, the injector temperature was set at 250°C; the oven was initially held at 150°C for 1 minute and then increased to 230°C (ramp 3°C/min) and held at that temperature for 10 minutes.

Nile Red fluorescence determination of neutral lipids was used as a means to verify results obtained by gravimetric quantification [25]. Specifically, 10 ml of the microalgal suspension

was stained with 40 µl of a solution of Nile Red in acetone (0.25 g/l). After 30 minutes, fluorescence scanning (excitation wavelength: 490 nm, emission wavelength: 520-750 nm) was performed using a Fluoromax-4 spectrofluorometer (Horiba, Ltd., Kyoto, Japan). Comparisons between plasmon-enhanced culture and control culture were then made after the subtraction of the auto-fluorescence of microalgal cells and the relative fluorescence of Nile Red solution from the overall fluorescence spectra [25].

### 2.2.7 Carbohydrate determination

Carbohydrate content was assessed using the phenol-sulfuric acid method proposed by Dubois *et al*. [26]. Specifically, 2 mg of dry microalgae was mixed with 2 ml of water. Subsequently, 50 µl of 80% phenol was added, followed by a rapid addition of 5 ml of concentrated sulfuric acid. The mixture was then placed in a 50°C water bath for 20 minutes. Following this, the absorbance at 490 nm was determined and compared to a standard curve based on glucose.

### 2.2.8 Microalgal pigment determination

Microalgal pigments were assessed by spectrophotometry. Specifically, an aliquot of 2 ml of culture was centrifuged (8,000 rpm, 5 min, 20°C) with the supernatant discarded and the pellets suspended in 2 ml of 95% ethanol. Then, the extraction was allowed to proceed on a rotary shaker (100 rpm) for 24 hours in the dark. Finally, the mixture was centrifuged (8,000 rpm, 5 min, 20°C) and the absorbance of the supernatant at 470, 649, 665 and 750 nm was determined. The quantification of total chlorophylls and total carotenoids were then made using the formulae of Lichtenthaler [27].

### 2.2.9 Statistical analyses

The microalgal culture experiment was repeated 15 times and for each run, all the analyses mentioned above were performed thrice. Statistical analyses over the 45 data points for each parameter were performed using Minitab software. The results with P-Value less than 0.05 (t-test) were considered statistically significant.

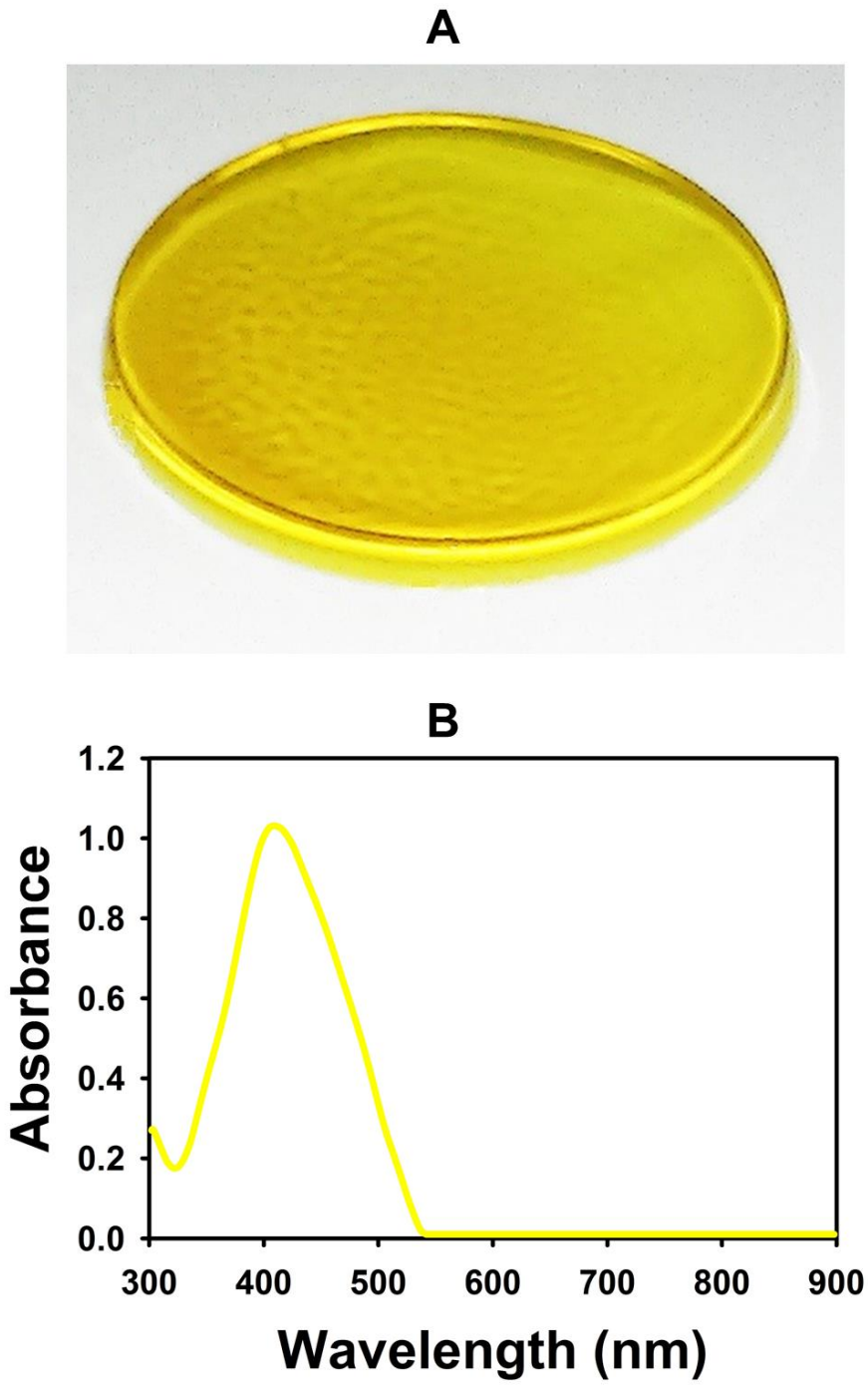


**Figure 2.1: Plasmonic film consisting of silver nanospheres.** Photograph of a plasmonic film after being peeled off from petri-dish (A) and absorbance spectrum of the plasmonic film consisting of spherical silver nanoparticles (B).

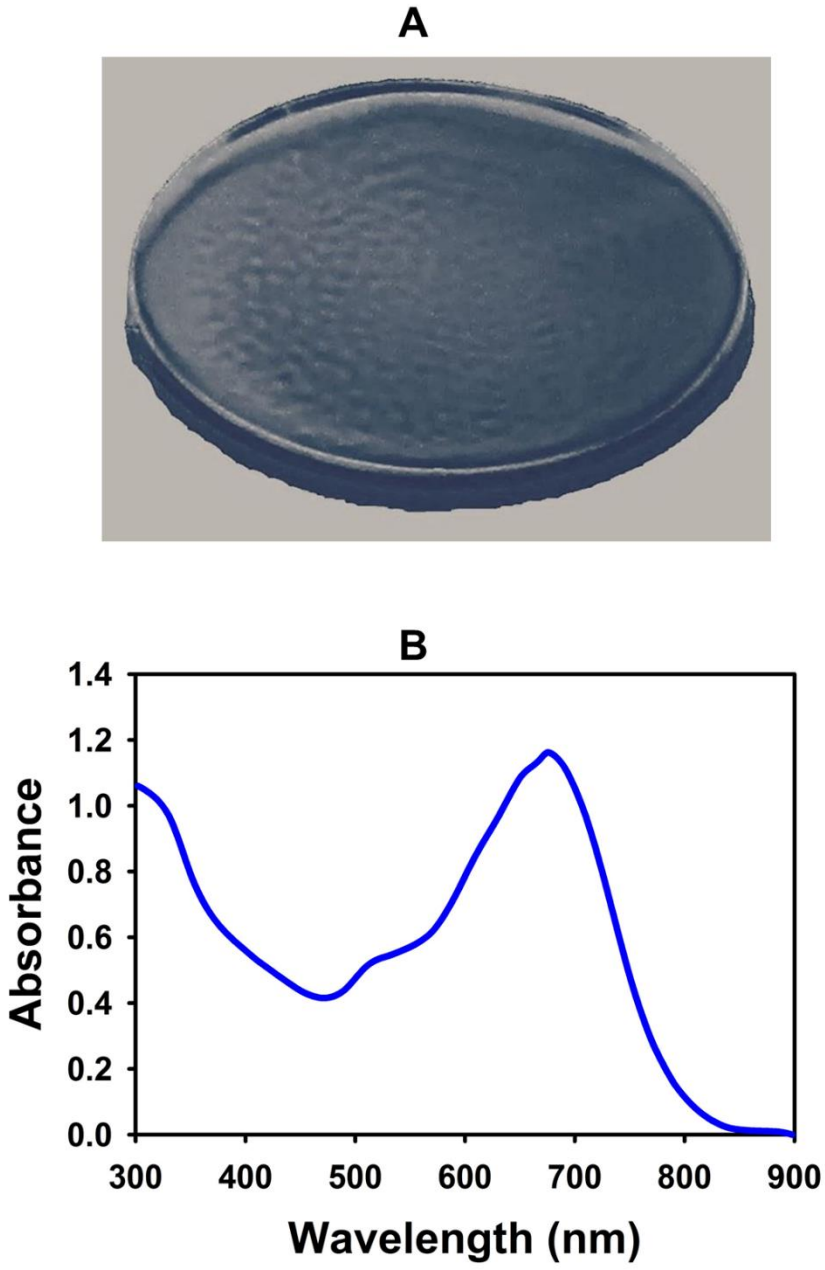


**Figure 2.2: Plasmonic film consisting of gold nanorods.** Photograph of a plasmonic film consisting of gold nanorods after being peeled off from petri-dish (A) and absorbance spectrum of the plasmonic film (B).

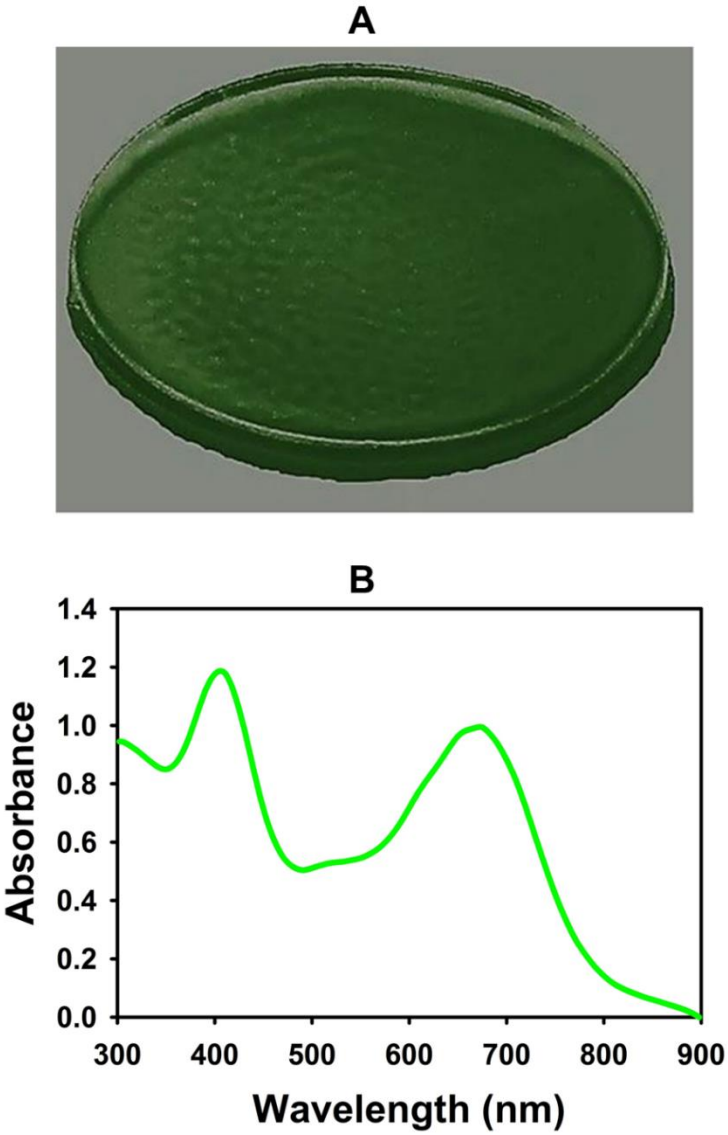


**Figure 2.3: Plasmonic film consisting of silver nanospheres and gold nanorods.** Photograph of a plasmonic film consisting of gold nanorods and silver nanospheres after being peeled off from petri-dish (A) and absorbance spectrum of the plasmonic film consisting of gold nanorods and silver nanospheres (B).

## 2.3 Results and discussion

### 2.3.1 Effect of plasmonic film filters consisting of silver nanospheres on microalgal biomass production

Wild type microalgae *Chlamydomonas reinhardtii* CC-124 growing on agar plates was used to prepare liquid subcultures, four days preceding the experiment. Then, a fresh culture was prepared and equally split in two flasks, one with the plasmonic film consisting of silver nanoparticles and the other without film used as the control. The optical density ($OD_{675}$) of the culture was monitored over ten days. From day 0 to day 4, there was no significant difference ($P > 0.05$) in optical density between the plasmon-enhanced culture ($OD_{675}$ increased from 0.05 to $0.14 \pm 0.02$) and the control ($OD_{675}$ increased from 0.05 to $0.12 \pm 0.02$). From day 4 to day 10, optical densities varied largely between the two settings (Figure 2.4A). Average $OD_{675}$ values increased from (0.14 to $1.18 \pm 0.06$) and (0.12 to $0.88 \pm 0.06$), respectively for the plasmon-enhanced culture and the control ($P < 0.05$). Gravimetric analyses, after ten days of cultivation, confirmed that microalgal biomass production was increased by more than 25 % ($P < 0.05$) when the plasmonic film filter was used (Figure 2.4B). This enhancement of microalgal biomass production is in good concordance with the theoretical model for the enhancement of light through plasmon resonance by spherical silver nanoparticles. It is also consistent with the results obtained with nanoparticle suspension where microalgal biomass production was increased by more than 30% [14].

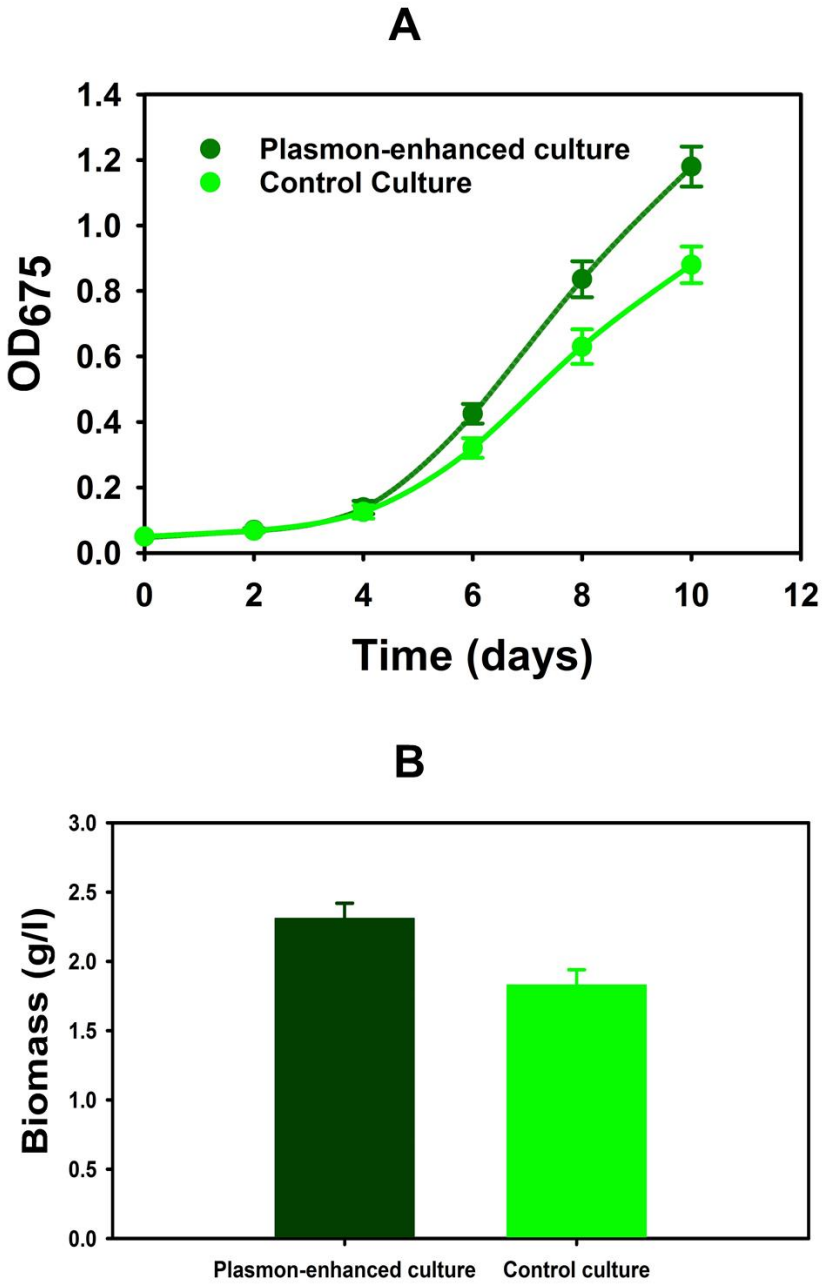


**Figure 2.4. Effect of plasmonic film filters consisting of silver nanospheres on microalgal biomass production.** Optical density at 675 nm ($OD_{675}$) at different culture times (A) and microalgal biomass production after ten days of cultivation period (B). Values represent means of 45 measurements and error bars are one S.D.

### 2.3.2 Effect of plasmonic film filters consisting of silver nanospheres on lipid production

Lipid production results from gravimetric measurements, after extraction using the modified Bligh and Dyer method, were consistent with results from fluorescence scanning using Nile Red dye. It was observed that more total lipid (0.252 ± 0.009 g/l with the film vs. 0.206 ± 0.009 g/l without the film) was obtained when the cells were cultured under plasmon-enhanced condition ($P < 0.05$) (Figure 2.5A). However, when normalized by dry biomass, there was no significant difference ($P > 0.05$) in the amount of lipid per unit cell mass (Figure 2.5B). This suggests that the higher lipid generation is not due to induction of lipid accumulation in microalgal cells, as observed under other light enhancement conditions [28]. Higher total lipid generation could normally be expected because of the increase in cell density. The FAME compositions of lipid extracts from plasmon-enhanced culture and control were very similar with a distribution predominantly between C18:1-3, C16:0, C16:1- 4, and C18:0 (Figure 2.5C). The FAME distribution is consistent with compositional screening of microalgae in other studies [29,30]. Together, these findings support the idea that lipid accumulation in individual microalgal cells is not affected by such plasmon-enhanced growth. This may be due to the fact that the difference in light intensity owing to plasmon resonance is not large enough to significantly influence the biochemical pathways of lipid production. In fact, significant effects are often seen with very high increase in light intensity (e.g. from 60 to 420 μE $m^{-2}$ $s^{-1}$) [27]. Light intensity effect on lipid production is also believed to be species-specific [27].

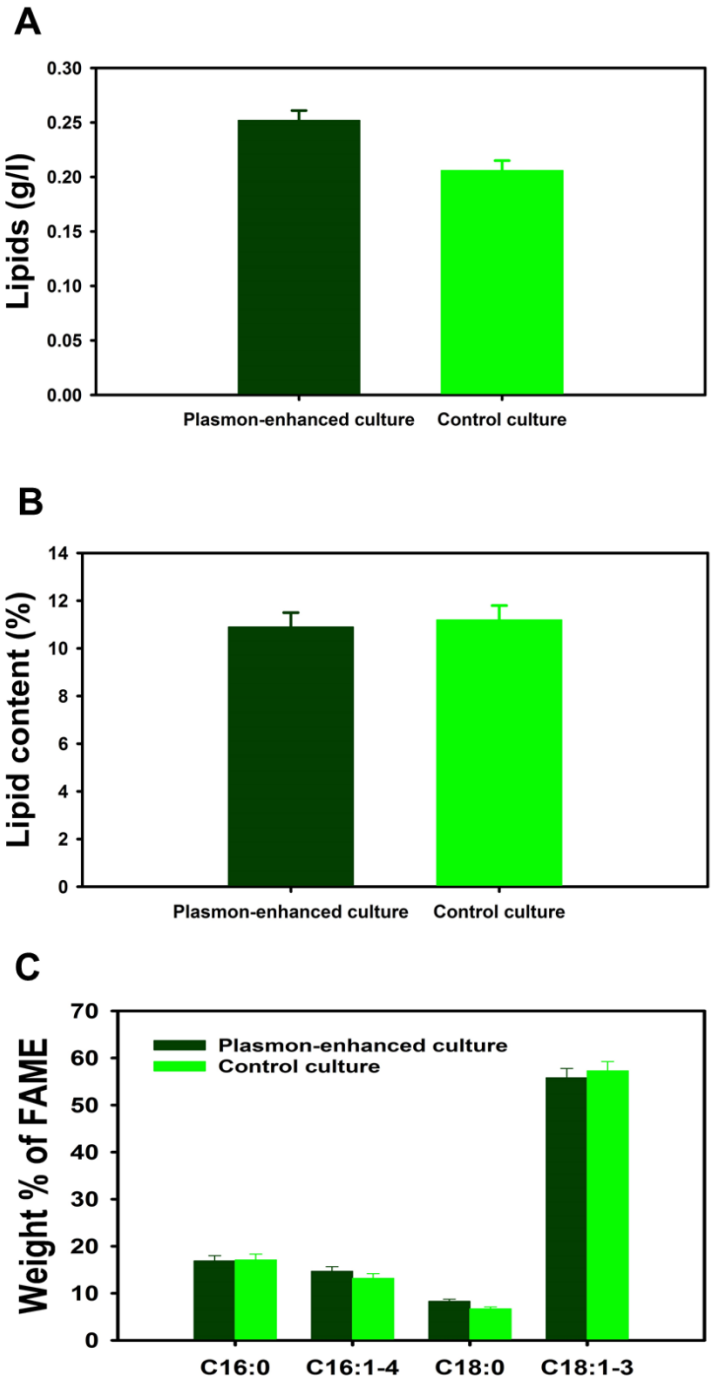


**Figure 2.5: Effect of plasmonic film filters consisting of silver nanospheres on microalgal lipid production.** Total lipid concentration (mg/l) (A), lipid in dry algal biomass (B) and fatty acid methyl esters distribution (C). Values represent means of 45 measurements and error bars are one S.D.

### 2.3.3 Effect of plasmonic film filters consisting of silver nanospheres on carbohydrate production

Similar to lipid production, there was an increase in total carbohydrate production (0.221 ± 0.008g/l with the film vs. 0.182 ± 0.008g/l without the film) (Figure 2.6A). However, when normalized by dry biomass the difference in carbohydrate contents was not significant ($P > 0.05$) (Figure 2.6B). This suggests that plasmon resonance does not increase carbohydrate accumulation in individual microalgal cells. Several other studies have shown increase in carbohydrate production with significantly larger increase in light intensity [31]. On the other end, other research results have suggested that there are no demonstrated impacts of increased light intensities on the accumulation of carbohydrate by microalgal cells. The work by Carvalho *et. al.* even reported that the carbohydrate content of microalgal cells was found to increase with increasing irradiance when experiments were performed at the temperature of 18°C, but decreased with increasing irradiance at when the culture experiments were performed 22°C [32]. In fact, a photochemical mechanism that explains a positive correlation between irradiance and carbohydrate accumulation has not yet been discovered [31].

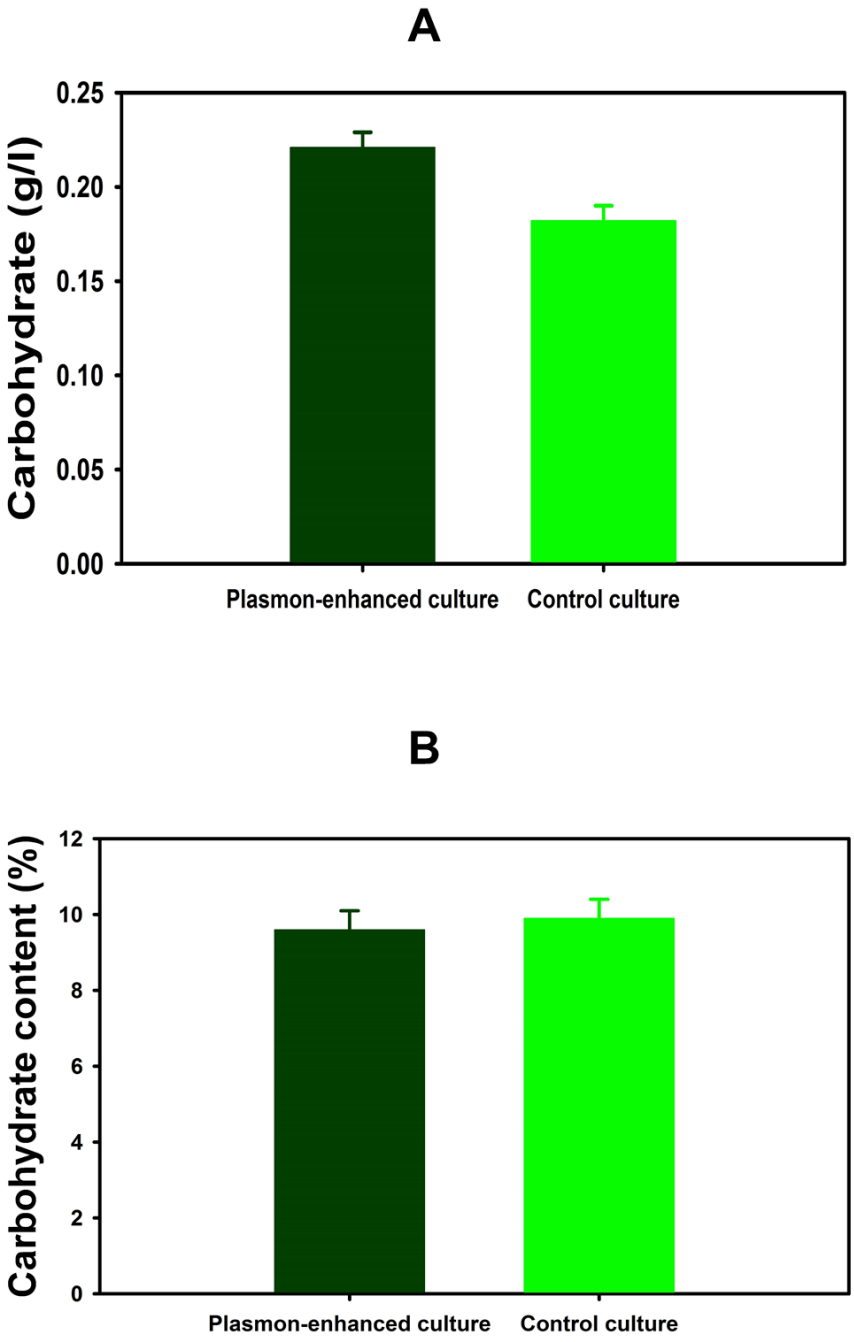


**Figure 2.6: Effect of plasmonic film filters consisting of silver nanospheres on microalgal carbohydrate production**. Carbohydrate concentration (g/l) (A) and weight percent of carbohydrate in dry microalgal biomass (B). Values represent means of 45 measurements and error bars are one S.D.

### 2.3.4 Effect of plasmonic film filters consisting of silver nanospheres on microalgal pigment accumulation

The effect of plasmon-enhanced growth on pigment accumulation was evaluated by measuring the total chlorophylls and the total carotenoids, after ten days of cultivation. The results show an increase in pigment concentration by more than 35% ($P < 0.05$) with the use of the plasmonic filter. The effect was more pronounced in total chlorophylls (15.3 ± 0.5 mg/l with the film vs. 10.7 ± 0.5 mg/l without the film) than total carotenoids (5.1 ± 0.2 mg/l with the film vs. 3.7 ± 0.2 mg/l without the film) (Figure 2.7A). This is likely due to the fact that the resonance peak of the plasmonic film is closer to the absorbance peak of chlorophyll a. Further, by normalizing with algal dry biomass, the pigment content per unit cell mass of the plasmon-enhanced culture exceeds the control by about 10% ($P < 0.05$) for carotenoids and 14% ($P < 0.05$) for chlorophylls (Figure 2.7B). These results show that plasmon resonance effectively improve pigment accumulation in microalgal cells.

The work by Bonente *et al.* reported a photo-acclimation phenomenon where under high light *Chlamydomonas reinhardtii* culture acclimated to an increase of growth and a decrease in pigment content per cell [33]. However, the irradiance referred to as high light in that work was 400 $\mu E/m^2/s$. This is about four times greater than the irradiance involved in this work. An alternative mechanism that corroborates our findings is chromatic acclimation as described by Mouget *et al.* where the additional blue light provided through plasmon resonance enhances growth rate, photosynthetic activity and pigment contents [21]. The experiments by Del Campo *et. al.* with a Chlorella species also with reported a significantly large increase of the cellular content of the carotenoid astaxanthin as the result of an increase in the culture light intensity [34]. The work by Eroglu *et al*. with nano-metallic suspensions also demonstrated that microalgal

pigment accumulation is enhanced by light enhancement owing to plasmon resonance although in our case the effect on carotenoid production was more pronounced [4]. This very last fact may be due to either the use of a different species (*Chlamydomonas reinhardtii* vs. *Chlorella vulgaris*) or the difference in incident light intensity (100 vs. 75 $\mu E/m^2/s$) which is a crucial factor in determining carotenoid accumulation [4].

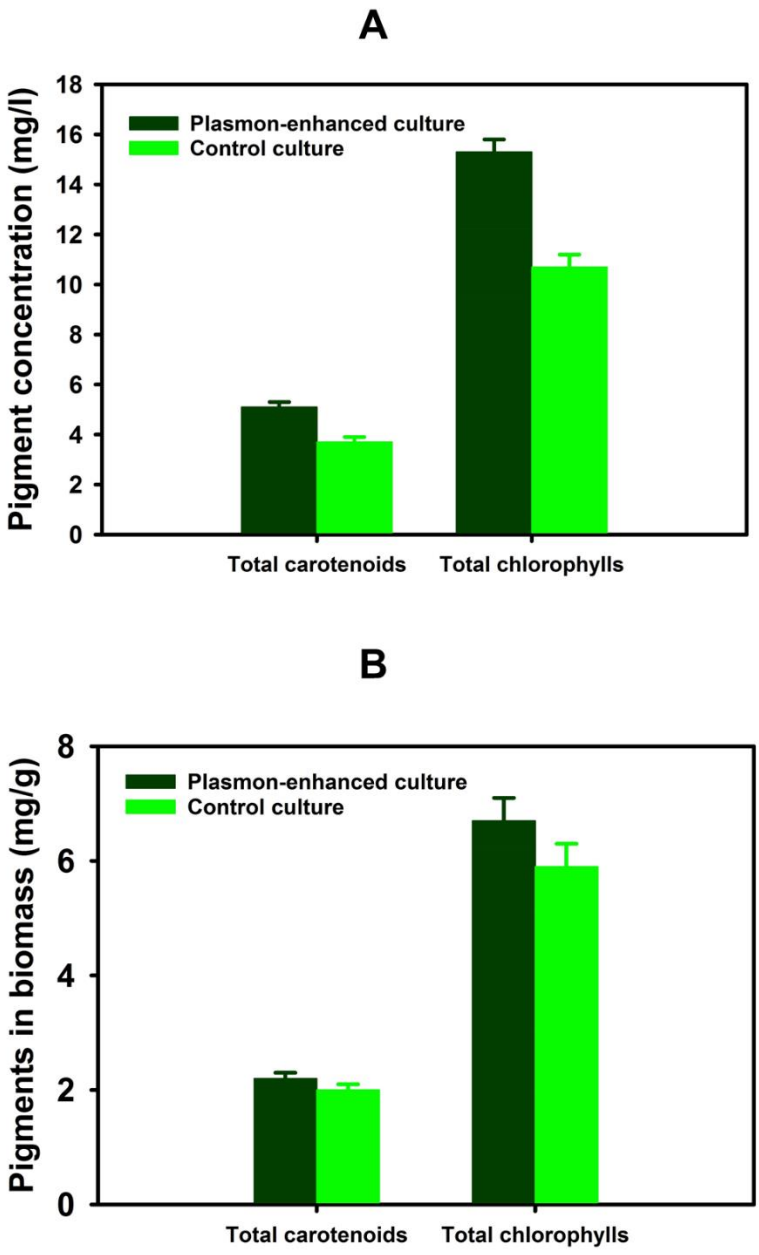


**Figure 2.7: Effect of plasmonic film filters consisting of silver nanospheres on microalgal photosynthetic pigment accumulation**. Pigment concentration (mg/l) (A) and pigment content in microalgal biomass (mg/g) (B). Values represent means of 45 measurements and error bars are one S.D.

### 2.3.5 Effects of plasmonic film filters with different backscattering wavelengths on microalgal growth and biomass composition

Different plasmonic films were tailored and fabricated to backscatter light at the specific wavelengths necessary for microalgal photosynthetic activities. Due to the proven effects of the plasmonic film consisting of silver nanoparticles on microalgal photosynthetic growth and carotenoids, chlorophyll a and chlorophyll b accumulation, the plasmonic films were tailored to enhance light in the violet-blue and red regions of the electromagnetic spectrum necessary for microalgal growth and accumulation of the related pigments. While the silver nanoparticles synthesized via the sodium borohydride methods have a fixed backscattering peak in the violet-blue region of the electromagnetic spectrum, the modified Nikoobakht & El-Sayed method can lead to gold nanorods of different aspect ratios and backscattering peaks at different wavelengths by changing the amount of seed solution or the amount of silver nitrate added in the nanorods growth mixture. We decided to synthesize gold nanorods with extinction spectra peak of 675 nm due to largely reported effects of light of such wavelength on microalgal growth, and total chlorophylls production [35].

Similar to the plasmonic film consisting of silver nanospheres, the gold nanorods based plasmonic films and the dual wavelength plasmonic films consisting of both silver nanospheres and gold nanorods were used to wrap the base of conical flasks containing the microalgal broths which were then covered with a black tape to ensure that light was only coming from the top of the microalgal culture. Microalgal growth in such cultivation systems was monitored through optical density measurements ($OD_{675}$) and compared against a control without plasmonic films. The growth curves constructed through regular optical density measurements exhibited a similar trend to that reported for enhancement of light in the blue region of the electromagnetic spectrum

by silver nanospheres, with more pronounced and increasing effects after the fourth cultivation day. However, by comparing the enhancement of light solely in the blue region of the electromagnetic spectrum using silver nanospheres to the enhancement of light solely in the red region of the electromagnetic spectrum using gold nanospheres, the effects were more pronounced for the last one leading to an increase in microalgal biomass production by 30% compared to 25% ($P< 0.05$) with silver nanospheres. This may be due to the fact that the absorption peak of the plasmonic film consisting of gold nanorods covers the red light absorption peaks of the photosynthetic microalgae better than the silver nanospheres based plasmonic film does in the blue region. Further, the gold nanorods based plasmonic films led to a total carotenoids and a total chlorophylls concentration of 4.9 mg/l and 15.1 mg/l respectively compared to the respective concentrations of 3.4 mg/l and 9.9 mg/l in the control culture. When normalized by the dry biomass, the pigment contents per dry biomass unit obtained with the gold nanorods based plasmonic film were 6.8 mg/g for total chlorophyll and 2.2 mg/g for total carotenoids as opposed to 5.9 mg/g and 2.0 mg/g obtained with the control culture which again corroborate a clear increase in pigment accumulation through plasmon enhancement. The dual wavelengths plasmonic led to a significantly higher biomass production by accusing a 50% increase in microalgal biomass production as compared to the control culture after the ten days cultivation period. The cumulative effects of dual enhancement of both violet-blue and red lights through plasmon enhancement also led to a very high pigment accumulation accusing up to a 78% increase in total chlorophyll production as compared to the control culture.

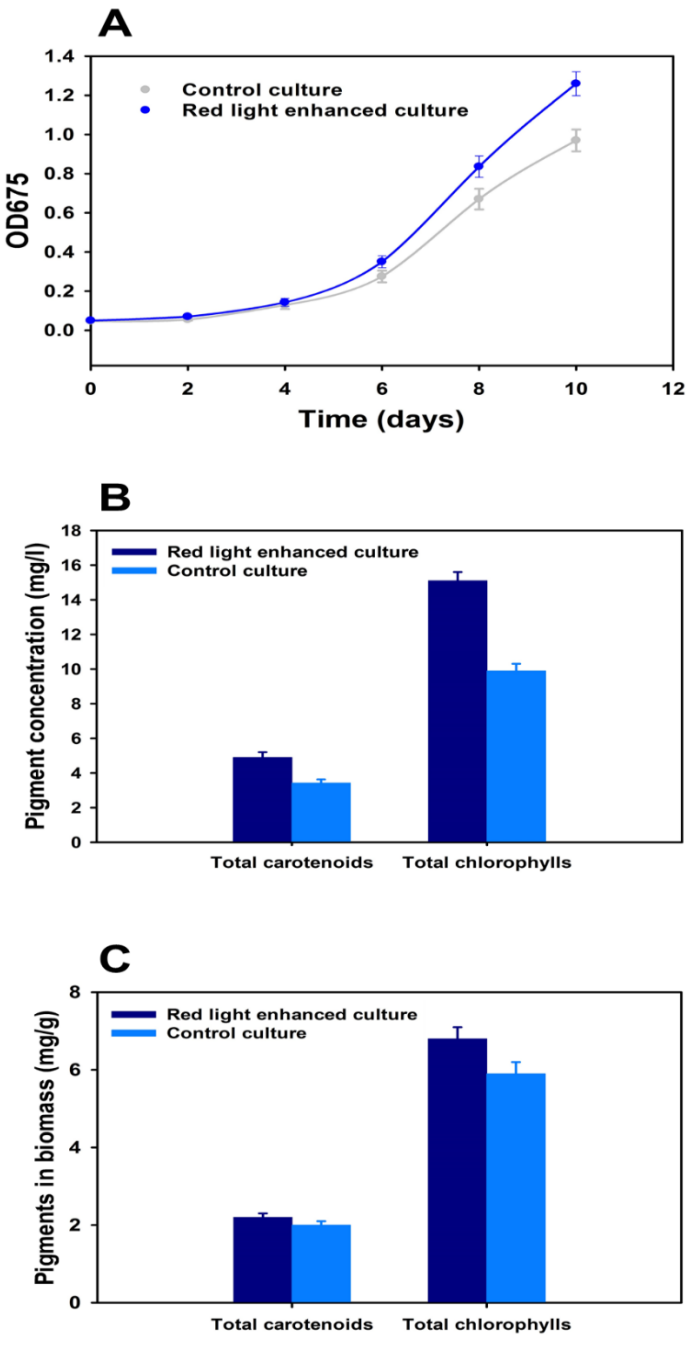


**Figure 2.8: Effects of plasmonic films consisting of gold nanorods on microalgal growth and biomass composition.** Microalgal growth curve (A), pigment concentration (g/l) (B) and pigment contents in dry microalgal biomass (mg/g) (C). Values represent means of 45 measurements and error bars are one S.D.

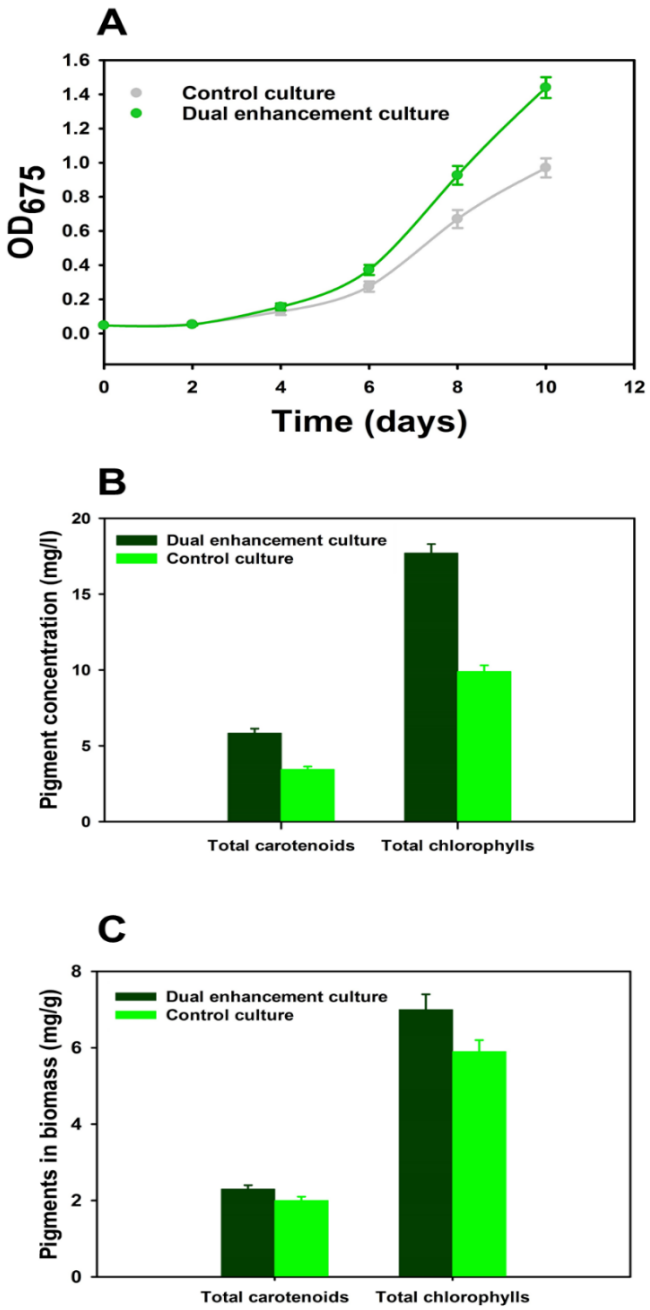


**Figure 2.9: Effects of plasmonic films consisting of gold nanorods and silver nanospheres on microalgal growth and biomass composition.** Microalgal growth curve (A), pigment concentration (g/l) (B) and pigment contents in dry microalgal biomass (mg/g) (C). Values represent means of 45 measurements and error bars are one S.D.

## 2.4 Conclusions

In summary, plasmonic films with different extinction wavelengths were fabricated and used as light filters that can selectively enhance light absorption in microalgal culture. First, through plasmon resonance by the spherical silver nanoparticles embedded in the film, blue light was backscattered and supplemented to a *Chlamydomonas reinhardtii* culture. This led to an increase in microalgal biomass production by more than 25% and an increase in total photosynthetic pigment generation by more than 35%, after ten days of cultivation. Light enhancement by plasmon resonance did not affect cellular carbohydrate and lipid accumulation although the increase in cell density resulted in enhanced overall lipid and carbohydrate production. The plasmonic film technology was then extended to plasmonic films consisting of nanoparticles of different shapes (e.g. rods, mixtures of spheres and rods) that have been tested for backscattering of blue and/or red lights to microalgal culture [36]. The results with plasmonic films containing both silver nanospheres and gold nanorods proved that biomass production and photosynthetic pigments accumulation can be increased by up to 50% and 78% respectively through light enhancement induced by the use of plasmonic film filters. With all these beneficial effects, this technology has significant potential to be scaled up and applied to large scale photobioreactors for microalgal culture. Although the implementation should be easier and safer compared to the use of nano-suspensions, further investigation is still needed to enable scale-up and optimization of the plasmonic film technology. One issue to consider during the application of this plasmonic film light filter technology to the design of large scale microalgal photobioreactors is the fact that the light enhancement through plasmon resonance is only effective over a few centimeters of light path length. Strategies to palliate this effect may include the development of short light path microalgal cultivation systems and also the

coordinated cycle of the microalgal cells to the zones with enhanced irradiance during the cultivation period. We will further discuss these two ideas in chapter 3 and chapter 5 of this dissertation. Another issue to overcome is to prevent microalgal adhesion and biofilm formation in the surface of the cultivation system that would hinder light penetration and decrease the effects of plasmon enhancement. This last issue is discussed in detail in chapter 4. Finally, efforts to optimize photosynthetic light utilization by microalgae, if successful, may mitigate the risk of efficiency loss through light saturation and potentially broaden the application of the plasmonic film technology[37].

**Acknowledgments**

We acknowledge Professor Mathew M. Maye (Chemistry Department, Syracuse University) for providing access to the Fluoromax-4 spectrofluorometer as well as Professors Jesse Q. Bond and Lawrence L. Tavlarides (Biomedical and Chemical Engineering Department, Syracuse University) for the use of the GC-MS and the GC-FID systems respectively. B.E. is grateful for help from graduate students Tao Cong (nanoparticle synthesis), Ali Adem Bahar (sonicator), Srujana Govindarajulu (algal culture), Yue Nan (GC-FID) and Anargyros Chatzidimitriou (GC-MS) and, financial support through a fellowship under the Syracuse University - Université d'Etat d'Haïti student exchange program.

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# CHAPTER 3

## Development of a short light path energy efficient microalgal cultivation and harvesting system using thermoreversible sol-gel transition

Part of this chapter has been published as Bendy Estime, Dacheng Ren, and Radhakrishna Sureshkumar. "Cultivation and energy efficient harvesting of microalgae using thermoreversible sol-gel transition". Scientific Reports. 7: 40725 (2017).

**Abstract**

Microalgae represent a promising source of renewable biomass for the production of biofuels and valuable chemicals. However, energy efficient cultivation and harvesting technologies are necessary to improve economic viability. Several issues related to the distribution and absorption of light in microalgal cultivation systems as well as the energy efficient harvesting of the biomass produced were addressed by developing a new microalgal cultivation and harvesting system. Specifically, a Tris-Acetate-Phosphate-Pluronic (TAPP) medium that undergoes a thermoreversible sol-gel transition was developed to efficiently culture and harvest microalgae without affecting the productivity as compared to that in traditional culture in a well-mixed suspension. After seeding microalgae in the TAPP medium in a solution phase at 15°C, the temperature was increased by 7°C to induce gelation. Within the gel, microalgae were observed to grow in large clusters rather than as isolated cells. The settling velocity of the microalgal clusters was approximately ten times larger than that of individual cells cultured in typical solution media. Such clusters were easily harvested gravimetrically by decreasing the temperature to bring the medium to a solution phase.

## 3.1 Introduction

The development of methods for high throughput cultivation and efficient harvesting of microalgae has, over the past decades, constituted an active field of research[1, 2]. Despite major advances, there is still a need to optimize and increase productivity in microalgal cultivation systems in order to make microalgal biofuels production a more viable option[3, 4]. It is also imperative to improve microalgal harvesting processes which currently account for about thirty percent of total production cost[5].

Many cultivation methods have been proposed to improve microalgal biomass production. For instance, growth medium modifications with high salt and nutrient deprivation have been used to enhance accumulation of specific chemicals such as lipids and carbohydrates[6,7]. Furthermore, biofilm and biofouling of microalgae that are often portrayed as challenges for suspended culture have recently been explored as cultivation methods for large scale microalgal biomass production[8]. Among many others, the large decrease in water consumption and the simplification of the harvesting process are considered as two major benefits of biofilm cultivation of microalgae[9,10]. As for suspended cultivation, constant mixing is usually necessary during the entire cultivation period and the current harvesting methods often involving centrifugation, pumping or electrophoresis techniques are largely energy intensive. The alternatives that have been proposed thus far are yet to resolve the energy consumption issue[11].

Motivated by the need for short light path microalgal cultivation systems and the need for energy efficient harvesting technologies, we aimed at exploring a microalgal cultivation and harvesting strategy using the thermoreversible copolymer pluronic. Pluronic is an amphiphilic

ABA type copolymer composed of both hydrophobic Polypropylene Oxide (PPO, B) block parts and hydrophilic Polyethylene Oxide (PEO, A) block parts known for its good biocompatibility and low toxicity[12]. The applications of this copolymer are highly diversified. For example, the copolymer pluronic F-127 is believed to be a good carrier for drug delivery and is therefore valuable in pharmaceutical formulations[13]. Pluronic has also largely been investigated for its potential in controlling biofouling[14]. Moreover, this copolymer is well known for its effectiveness in producing stable surface patterns and can be useful in long term single-cell culture[15,16]. Note that single-cell cultivation of microalgae has been proposed as a good method for preparing colonies of promising strains for large scale cultivation[17].

The temperature dependent sol-gel transition behavior of the copolymer pluronic along with the largely reported biocompatibility inspired its use in microalgal cultivation. An aqueous solution of pluronic would robustly undergo a phase transition to an elastic gel when heated above a gelation temperature $T_g$. This gelation process is induced by a thermodynamic self-assembly of the copolymer molecules into an inter-connected micellar network and is reversible, i.e., the gel can be brought back into the solution phase by cooling it below $T_g$[18]. Depending on the concentration of the pluronic polymer in the aqueous phase, $T_g$ values range between 15°C to 30°C[19, 20]. This intersects with the temperature range often used for microalgal cultivation.

The use of pluronic in the development of this medium may help solve several issues related to the distribution and absorption of light in microalgal cultivation systems. First, because this medium would allow microalgae to grow in a confined environment, light delivery to these photosynthetic microorganisms can better be controlled. Second, the very confinement would prevent microalgae from sticking to the wall of photobioreactors which often hinders light penetration in photobioreactors. Ultimately, the gel structure of the medium during the growth

period offers a lot of flexibility to divide the systems into short light path layers and also would offer a lot of flexibility when it comes to the orientation and inclination of the cultivation systems to receive the optimal irradiance.

Herein, a thermoreversible Tris-Acetate-Phosphate-Pluronic (TAPP) medium for energy efficient cultivation and harvesting of microalgae is presented. The thermorheological properties of the pluronic-based TAPP medium were systematically characterized to determine the optimal operating conditions for this novel system. These same properties were also characterized after microalgal cultivation to verify whether such properties would not alter significantly due to microalgal proliferation in the system. Further, cultivation experiments are performed using microalga *Chlamydomonas reinhardtii* and microalgal biomass production in the TAPP medium is assessed both qualitatively and quantitatively. Furthermore, a framework is proposed to efficiently harvest the microalgal biomass produced through relatively small variations of temperature (Figure 3.1). Finally, the microalgal biomass harvesting parameters are characterized and the harvesting efficiency is quantified using the experimental results.

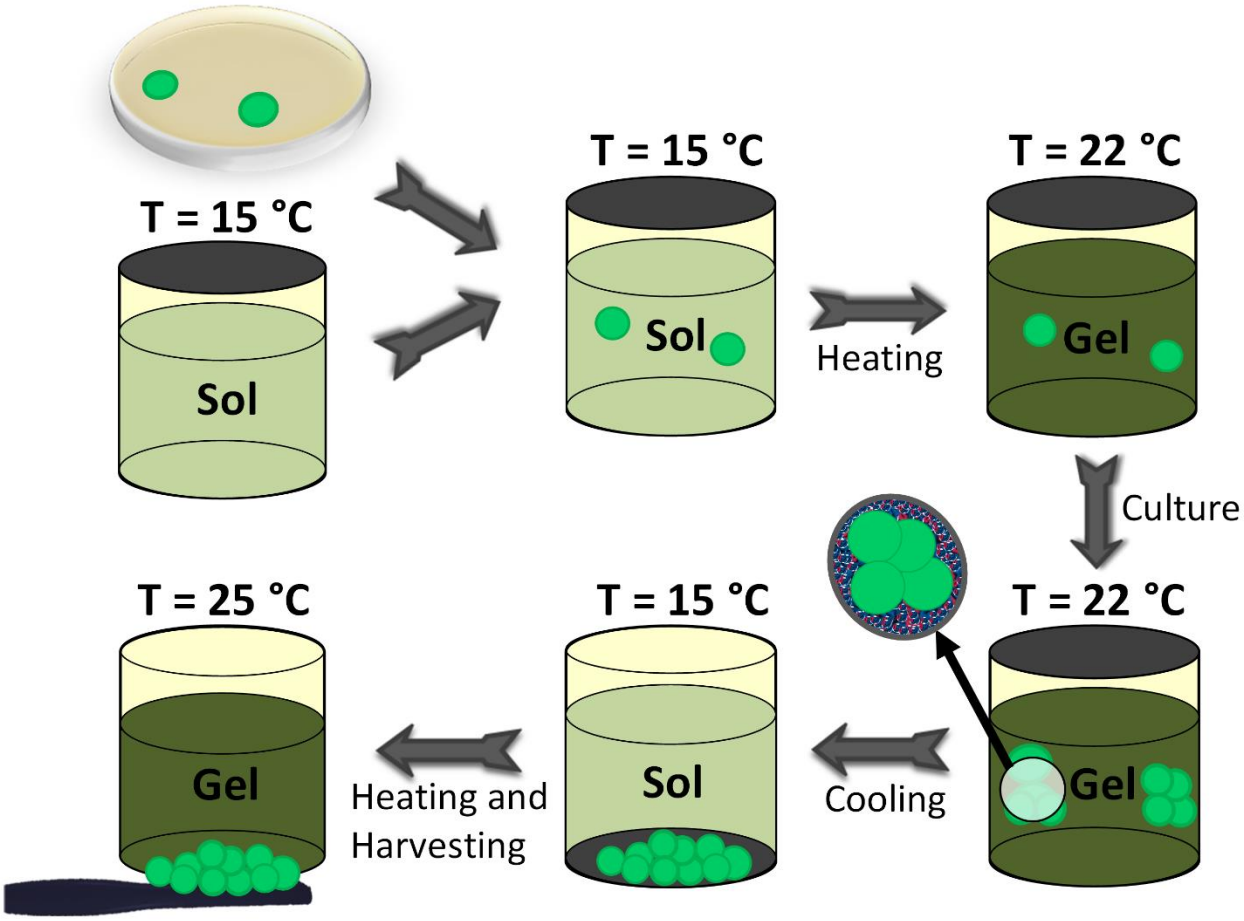


**Figure 3.1: Schematic of microalgal cultivation and harvesting process using thermoreversible sol-gel transition.** Microalgal cells are seeded in the TAPP medium in solution phase at 15°C. Then, the temperature is raised at 22°C for gelation of the medium and entrapped microalgal cultivation. After the cultivation period, the temperature is decreased to 15°C allowing microalgal clusters to gravimetrically settle at the bottom. The temperature is finally raised to 25°C and settled microalgal clusters are scraped off the TAPP surface.

## 3.2 Materials and Methods

### 3.2.1 TAPP medium preparation and culture conditions

Pluronic F-127 was obtained from BASF (Ludwigshafen, Germany) and was used without further purification. The chemical composition for this pluronic type is PEO100PPO65PEO100 and the total molecular weight is 12600 g $mol^{-1}$. PEO and PPO ratio is approximately 2:1 by weight. The pluronic-based growth medium was prepared in a way that maintains a concentration of nutrients similar to the traditional Tris-Acetate-Phosphate (TAP) medium with the addition of pluronic (TAPP medium) that confers the thermoreversible sol-gel transition properties.

The concentrations of chemicals in the TAPP medium were therefore as follows: Tris (19.98 mM), $NH_4Cl$ (7.011 mM), $MgSO_4.7H_2O$ (0.406 mM), CaCl2.2H2O (0.34 mM), $K_2HPO_4.3H_2O$ (0.47 mM), $KH_2PO_4$ (0.40 mM), acetic acid (0.1 % vol), Hutner's trace (0.1 % vol). Pluronic F-127 powder was dissolved in the medium at 4°C for 5 hours and under vigorous stirring. Note that in the new Tris-Acetate-Phosphate-Pluronic medium, just like in the traditional Tris-Acetate-Phosphate medium, acetate is used as the carbon source for microalgal growth. Final concentrations of pluronic in TAPP media were selected to be 18%, 20% and 22% (weight percent) in order to obtain a range of $T_g$ suitable for the microalgal cultivation and harvesting application.

Cultivation of the wild type microalgae *Chlamydomonas reinhardtii* CC-124, obtained from the Chlamydomonas Resource Center (University of Minnesota, St. Paul, Minnesota), was performed in the TAPP medium as follows: Aliquots of 0.5 ml from liquid subcultures prepared five days preceding the experiment were mixed with 50 ml of TAPP medium at 15°C (below $T_g$

for all the samples). Subsequently, vials containing microalgal culture were placed on a rotary shaker in a room continuously illuminated by full spectrum compact fluorescent lamps (CFL 60W, Fancierstudio, San Francisco, California) with the photosynthetic active radiation at the top surface of the culture at 100 ± 5 μE $m^{-2}$ $s^{-1}$ and the temperature at 22 ± 1°C. After seven days of cultivation, the resulting TAPP-microalgae matrix was used for thermorheological characterization and biomass production analyses.

### 3.2.2 Rheometry

The rheological experiments to characterize the properties of the TAPP medium were performed using a Combined Motor and Transducer AR-G2 rheometer from TA instruments (New Castle, Delaware). The cone-and-plate geometry with a diameter of 40 mm and a cone angle of 0° 59' 49" was used for all the measurements. The temperature control was achieved by a peltier plate using thermoelectric effects to control the temperature accurately and water circulation for rapid heating and cooling over a temperature range of 0 to 100°C. Strain sweep measurements were performed on the TAPP media samples in order to determine the experimental parameters for the linear viscoelastic (LVE) regime in which the elastic and loss moduli were measured using small amplitude oscillatory shear flow experiments. It was found that for all the samples, a 0.5% strain at a frequency of 1 Hz was appropriate for measuring the LVE properties.

### 3.2.3 Characterization of microalgal biomass production and harvesting

The effects of pluronic presence on microalgal cultivation and biomass production were assessed using different analytical techniques. Microalgal biomass production was evaluated through optical density measurements and gravimetric quantification. Microalgal biomass

carbohydrate content was assessed using the phenol-sulfuric acid method[21]. Specifically, 2 mg of dry microalgae was mixed with 2 ml of water. Subsequently, 50 µl of 80% phenol was added, followed by a rapid addition of 5 ml of concentrated sulfuric acid. The mixture was then placed in a 50°C water bath for 20 minutes. Following this, the absorbance at 490 nm was determined and compared to a standard curve based on glucose.

Lipids content was assessed through a modified Bligh and Dyer method and fluorescence scanning using Nile Red dye[22,23,24]. Briefly, each microalgal sample was centrifuged (4,800g, 5 min, 20°C) to remove excess water. Cell lysis for lipid release was assured by sonication (Q500 Sonicator, Qsonica, LLC., Newtown, Connecticut) with a 20/10 seconds ON/OFF cycle for 2 minutes; 5 ml of chloroform, 10 ml of methanol and 5 ml of deionized water were then added to the sonicated samples. To facilitate phase separation, the samples were placed on a rotary shaker (100 rpm) for 6 hours and 5 more ml of chloroform and deionized water were then added prior to centrifugation (4,800g, 5min, 20°C). Following this, the lipid rich chloroform layer was removed and dried for gravimetric measurement.

Nile Red fluorescence determination of neutral lipids was also used as a means to verify results obtained by gravimetric quantification. Specifically, microalgal cells were first harvested from the growth medium via centrifugation (4,800g, 5 min, 20°C) and resuspended in water. Following this, 5 ml of this microalgal suspension was stained with 20 µl of a solution of Nile Red in acetone (0.25 g/l). After 30 minutes, fluorescence scanning (excitation wavelength: 490 nm, emission wavelength: 520-750 nm) was performed using a Fluoromax-4 spectrofluorometer (Horiba, Ltd., Kyoto, Japan).

The impacts of the pluronic-based environment on shape and size of microalgal cells were systematically analyzed using an Axio Imager M1 microscope and a ZEN pro software (Carl Zeiss Inc., Berlin, Germany). These same tools were also used to characterize the distribution of microalgal cells (clusters etc.) in TAPP media with different pluronic concentrations as well as the traditional TAP medium used as control. The shape and size of microalgal clusters were characterized in order to predict the settling velocity for harvesting. The form factor (*FF)* characterizing the deviation from a circle and the equivalent diameter (*De*) of microalgal clusters were computed as presented by Grijspeerdt and Verstraete[25]:

$$FF = \frac{4*\pi*Area}{Perimeter^2} \quad (1)$$

$$De = 2 * \sqrt{\frac{Area}{\pi}} \quad (2)$$

The settling velocity (*V*) of microalgal clusters during the harvesting process could be approximated using Stokes' law as long as the form factor concurred with a spherical shape and the Reynold number fell within the Stokes' regime. Under such conditions the settling velocity was calculated as[26]:

$$V = \frac{(\rho s - \rho l)*g*De^2}{18\mu} \quad (3)$$

where $\rho s$ and $\rho l$ are solid and liquid densities, $g$ the acceleration due to gravity, *De* the equivalent diameter and $\mu$ the solvent viscosity. The average settling rate was then experimentally monitored by allowing microalgae to settle at 15°C in columns with 10 cm working height and by taking regular optical density measurements ($OD_{675}$) of the broths. To estimate the settling rate, the percent recovery at each measurement time was computed as follows[27]:

$$\% \, Recovery = \left(1 - \frac{OD_{675}(t)}{OD_{675}(to)}\right) * 100 \quad (4)$$

where $OD_{675}(t)$ is the optical density of the broth after a settling time ($t$) and $OD_{675}(to)$ the optical density at the beginning of the settling experiment or time ($to$).

### 3.2.4 Statistical analyses

The microalgal cultivation and harvesting experiments and the related biochemical and rheological analyses mentioned previously were repeated at least 10 times with triplicate measurements for each run. Statistical analyses over the data collected were performed using Minitab software. The results with P-Value less than 0.05 (t-test) were considered statistically significant.

## 3.3 Results and discussion

### 3.3.1 Rheological characterization of the TAPP medium

In order to obtain a range of pluronic concentrations that can confer the suitable properties necessary for the proposed thermoreversible microalgal cultivation and harvesting system, TAPP media with different pluronic concentrations were prepared and were subjected to rheological testing. Specifically, the linear viscoelastic properties, namely the storage (G') and loss (G") moduli were measured as a function of temperature T using a small amplitude oscillatory shear flow experiment. The storage and loss moduli are material functions characterizing the response from a small amplitude oscillatory shear flow. The storage modulus (G') relating the elastic property of the material represents the portion of the stress wave that is in phase with the strain wave whereas the loss modulus (G") relating the viscous property of the material represents the portion of the stress wave that is out of phase with the strain wave. Therefore, when the TAPP medium is in the viscous solution phase, G" >> G'. The gel point $T_g$ (or the critical micellation temperature, CMT) is defined as the temperature for which G' = G"[28]. For $T > T_g$, G' exceeds G" and reaches a plateau. Further, the zero-shear viscosity $\eta_0$, i.e., viscosity of the samples under very small shear rates (near-equilibrium conditions), was also measured. Gelation is accompanied by a sharp increase in $\eta_0$.

Typical results for G', G" and $\eta_0$ obtained from dynamic temperature ramp experiments are reported in Figure 3.2. For low T, the moduli of pluronic-based media are relatively low. It is in fact known that, at low T, pluronic in water solution tends to adopt the form of a unimer[29]. Therefore, low entanglement between the chains would lead to low moduli. As the temperature was increased, the breakage of hydrogen bonds and aggregation of hydrophobic PPO blocks

stimulated gelation and led to sharp increases in the moduli (by up to six orders of magnitude)[30]. The gel points $T_g$ were found to be 20.1°C, 21.8°C and 23.9°C respectively for the 22, 20 and 18 weight percent pluronic in TAPP media samples (Figure3.2a). The decrease in $T_g$ with increasing concentration can be attributed to an increase in molecular entanglements with increasing concentration[31]. The viscosity profiles of the pluronic-based media exhibited a behavior similar to that of the moduli during the temperature ramp experiment where the viscosity increases significantly for $T > T_g$ (Fig. 2b). This is likely due to the fact that with an increase in temperature the polymer chains dehydrate and begin to cross-link leading to a closely packed polymeric network[32,33]. Accordingly, the mesh size of the network decreases with increasing temperature and increasing pluronic concentrations with values ranging from tens to thousands of nanometers[34].

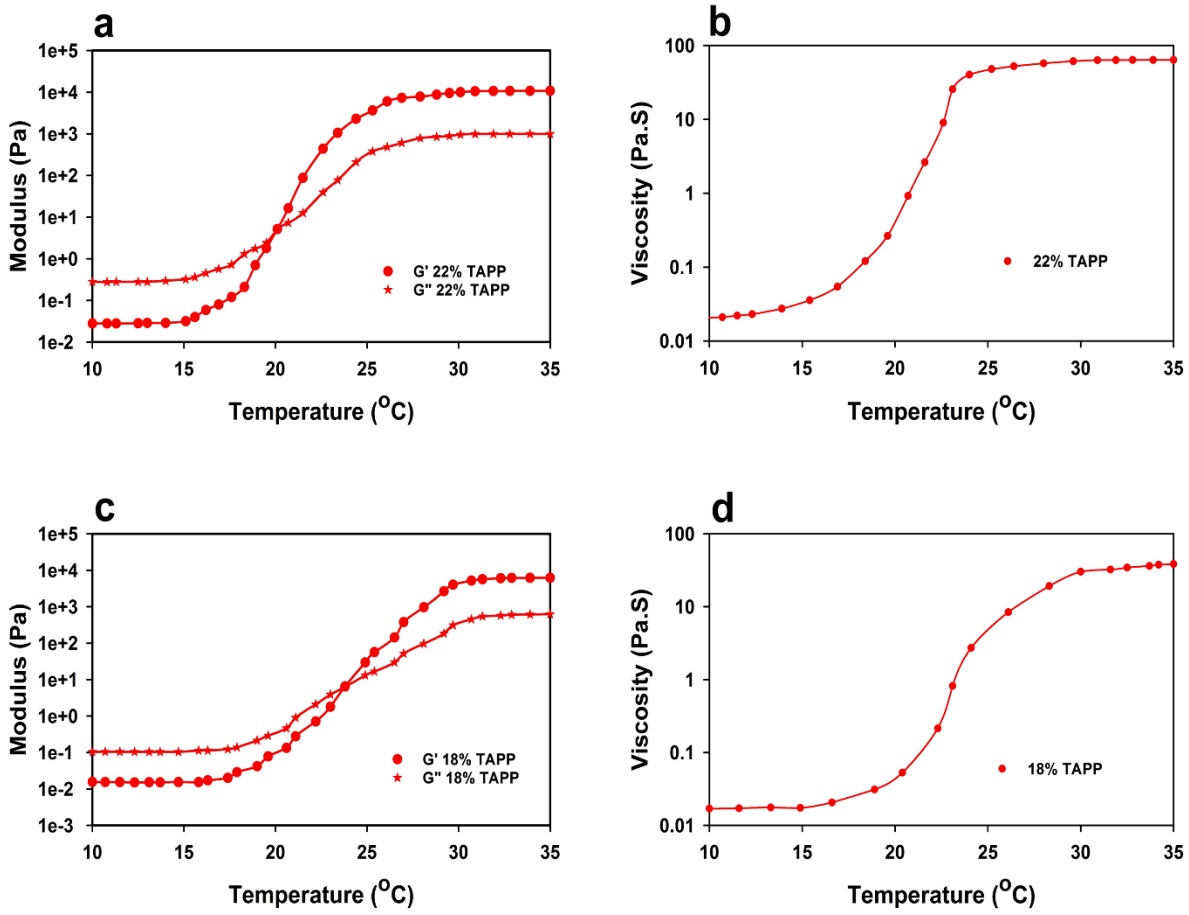


**Figure 3.2: Thermorheological properties of the TAPP medium.** (**a**) Storage modulus (circle) and loss modulus (star) of the 22% pluronic in TAPP sample as a function of temperature, (**b**) viscosity profile of the 22% pluronic in TAPP sample as a function of temperature, (**c**) storage modulus (circle) and loss modulus (star) of the 18% pluronic in TAPP sample as a function of temperature and (**d**) viscosity profile of the 18% pluronic in TAPP sample as a function of temperature.

### 3.3.2 Microalgal biomass production in the TAPP medium

The wild type microalga *Chlamydomonas reinhardtii* CC-124 was allowed to grow in the TAPP media with 3 different pluronic concentrations (18, 20 and 22 weight percent) and a traditional well-mixed TAP medium culture, used as a control. Therefore, at the operating temperature (22°C), the 18% TAPP medium would still be in early micellation stage (i.e., a viscous liquid), the 20% TAPP medium would be close to $T_g$ (i.e., a soft gel) and the 22% TAPP sample in an elastic solid-like state. The initial microalgal biomass concentration for all the samples was set to 0.1 g $l^{-1}$. Microalgal growth was assessed through optical density ($OD_{675}$) analyses and gravimetric measurements. Microalgal biomass concentrations, after a seven day cultivation period, were found to be 3.1 ± 0.2 g $l^{-1}$, 2.9 ± 0.3 g $l^{-1}$, 2.8 ± 0.4 g $l^{-1}$ and 2.9 ± 0.4 g $l^{-1}$, respectively for the well-mixed TAP medium control, the 18% TAPP, the 20% TAPP and the 22% TAPP samples ($P > 0.05$) (Figure 3.3a). The content in lipid and carbohydrate of the microalgal biomass generated did not vary significantly ($P > 0.05$) under these four conditions (Figure 3.3b). These results indicate that microalgae grow well in the TAPP medium with no statistically significant variations in the composition of the generated biomass unlike those commonly observed in many other experiments with growth medium modification[6]. Microalgal growth was also tested in a pluronic-based medium with an inorganic carbon source. Towards this end, microalgae were grown in a minimum medium enriched with 20 mM of sodium bicarbonate ($NaHCO_3$) and 22% (weight) pluronic. Note that this medium had the same composition as the TAPP medium except that in this case sodium bicarbonate was used as the carbon source in lieu of acetic acid. Under the same culture conditions, the pluronic-based medium with inorganic carbon source led to a microalgal biomass production of 1.7 ± 0.3 g $l^{-1}$ whereas a control minimum medium enriched with 20 mM of sodium bicarbonate not containing

pluronic led to a microalgal biomass production of $1.9 \pm 0.2$ g $l^{-1}$ ($P>0.05$). This proves that pluronic-based media with organic as well as inorganic carbon sources are conducive for the growth of microalgae.

The shape and size of microalgal cells confined in the TAPP media were assessed through microscopic analyses. While there was no significant change ($P > 0.05$) in the shape of the cells, their size decreased significantly ($P < 0.05$) when growing in the TAPP medium. Average cell diameters were found to be $8.1 \pm 0.6$ μm, $6.9 \pm 0.5$ μm, $6.7 \pm 0.4$ μm and $6.3 \pm 0.5$ μm, respectively for growth in the well-mixed TAP medium control, the 18% TAPP, the 20% TAPP and the 22% TAPP samples (Figure 3.3c). This decrease in cell size could be attributed to confinement of the microalgal cells by the surrounding polymeric network. In fact, at the operating conditions, the three TAPP samples were at different micellation stages and their elastic moduli and mesh sizes exhibited significant differences, which corroborate the observed variation in cell size[34]. Nonetheless, the decrease in cell size does not undermine the fact that the thermoreversible polymer pluronic F-127 can be used in microalgal cultivation since the biomass productivity and composition did not exhibit any significant differences from the control.

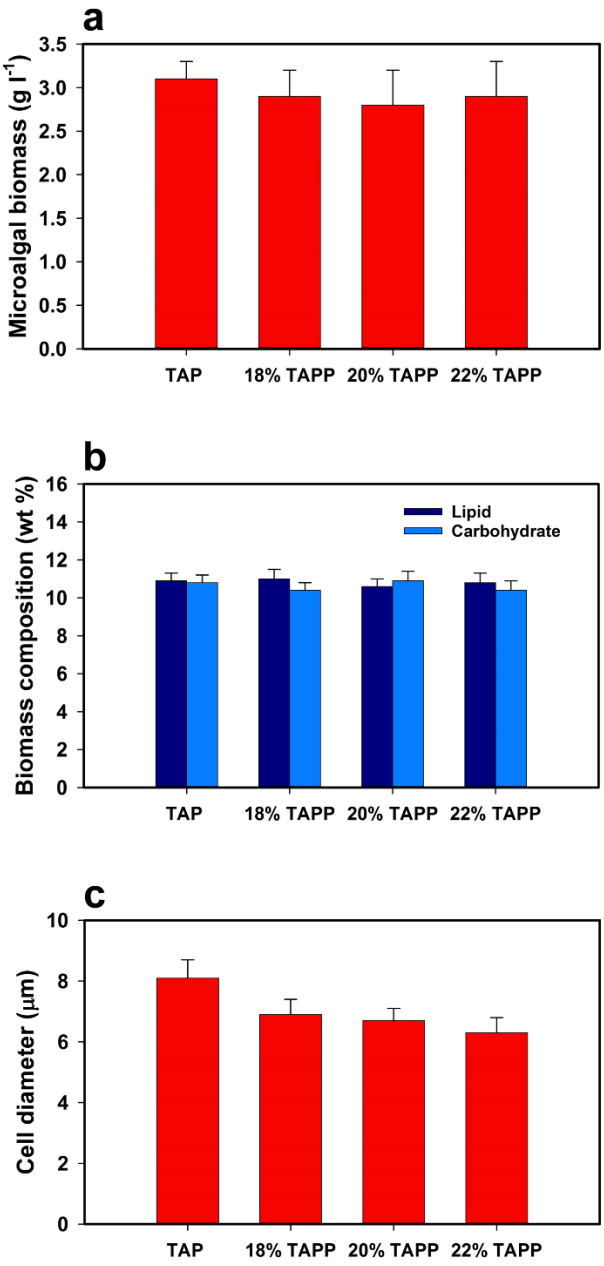


**Figure 3.3: Microalgal biomass production in the TAPP medium.** (**a**) Microalgal biomass generation (g $l^{-1}$), (**b**) weight percentage of lipid and carbohydrate in microalgal biomass and (**c**) average diameter of microalgal cells growing in the well-mixed TAP control, the 18% TAPP, the 20% TAPP and the 22% TAPP. Bars represent means of 30 measurements and error bars are one S.D.

### 3.3.3 Influence of microalgal proliferation on the thermorheological behavior of the TAPP medium

The effects of proliferation of microalgal cells on the thermorheological properties of the TAPP medium were also assessed since large variations on such properties might impact its applicability in microalgal cultivation and harvesting. For this reason, rheological measurements were made on the microalgae-TAPP matrix resulting from the seven day cultivation period. It was observed that, with all three pluronic concentrations (22, 20 and 18 weight percent pluronic), there was a slight decrease in $T_g$ in presence of microalgal cells. Specifically, $T_g$ decreased from 20.1°C to 19.2°C, 21.8°C to 20.9°C and 23.7°C to 22.8°C respectively for the 18% TAPP, the 20% TAPP and the 22% TAPP samples (Figure 4a, 4b). This is likely due to the hydrophobic interactions between microalgal cell walls and the hydrophobic PPO blocks of the pluronic molecules resulting in an acceleration of the micellation process[35]. Nonetheless the decrease in the $T_g$ is relatively small and does not affect the microalgal cultivation and harvesting.

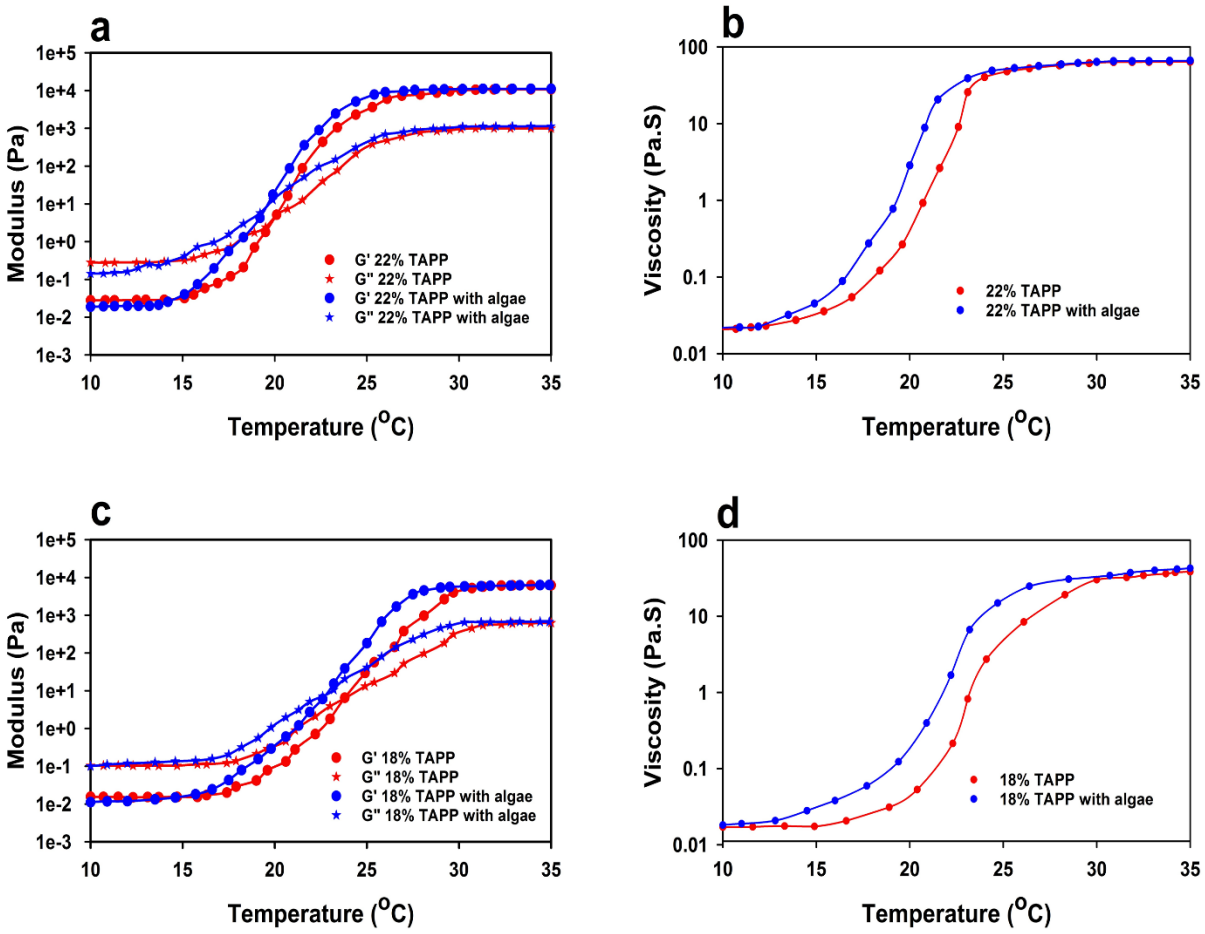


**Figure 3.4: Effects of microalgal proliferation on the thermorheological behavior of the TAPP medium.** (**a**) Storage modulus (circle) and loss modulus (star) of the 22% pluronic in TAPP sample, with (blue) and without (red) microalgae, as a function of temperature, (**b**) viscosity profile of the 22% pluronic in TAPP sample, with (blue) and without (red) microalgae, as a function of temperature, (**c**) storage modulus (circle) and loss modulus (star) of the 18% pluronic in TAPP sample, with (blue) and without (red) microalgae, as a function of temperature, (**d**) viscosity profile of the 18% pluronic in TAPP sample, with (blue) and without (red) microalgae, as a function of temperature.

### 3.3.4 Harvesting and recultivation of microalgae using thermoreversible sol-gel transition

One of the major advantages of the TAPP medium is the potential for a simple and efficient microalgal harvesting process resulting from the robust thermoreversible sol-gel transition. Specifically, the fact that this transition is completely reversible allows one to control confinement and/or settlement of microalgae through relatively small changes in the system temperature. As illustrated in the schematic (Figure 3.1), the cultivation and harvesting experiment involved seeding microalgae at a temperature where the TAPP medium is still in a solution phase and increasing the temperature beyond (or around) $T_g$ to allow microalgal cells to grow in a confined environment. After a fixed cultivation period, the temperature was decreased below $T_g$ and microalgae settled to the bottom of the cultivation chamber, thereby allowing for a *gravimetric separation of* the microalgal cells from the culture medium. Subsequently, the temperature was increased to a temperature above $T_g$ to jellify the supernatant. At this time, the harvesting of the microalgal clusters settled at the bottom would simply require to open the bottom cap and scrape the microalgal flocs off the TAPP surface.

The distribution of microalgal cells within the TAPP medium was an important parameter for the proposed harvesting technique. We hypothesized that under the selected cultivation conditions microalgal cells would be distributed in clusters due to confinement effects as opposed to randomly distributed cells as it is the case in a well-mixed TAP suspension. The morphology of the clusters would impact the velocity with which they settle during the gravimetric separation stage of the process with spherically shaped clusters settling faster than anisotropic ones[25]. Furthermore, there is a direct correlation between the size of the clusters and

the settling velocity, both according to Stokes' law or the empirical formulae often used to determine settling velocity beyond the Stokes regime[26].

The distribution of microalgal cells was assessed through random selection of images captured with an Axio Imager M1 microscope (Carl Zeiss Inc., Berlin, Germany) on each batch of microalgal culture. The images were then processed and the shape and size of cells and clusters were characterized with a Zen pro software (Carl Zeiss Inc., Berlin, Germany). As hypothesized, microalgal cells from the TAPP system were observed to be organized into clusters (Figure 3.5c) whereas those from the control (TAP medium) were visualized as randomly dispersed cells (Figure 3.5b). The average form factor of the microalgal clusters was found to be 0.98 ± 0.02 µm which suggests nearly-spherical clusters and justifies the use of Stokes' law to predict the settling velocity. The average equivalent diameter of the clusters was found to be 78 ± 9 µm compared to an 8.1 ± 0.6 µm average diameter for isolated cells in the well-mixed TAP medium. Considering the viscosity measured during the rheological characterization, the settling velocity was calculated according to Stokes' law and averaged a value of 2.6 ± 6 m $day^{-1}$ which is about ten times greater than the estimated settling velocity of isolated microalgal cells in TAP medium (0.27 ± 0.03 m $day^{-1}$).

To verify the settling rate experimentally, microalgae were allowed to settle at 15°C in tubes with 10 cm working height after the seven day cultivation period. The optical density ($OD_{675}$) of the TAPP broth was measured at regular intervals and the variation in microalgal biomass concentration was compared against a TAP broth used as control. The optical density measurements during the settling assay were used to compute the percentage recovery through settling over time. It was found that over a 2-hour period, 89 ± 5% of microalgal clusters in the TAPP broth were recovered through settling compared to 34 ± 3% for isolated cells in the TAP

broth for the 10 cm working height (Figure 3.5a). It is clear that these experimental results exhibited slower biomass recovery compared to the predictions using Stokes' law. Similar deviations of experimental data from predicted settling rate are often reported and may be due to the large heterogeneity of cluster and cell sizes[36] as well as due to hydrodynamic interactions among the clusters that modify the underlying flow field in the settling column. Nonetheless our experiments offer clear corroboration for a higher settling velocity of microalgal clusters in the TAPP medium compared to that of dispersed microalgal cells in the TAP medium.

To evaluate the harvesting efficiency through scraping of microalgae off the surface of the jellified TAPP system, two-capped containers may be used or one-capped containers may be flipped upside down before the decrease in temperature for clusters settling. The percentage of microalgae harvested was assessed through gravimetric measurements on the harvested biomass and also through optical density measurements on the remaining broth. The harvesting efficiency was then computed as the percent difference between microalgal concentrations of the broths prior and after harvesting. It was found that 89 ± 2%, 88 ± 3% and 86± 2% of microalgae were harvested respectively for the 22% TAPP, the 20% TAPP and the 18% TAPP samples.

Another advantage of the TAPP medium is the potential for recycling and reuse of the medium for recultivation of microalgae after the harvesting process. The thermoreversible sol-gel transition properties of the TAPP medium are not altered after cultivation and harvesting of microalgae. Therefore, recultivation of microalgae in the recycled TAPP medium simply requires a replenishment of nutrients based on the nutrient uptake in the preceding microalgal culture[37,38,39]. This is very important and would ensure a sustainable use of pluronic but also would decrease the operating cost associated with using the TAPP system. This recycling

potential of the medium was confirmed by recycling and reusing the TAPP medium for the recultivation of microalgae in a three cultivation cycles experiment.

After each cultivation cycle, the microalgal biomass was quantified and harvested and the TAPP growth medium was recycled and reused for another cultivation. Starting with the same initial biomass concentration in each microalgal cultivation cycle, final microalgal biomass concentrations were found to be $2.8 \pm 0.2$ g $l^{-1}$, $2.3 \pm 0.3$ g $l^{-1}$ and $2.5 \pm 0.3$ g $l^{-1}$ respectively for the first, second and third cultivation cycles.

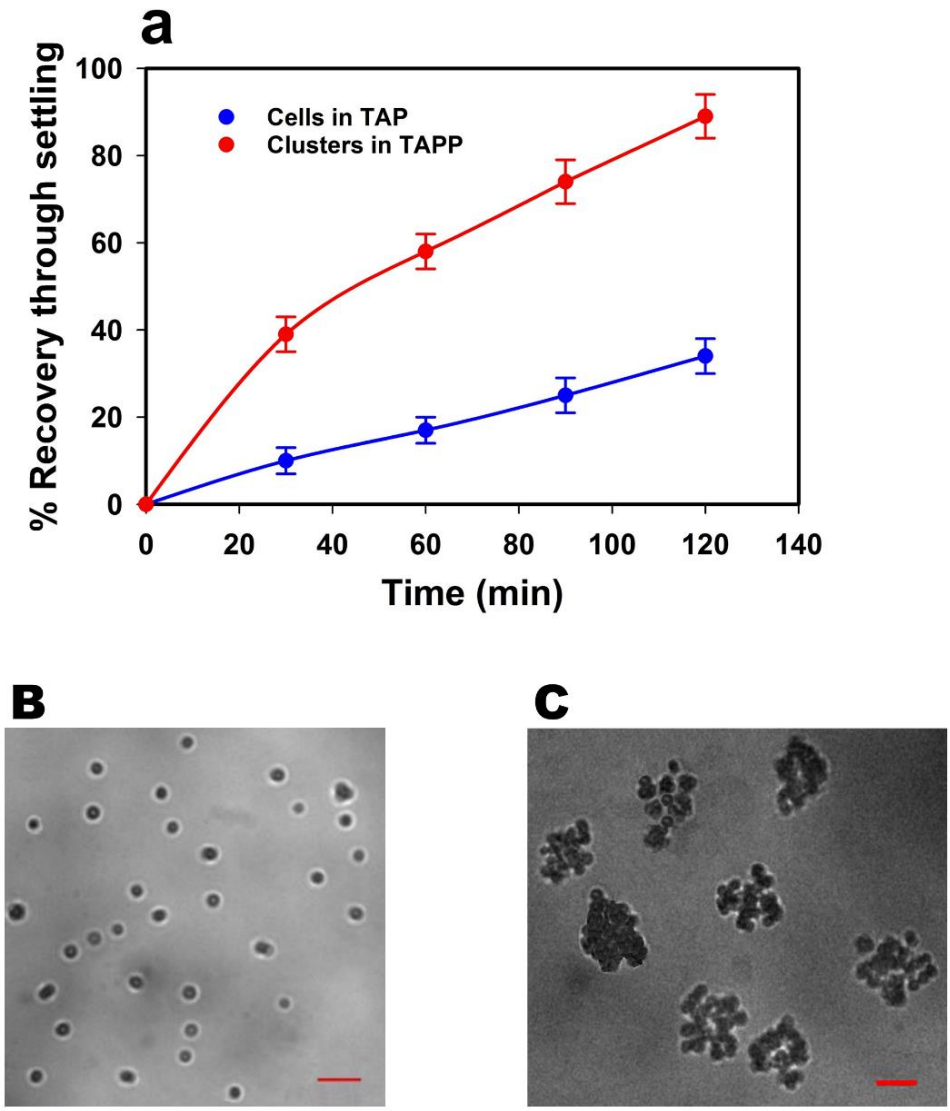


**Figure 3.5: Characterization of microalgal settling.** (**a**) Percentage of microalgal biomass recovery through settling determined through results from optical density measurements at regular intervals. Data points represent means of 30 measurements and error bars are one S.D. (**b**) Image of microalgal cells in the well-mixed TAP medium and (**c**) image of microalgal cell clusters in the TAPP medium. Scale bars are 50 μm.

## 3.4 Conclusions

In summary, a Tris-Acetate-Phosphate-Pluronic medium with thermoreversible sol-gel transition properties was developed for energy efficient cultivation and harvesting of microalgae. The thermorheological properties of the medium and the effects of microalgal proliferation on such properties were experimentally characterized to offer a framework for designing of robust microalgal cultivation and harvesting systems using the thermoreversible copolymer pluronic. Microalga *Chlamydomonas reinhardtii* was successfully cultivated in the TAPP medium and led to production of microalgal biomass with similar productivity, lipid and carbohydrate composition to that obtained from cultivation in a traditional well-mixed TAP suspension medium. In fact, starting with a same initial biomass concentration of 0.1 g $l^{-1}$, microalgal biomass concentrations of 3.1 ± 0.2 g $l^{-1}$, 2.9 ± 0.3 g $l^{-1}$, 2.8 ± 0.4 g $l^{-1}$ and 2.9 ± 0.4 g $l^{-1}$, were obtained respectively with the well-mixed TAP medium control, the 18% TAPP, the 20% TAPP and the 22% TAPP samples after a seven day cultivation period ($P > 0.05$). The harvesting process of the microalgal biomass produced was highly simplified with the TAPP system. In fact, confinement of microalgal cells in the pluronic matrix led to a near-spherical cluster distribution that could increase their settling velocity by a factor of ten as compared to that of individual cells in well-mixed TAP medium. This allowed for an *energy efficient gravimetric separation* of the biomass from the culture medium. Through thermal cycles with relatively small variations in temperature within 7°C, microalgal clusters were allowed to settle and harvesting of microalgae simply involved scraping the microalgal clusters off the surface of the jellified TAPP medium. These findings confirm that microalgae can be efficiently cultured in the TAPP system which does not require constant mixing like in typical solution broths. Moreover, this new process is capable of efficiently harvesting about ninety percent of the microalgal

biomass produced with little energy input and is biocompatible. All of the above cultivation and harvesting features were also successfully verified with a pluronic-based medium with an inorganic carbon source to demonstrate that such thermoreversible media can be used for photoheterotrophic as well as photoautotrophic cultivation of microalgae. Further, this new way of growing microalgae has the potential to greatly improve light absorption in microalgal photobioreactor by preventing microalgae from sticking to the wall of photobioreactors which often hinders light penetration, by allowing the development of short light path systems or by allowing flexible orientation of the cultivation systems for optimal light absorption. It is envisioned that thermoreversible TAPP-like systems could find further applications in microalgal biomass production for biofouling control and single-cell cultivation[14,15,16]. Future studies to continue this work may include potential functionalization of the copolymer in order to minimize the concentration necessary to confer the required thermorheological properties. Other studies may also be undertaken to elucidate the interactions of different microalgal species and strains with the pluronic matrix and to further characterize the microalgal clusters obtained through confinement in the TAPP matrix.

## 3.5 References


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# CHAPTER 4

## Controlling light delivery through manipulation of microalgal cells attachment on the surface of photobioreactors

**Abstract**

The ability to manipulate the adhesion of microalgal cells to the surface of photobioreactors would be largely beneficial to large scale microalgal biomass production whether for enhanced microalgal adhesion on biofilm-based photobioreactors or for a decrease of biofilm formation that hinders light penetration in enclosed and suspension-based microalgal cultivation systems. In this work, the adhesion of microalga *Chlamydomonas reinhardtii* on micropatterned surfaces was studied. Based on the results of our experiments it can be inferred that the adhesion of such microalgae was largely decreased on surfaces that were patterned with protruding squares of length smaller than the diameter of the cells and a valley distance largely smaller than the diameter of the cells, as compared to a smooth surface. It was also proven that microalgal adhesion was greatly increased when the size of the protruding squares and the valley length were slightly bigger than the diameter of the cells. An adhesion enhancement factor was computed to specify how surface micropatterning affects the total microalgal adhesion and it was found that for valley distance larger than the cells diameter, this adhesion enhancement factor decreased with increasing valley distances between protruding squares and the effects of surface micropatterning became almost non-significant when the valley distance approached 100 μm. Further, no critical protruding square size for maximal adhesion over the top of such protruding squares was found, like it has been reported for rod-like shaped *E. coli* cells.

## 4.1 Introduction

Bioadhesion to solid surfaces has for decades been extensively studied [1]. The interest in this topic has risen due to the largely reported adverse impacts of biofouling and biofilm. The contamination of medical devices and the resulting effects on human health due to bacterial biofilm formation have raised big concerns since the early 1980s [1-3]. Algal biofouling in marine environment causes corrosion or other functioning problems to human-made structures encompassing ships, fishing nets, bridges, pipelines and hydrotechnical constructions [4]. Microbial biofilm formation has also been portrayed as an industrial challenge affecting, among others, fluid transport and heat transfer efficiency [5].

In the context of microalgal biomass production, adhesion of microalgae to surfaces can be perceived as desirable or undesirable depending on the cultivation mode. In enclosed and suspended microalgal cultivation systems, biofilm development hinders light penetration and therefore decreases the growth rate and the production of valuable photosynthetic compounds [6]. It can further lead to mechanical functioning issues to the microalgal biomass production installations [5]. On the other hand, microalgal biofilm has recently been largely investigated as a good cultivation method for large scale microalgal biomass production. With the biofilm cultivation systems, the light path is much shorter, a higher biomass concentration is often obtained, less volume of water is used and harvesting of the microalgal biomass produced is less complicated and more energy efficient [7,8]. Therefore, efforts to study bioadhesion, biofouling or biofilm formation related to microalgal biomass production should be carried out with the goal of acquiring the ability to control the enhancement or the inhibition of bioadhesion as desired based on the cultivation mode.

There is a long history of strategy development to decrease bioadhesion to solid surfaces. One of the earliest technique consisted of the use of antifouling coatings to protect marine vessels [9]. Environmental concerns raised by the effects of antifouling substances containing lead, arsenic, mercury and some harmful organic compounds soon necessitated legislations to restrict the use of certain coating materials [10,11]. Although nowadays several environmentally benign and natural compounds to prevent bioadhesion have been proposed, their use in large scale industrial application is yet to be a reality [12]. Another strategy to prevent bioadhesion involves the use of modern microbiological techniques to understand the physiological and biochemical activities governing the biofouling processes. This allows the creation of enzymes that degrade biofilm-forming quorum sensing signals, inhibit microbial signaling and regulatory cascades or degrade the biofilm matrix [13-15].

Another approach to control bioadhesion takes into account the size, shape and surface properties of the adhering organism as well as the surface properties of the solid materials [16]. There is a wide range of studies that have proven the impacts of surface modification on bioadhesion [17]. Some of the experiments use biomimetics to inspire from how marine organisms protect their surfaces against biofouling. For instance, Chung *et. al.* demonstrated that sharklet microtopography successfully disrupted *S. aureus* biofilm formation [18].

A few theories have been developed or extended to design surface microtopographic patterns for controlling bioadhesion. For example, the Wenzel theory and the Cassie theory describing respectively the adhesion or non-adhesion of a water droplet, based on the surface roughness, have been extended to the study of bioadhesion to surfaces [19-21]. The attachment point theory has also been largely used. This last theory preconizes that cell attachment would be

reduced on surfaces with texture below the size of the cell while attachment would be increased when the surface texture provides multiple numbers of attachments [22].

More recently, Gu *et. al*. based on their work with *E. coli* to propose four principles for designing antifouling surface topography [23]. The first one recommends a minimum topographic feature's height to prevent cells' flagella from reaching into the grooves of patterned surface. The second suggests a critical area of the plateaus that can prevent significant biofilm formation on top of the plateaus. The third principle proposes a certain gap between the topographic features to prevent cells from falling in the grooves or bridging over the gap. Finally, the last principle preconizes the use of features with more edges or specific curvatures to minimize the interactions between the cells that get in the grooves.

The study herein aimed at applying some of these microtopographic strategies to study the adhesion of microalgae to surfaces in order to propose microtopographic design guidelines to decrease or enhance microalgal biofilm formation as needed. The microalgal species *C. reinhardtii* was selected because its characteristics have been well studied and it has shown great potential for genetic engineering manipulations to booster the production of targeted compounds for biofuels production. Strains with different characteristics were considered and their adhesion on PDMS patterned surfaces were analyzed and discussed to propose some cues to control bioadhesion of such species through surface engineering.

## 4.2 Materials and methods

### 4.2.1 Microalgal strains and culture conditions

The wild type microalga *C. reinhardtii* CC-124, the motility impaired mutant CC-1036 and the cell wall deficient mutant CC-503 used in this study were all obtained from the Chlamydomonas Resource Center (University of Minnesota, St. Paul, Minnesota). Prior to the adhesion experiments, these strains were inoculated with a Tris-Acetate-Phosphate (TAP) medium in conical flasks placed on a rotary shaker (150 rpm). The culture room was continuously illuminated by full spectrum compact fluorescent lamps (CFL 60W, Fancierstudio, San Francisco, California) with the photosynthetic active radiation at the top surface of the culture at 75 ± 5 μE $m^{-2}$ $s^{-1}$ and the temperature at 22 ± 1°C. The microalgal growth was monitored through optical density ($OD_{675}$) measurements. At early indication of the exponential growth phase (usually the fourth day), aliquots of microalgal culture were withheld and used for the adhesion experiments.

### 4.2.2 Preparation of PDMS surfaces with microtopographic features

Silicon wafers with patterned features fabricated via photolithography and soft lithography were used to prepare the PDMS (Poly di methyl siloxane) patterned surfaces. A detailed description of the fabrication procedures of these patterned wafers was presented by Hou *et. al.* [24]. In this study, patterned surfaces with different protruding square sizes (5, 10, 15, 20, 30, 40, 50, 100 μm) were considered to study the effects of protruding square size on microalgal adhesion. For each size of protruding square, the distance between protruding squares was varied (2, 5, 10, 15, 20, 30, 40, 50, 100 μm) to explore how the gap between protruding features impacts the adhesion of microalgal cells. PDMS patterned surfaces were prepared by mixing the silicone elastomer base and the curing agent at a 10:1 ratio. This mixture was

degassed and then poured onto the patterned wafer and was incubated at 50 ℃ for 24 hours. Following this, the wafer containing the patterned PDMS surface was cooled down at room temperature and the PDMS surface was peeled off and stored for the adhesion experiment.

### 4.2.3 Microalgal adhesion analysis

For the microalgal adhesion experiment, the prepared PDMS surfaces were soaked in ethanol for 24 hours. Then, the PDMS surfaces were placed in Petri-dishes and dried at 50℃ for five hours. Subsequently, 25 ml of TAP medium was added to each petri-dish containing a sterile PDMS surface and 0.3 ml of microalgal subculture was added. The desired microalgal strain was allowed to grow for five days to allow enough time for significant growth and microalgal adhesion to the surface. Then, the PDMS surfaces were washed cautiously in 0.85% NaCl buffer to remove the cells that are not attached to the surface. Finally, the PDMS surfaces were placed on microscopic glass slides and microalgal adhesion was analyzed using an Axio Imager M1 microscope and a ZEN pro software (Carl Zeiss Inc., Berlin, Germany).

Eight different protruding square sizes (5, 10, 15, 20, 30, 40, 50, 100 μm) and nine gap distances (2, 5, 10, 15, 20, 30, 40, 50, 100 μm) were considered. This gives a total of 72 combinations (protruding square/gap). To elucidate potential adhesion trends and compare attachment over the top of protruding squares and on the valley between protruding squares, five pictures were randomly taken for each configuration and the number of cells attached over the top of the protruding squares or over the valley between them were hand-counted and normalized by the surface area considered. Each experiment was repeated 3 times. Thus a total of 1080 pictures were analyzed for each microalgal strain.

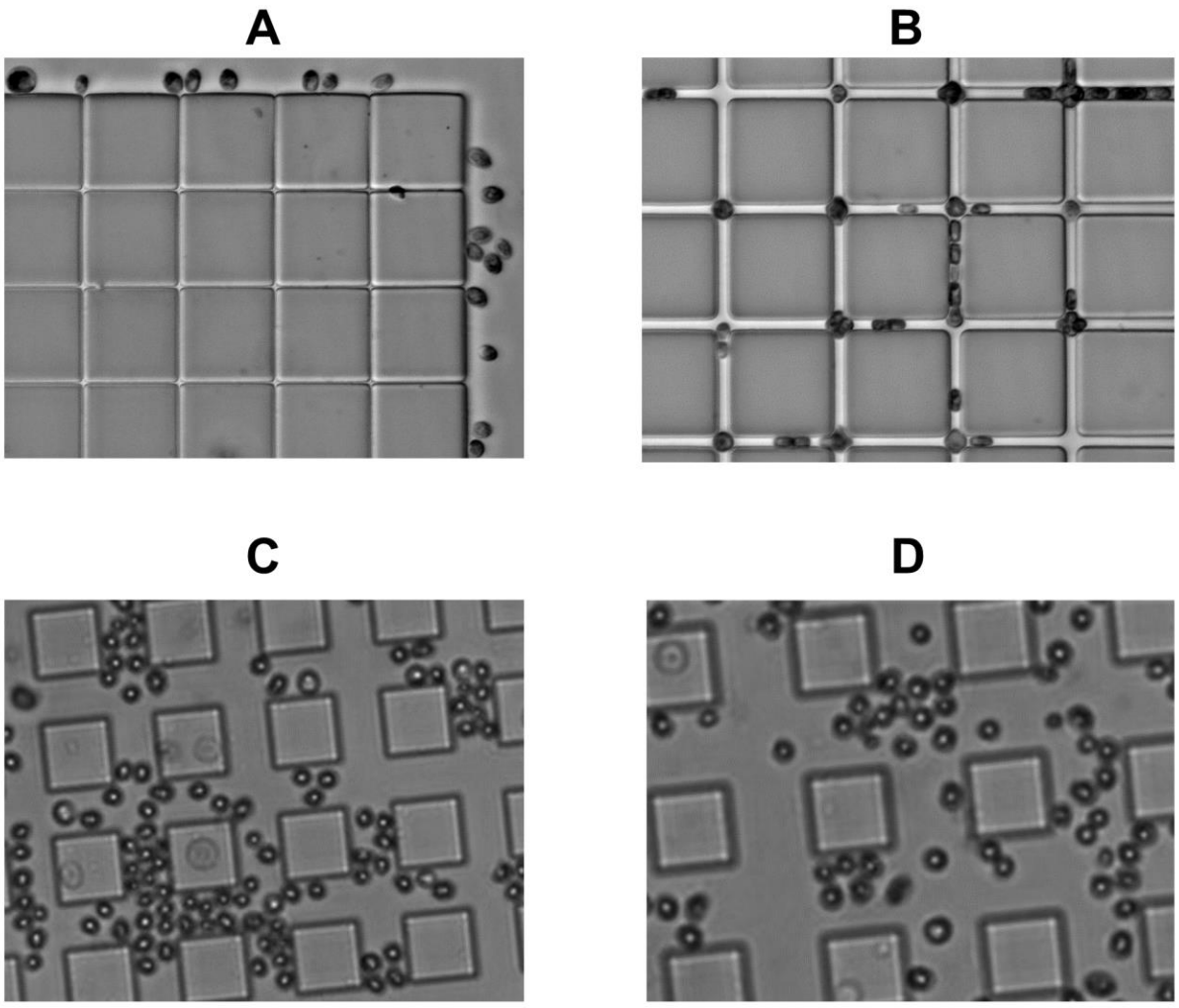


**Figure 4.1. Image of microalgae attached on patterned surfaces.** (A) Adhesion of microalgae on patterned surfaces with protruding squares of 30 µm length and 2 µm valley width, (B) adhesion of microalgae on patterned surfaces with protruding squares of 30 µm length and 5 µm valley width, (C) adhesion of microalgae on patterned surfaces with protruding squares of 30 µm length and 20 µm valley width and (D) adhesion of microalgae on patterned surfaces with protruding squares of 30 µm length and 30 µm valley width.

## 4.3 Results and discussion

The adhesion of microalgal cells was investigated using micropatterned surfaces fabricated using a same ratio of poly dimethyl siloxane and its curing agent. This allowed to study the effects of surface texture at a microscopic scale on microalgal adhesion while the surface chemistry was maintained as a fixed parameter. The micropatterned surfaces with protruding squares of different sizes and different valley distances between the protruding squares were compared to smooth PDMS surfaces used as control. The number of microalgal cells attached on the surface divided by the unit surface area considered was used as a metric to characterize microalgal adhesion over the top of protruding squares, over the valley of patterned surfaces or over the smooth surfaces.

Before assessing the impacts of different pattern features on microalgal adhesion to PDMS surfaces, it was important to characterize certain features of the microalgal cells used since they could potentially impact the adhesion. Specifically, the wild type *C. reinhardtii* CC-124 is a spherical microorganism with a rigid cell wall [25]. At the exponential growth phase, the diameter of microalgal cells was measured using an Axio Imager M1 microscope and a ZEN pro software (Carl Zeiss Inc., Berlin, Germany). The average diameter of the wild type *C. reinhardtii* CC-124 measured in such conditions was found to be 8.1 ± 0.6 µm.

The impact of patterned surfaces was studied by assessing the impact of valley distance and protruding square sizes separately. Before evaluating the role the valley distance and the protruding square size play in the adhesion of microalgal cells on patterned surfaces compared to smooth surfaces, it is important to state the preferential adhesion trend observed through the experiments with patterned surfaces. As it can be seen in figure 4.1, when the valley distance of the patterned surfaces was large enough for microalgal cells to penetrate, there was a net

prevalence of adhesion on the valleys of the patterned surfaces as opposed to the tops of the protruding squares. This was proven to be true for every distance of the valleys and independently of the size of the protruding squares over the range of values considered in this work. The preferential adhesion of organisms on the valley of microtopographic features has been reported for different organisms or microtopographic features and is often attributed to the lower energy requirement for adhesion on valleys of microtopographic features[26].

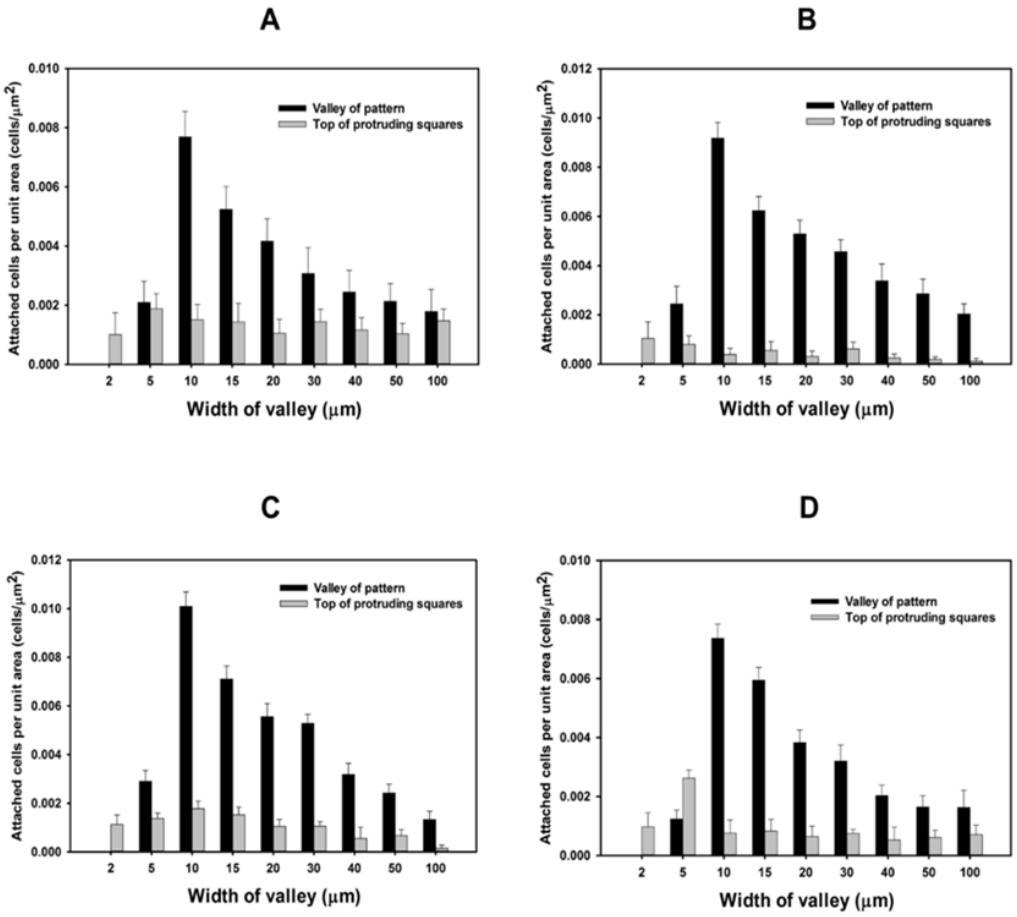


**Figure 4.2. Preferential adhesion on valleys of patterned surfaces.** (A) Protruding squares of 15 μm length with valley distances of 2 μm, 5 μm, 10 μm,15 μm, 20 μm, 30 μm, 40 μm, 50 μm and 100 μm, (B) Protruding squares of 30 μm length with valley distances of 2 μm, 5 μm, 10 μm,15 μm, 20 μm, 30 μm, 40 μm, 50 μm and 100 μm, (C) Protruding squares of 50 μm length with valley distances of 2 μm, 5 μm, 10 μm,15 μm, 20 μm, 30 μm, 40 μm, 50 μm and 100 μm, (D) Protruding squares of 100 μm length with valley distances of 2 μm, 5 μm, 10 μm,15 μm, 20 μm, 30 μm, 40 μm, 50 μm and 100 μm. Bars represent the average of 15 measurements and errors are one S.D.

### 4.3.1 Effects of valley width on microalgal adhesion over the valleys of patterns

To study the effects of valley distances on microalgal adhesion over the valleys of the patterned surfaces, valley distances of 2 µm, 5 µm, 10 µm, 15 µm, 20 µm, 30 µm, 40 µm, 50 µm and 100 µm were considered separately with different protruding square sizes. Note that the average microalgal cell diameter was 8.1 ± 0.6 µm. Figure 4.3 illustrates the adhesion of microalgae with different valley distances. The average adhesion of microalgae on smooth surfaces is also presented as a control to illustrate the role that the valley distance plays in the adhesion. First, for a valley distance of 2 µm, the adhesion over the valleys of the patterned surfaces was suppressed because microalgal cells could not penetrate into the valley. Later on, the impact of such valley size on the total adhesion, *i,e,* the adhesion over the valleys of the pattern and the adhesion over the top of the protruding squares combined, will be analyzed through the total adhesion enhancement factor considerations.

For the valley distance of 5 µm, although the average microalgal cell diameter (8.1 ± 0.6 µm) measured at the exponential phase was greater than the valley distance, there was significant microalgal adhesion in the valley. The average adhesion per unit of valley area was 0.002± 0.0006 cells/µm$^2$ whereas the average adhesion unit area on a smooth surface was 0.0015 ± 0.0006 cells/µm$^2$. An interesting observation was the consistent change on the shape of the microalgal cells attached in the 5 µm valley (figure 4.1). The size of these microalgal cells were measured and the form factor (*FF)* characterizing the deviation from the typical spherical shape along with the equivalent diameter (*De*) of the microalgal cells were computed as presented by Grijspeerdt and Verstraete [27]:

$$FF = \frac{4*\pi*Area}{Perimeter^2} \quad (1)$$

$$De = 2 * \sqrt{\frac{Area}{\pi}} \quad (2)$$

The average form factor was found to be 0.82 ± 0.02 which corroborates a clear change in the shape of these microalgal cells compared to the spherical cells attached elsewhere with a form factor of approximately 1.00. There are two potential explanations of the adhesion of microalgal cells on such small valley distance and the non-spherical shape of the adhering microalgal cells. First, due to the large heterogeneity and the growth dependency of the microalgal cell size, cells with diameter below the average diameter size and close to the valley distance could penetrate the valley. A stronger explanation is that young cells who have not fully developed to their full size could penetrate the valley and become entrapped and forced to grow in the confined environment leading to the non-spherical shape.

For valley distance larger than the microalgal cell size, adhesion in the valley increased significantly as compared to smooth surface. The adhesion coverage decreased consistently with increasing valley distance. When the valley distance reached 100 µm, the adhesion coverage in the valley of patterned surfaces was close to the number of adhesion on smooth surfaces. This adhesion behavior on valley distances greater than the cells size can also be explained by the attachment point theory where the increase in contact points thermodynamically favors adhesion.

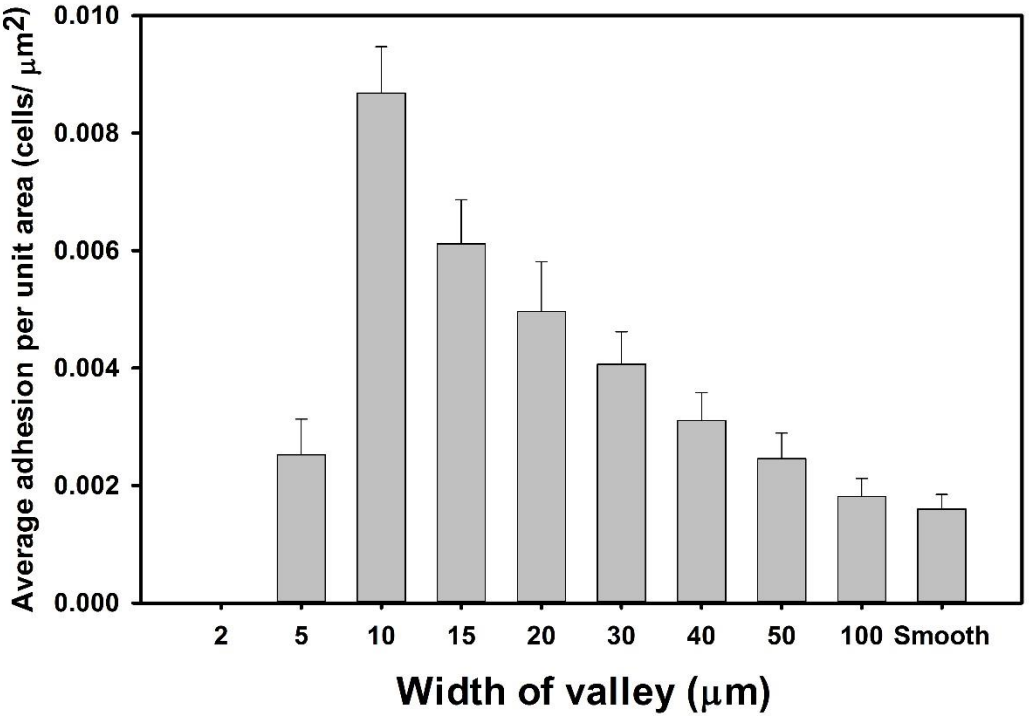


**Figure 4.3: Average adhesion density on valleys of patterns based on the valley width**. Bars represent the average of 15 measurements for adhesion in the valleys at each valley distance (2 µm, 5 µm 10 µm, 15 µm, 20 µm, 30 µm, 40 µm, 50 µm and 100 µm) and for a smooth surface. Errors bars are one S.D.

### 4.3.2 Effects of protruding square size on microalgal adhesion over the top surface of the protruding squares

To study how the size of the protruding squares impacts the adhesion over the top surface of the protruding squares, microalgal adhesion over the top surface of protruding squares of 5 µm, 10 µm, 15 µm, 20 µm, 30 µm, 40 µm, 50 µm and 100 µm lengths was evaluated. Beside the protruding square of 5 µm where the top surface is apparently too small to support the microalgal cells and the adhesion was very small, the adhesion of microalgal cells over the top surface of protruding squares did not follow any trend and did not depend on a critical distance, for the range of square length considered. This is different compared to the observation by Gu *et. al.* where a critical length of protruding squares was found for significant adhesion of *E. coli* over the top surface of protruding squares. Their study reported a significant adhesion of rod-like shaped *E. coli* cells on the top of the protruding squares when the protruding square length was greater than 20 µm [24]. While there is not enough information to propose a clear explanation of this big difference between the adhesion of the *C. reinhardtii* cells used in this study and that of the *E. coli* cells reported by Gu *et. al.*, it is important to mention that these two microorganisms have very different sizes and shapes with the first having a spherical shape and the second being rod-shaped. Another study by Gu *et. al.* provides a systematic analysis that provides insights on the mechanism of adhesion of these rod-shaped *E. coli* cells to surfaces[23]. Analogous studies on *C. reinhardtii* and similar microalgae can help further elucidate their adhesion mechanism.

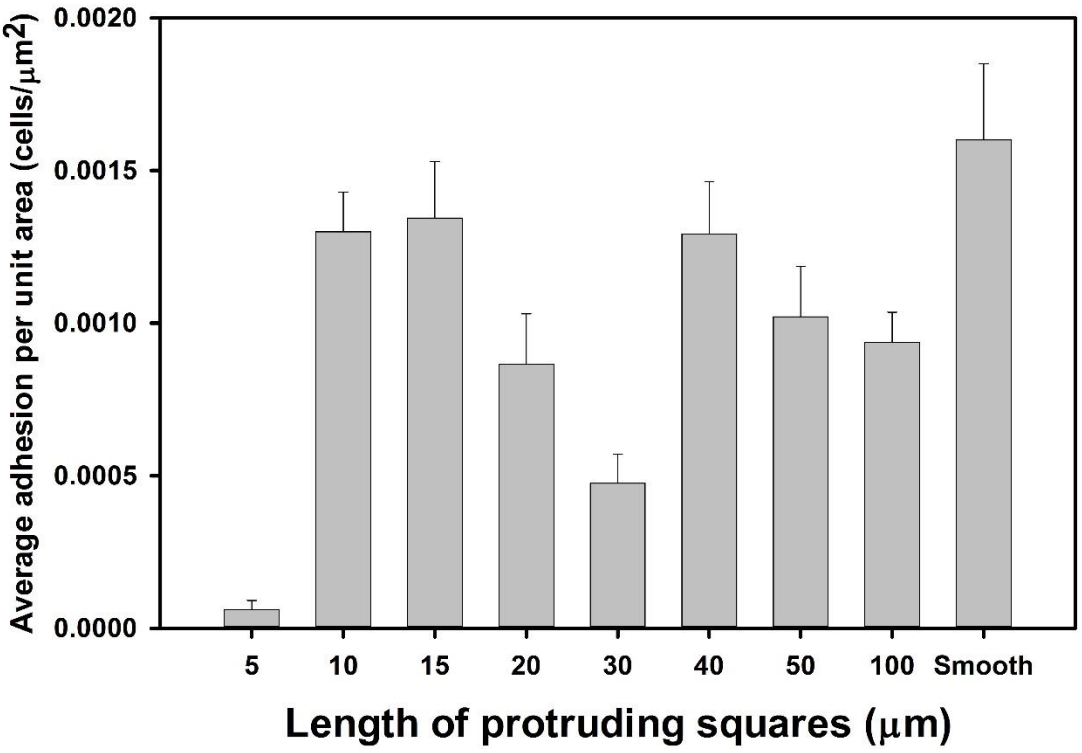


**Figure 4.4: Average adhesion density over the top surface of protruding squares.** Bars represent the average of 15 measurements for adhesion on the top surface of protruding squares at each protruding square length (5 μm 10 μm, 15 μm, 20 μm, 30 μm, 40 μm, 50 μm and 100 μm) and for a smooth surface. Errors bars are one S.D.

### 4.3.3 Construction of an adhesion enhancement factor chart

In order to specify how surface micropatterning affects the total microalgal adhesion as compared to a smooth surface, an adhesion enhancement factor was computed and tabulated using each of the 72 combinations of protruding square size/valley distance. To calculate such adhesion enhancement factor, the total number of cells attached both over the top of protruding squares and over the valleys of the patterned surfaces was counted and divided by the area of the patterned surface, the number of cells attached on the smooth control surface was also counted and divided by the area of the smooth surface. The adhesion enhancement factor was then computed as the ratio of cell adhesion density on the patterned surfaces to that of the smooth control surface. Therefore, a specific micropatterning configuration leading an adhesion enhancement factor with a value greater than one would mean that the adhesion is enhanced with this specific micropatterning configuration. Corollary, a specific micropatterning configuration leading to an adhesion enhancement factor smaller than one would mean that microalgal adhesion is minimized.

The average adhesion enhancement factors for the 72 combinations of protruding square size and valley distance are presented in table 4.1. The highest adhesion factor (4.49) was obtained with the pattern configuration of a 10 µm protruding square side length and a 10 µm valley distance. The lowest adhesion enhancement factor (0.02) was obtained with the pattern configuration of a 5 µm protruding square side length and a 2 µm valley distance. The decrease in adhesion with the 5 µm protruding square side length and a 2 µm valley distance pattern can be explained by the contact point the theory. In fact, the cell size ($8.1 \pm 0.6$) was far greater than the valley distance, the cells could not penetrate into the valleys and the cells would rest above the distance between protruding squares where the contacts with the surface became contact

points and the attachment of microalgal cells became thermodynamically unfavorable [21]. moreover, the side length of the protruding square was smaller than the cell diameter and became less favorable for adhesion over the top surface of the protruding squares. This makes the 5 µm protruding square side length and 2 µm valley distance pattern a good combination to minimize adhesion. The largest adhesion enhancement factor with the 10 µm protruding square length and a 10 µm valley distance pattern can also be explained by the contact point theory where such side length of protruding squares and valley distance slightly larger than the average diameter of the cells provide the maximum contacts possible. The adhesion enhancement factor chart also confirmed the trend for decreasing adhesion for increasing valley distance and did not show any particular trend for variation of the side length of protruding squares.

**Table 4.1: Adhesion properties of microalga *C. reinhardtii*.** Adhesion enhancement factor of patterned surfaces with different combination of valley widths and protruding square sizes

| **Length of protruding squares (μm)** | **Width of valley (μm)** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **2** | **5** | **10** | **15** | **20** | **30** | **40** | **50** | **100** |
| **5** | **0.02** | 1.04 | 3.61 | 2.76 | 2.29 | 1.76 | 1.43 | 1.27 | 1.09 |
| **10** | 0.47 | 1.53 | **4.49** | 3.48 | 3.32 | 2.72 | 2.15 | 1.74 | 1.45 |
| **15** | 0.51 | 1.23 | 3.04 | 2.73 | 2.29 | 1.83 | 1.48 | 1.31 | 1.12 |
| **20** | 0.62 | 0.67 | 2.92 | 2.90 | 2.62 | 1.90 | 1.73 | 1.25 | 1.22 |
| **30** | 0.58 | 0.74 | 2.29 | 2.26 | 2.29 | 2.32 | 1.82 | 1.60 | 1.24 |
| **40** | 0.61 | 1.13 | 2.60 | 2.57 | 2.37 | 2.51 | 2.49 | 2.00 | 0.96 |
| **50** | 0.66 | 1.01 | 2.47 | 2.56 | 2.25 | 2.49 | 1.60 | 1.33 | 0.78 |
| **100** | 0.59 | 1.57 | 3.12 | 1.47 | 1.12 | 1.22 | 0.88 | 0.81 | 0.90 |

## 4.4 Conclusions

In summary, this work provides some microtopographic cues to manipulate the adhesion of microalga *C. reinhardtii* (and probably, other microorganism with similar shape) to surfaces. Experiment with PMDS surfaces micropatterned with protruding square features showed a net preferential adhesion over the valley between the protruding squares as opposed to the top surface of the protruding squares. The experimental results supported by the contact point theory confirmed that the adhesion of microalgal cells to patterned surfaces was greatly minimized when the valley distance between protruding squares was significantly smaller than the diameter of the cells and when the length of the protruding square was also significantly smaller than the diameter of the cells. On the contrary, total microalgal adhesion was greatly enhanced for patterned surfaces with protruding square length and valley distances slightly larger than the microalgal cell diameter as compared to smooth surfaces. Further, over the range of protruding square lengths considered, a critical protruding square length for maximal microalgal was not found like it has been reported for rod-like shaped *E. coli* cells. The effects of surface micropatterning became less significant when the valley length approached 100 μm. These adhesion trends observed with the wild type *C. reinhardtii* CC-124 were also observed for the motility impaired mutant *C. reinhardtii* CC-1036 and the cell wall deficient mutant *C. reinhardtii* CC-503. These microtopographic cues can be exploited to influence microalgal adhesion on the surface of photobioreactors with the goal of improving the absorption of light by microalgae. Future studies to continue this work may include the use of different protruding feature shapes and also the use of microalgal species with non-spherical shape or with different surface properties.

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# CHAPTER 5

## Rheological characterization and flow manipulation to improve light absorption in microalgal photobioreactors

**Abstract**

While light is a critical parameter for microalgal growth, the distribution of light is non-homogeneous inside typical photobioreactors primarily because of photobioreactors configuration and shading effects by the microalgal cells. Without proper mixing, microalgal cells within a same cultivation chamber could experience three different realities: either they are located in a section with optimal irradiance where they grow effectively, in a section with poor irradiance where they fail to grow or in a zone with excessive irradiance where they are exposed to photoinhibition. The periodical motion of microalgal cells from well illuminated to poorly illuminated sections of a photobioreactor can be a good way to optimize light absorption by the microalgal cells. However, this requires a deep understanding of the hydrodynamic properties of typical microalgal culture. In this study, we characterized the rheological properties of suspensions of microalga *Chlamydomonas reinhardtii* and found, among other properties, that such suspensions exhibited Newtonian behavior at low microalgal concentration and non-Newtonian shear thinning behavior at concentrations above 50 g/l. The rheological insights obtained from the experimental analyses were then used as tools to control the cycle to which an average microalgal cell would travel from high light to low light zones within a typical photobioreactor.

## 5.1. Introduction

The optimization of microalgal biomass production and microalgal biorefining requires a thorough understanding of some inherent parameters and their impacts on the biomass productivity and/or on the energy consumption by such processes. While some of the parameters such as nutrient concentration, salinity, photobioreactor configuration etc. have for long been extensively studied, it is only recently that the hydrodynamic properties of microalgal broths and microalgal slurries have received great consideration as an optimization parameter for enhanced microalgal biomass production[1-4].

The hydrodynamic characteristics of microalgal broths have direct impacts not only on the microalgal cultivation in typical microalgal photobioreactors but also on the harvesting of the biomass generated. In fact, constant mixing in photobioreactor operations is often deemed necessary to keep the microalgal cells in suspension, to help nutrient distribution, to facilitate gas exchange and mass transfer etc.[5] On the other hand, excessive mixing can have bad effects on microalgal culture that can even lead to cell damage [6]. Some experimental studies using hydrodynamic manipulation for proper mixing of microalgal culture have known great success [7]. Further, the liquid velocity affecting, among others, the residence time and the rate of oxygen removal in most photobioreactors can easily be controlled using the hydrodynamic properties [8]. Adding to these, the harvesting and dewatering of microalgal biomass is known to be an energy intensive process. Some studies have focused on characterizing the hydrodynamic properties of microalgal slurries for an efficient design of harvesting units and for the minimization of the energy requirement [9]. The need for hydrodynamic optimization in microalgal cultivation systems is becoming more and more crucial as higher biomass concentrations are becoming more and more achievable and practical in the microalgal biorefining industry.

The nature of microalgal slurries makes the study of the hydrodynamic properties of such systems very tedious. Although water is the main constituent, the presence of micronutrients, dissolved salts, polymeric substances and microalgal cells makes microalgal broths complex fluids [10]. The characterization of the hydrodynamic properties of microalgal broths is even more difficult because the metabolism of microalgal cells has real time impacts on the nature of the fluids not only in terms of increased biomass concentrations due to proliferation of microalgal cells but also through motion of the microorganisms and potential bio-secretions in the medium [11]. Moreover, the large heterogeneity of microalgal species and strains used in microalgal biomass production and the largely reported morphological and physiological differences make it impractical to define broad hydrodynamic characteristics of microalgal broths. For instance, the work by Zhang *et. al.* reported the influence of different cell properties on the rheological characterization of microalgal suspensions [12].

In this work, we focus on the characterization of suspensions and culture of microalga *C. reinhardtii*. *C. reinhardtii* has been largely investigated for microalgal biomass production, among other reasons, because of its large potential for genetic engineering manipulations[13]. *C. reinhardtii* is a unicellular microorganism mainly spherical with diameter around ten micrometers. As microswimmers, they are classified among the pullers. As opposed to the micropushers such as *E. coli* that use a rotating flagellar bundle to push the fluid behind them, micropullers propel themselves through breaststroke-like motion with a pair of flagella attached at the front of their body. While it has been very challenging to characterize the macroscopic behaviors of micropushers, a few rheological studies on the collective properties of micropullers have been very promising [14]. Some of these studies have reported certain similarities between the behavior of micropullers in water to that of passive suspensions comprising of rigid spheres at

low concentration while others have reported shear thinning behavior over larger concentrations [9,10].

We aimed at studying the collective rheological behavior of broths and slurries of the microalga *C. reinhardtii.* A clear understanding of such behavior may serve to different applications including effective mass transfer and optimal mixing. Specifically, in the context of efficient distribution and absorption of light in microalgal photobioreactors, these results can particularly be used to control the exposure of microalgal cells to optimal irradiance. It has, in fact, been made clear that with the current microalgal biomass production technologies, not all sections of the microalgal cultivation systems receive the optimal irradiance for biomass productivity [15]. To mitigate the risk of photoinhibition due to excessive irradiance in certain sections of the photobioreactor and that of limited growth due to poor illumination in other sections, shuttle motion of microalgal cells is often deemed necessary. Further, a certain rotation of microalgal cells shifting between light and darkness periodically has been reported as largely beneficial for photosynthetic activities [16]. The collective rheological features of the microalgal culture experimentally characterized in this study are used to control the optimal light/darkness cycle duration not only to palliate the above listed issues but also to enhance microalgal photosynthetic activities.

## 5.2. Materials and methods

### 5.2.1. Microalgal culture and sample preparation

The wild type microalga *C. reinhardtii* CC-124, the motility impaired mutant CC-1036 and the cell wall deficient mutant CC-503 obtained from the Chlamydomonas Resource Center (University of Minnesota, St. Paul, Minnesota) were used in this study. Prior to the rheological characterization experiments, these strains were inoculated with a Tris-Acetate-Phosphate (TAP) medium in conical flasks placed on a rotary shaker (150 rpm). The microalgal culture duration was varied between 7 to 22 days in order to monitor any influence of culture time on the rheological properties. The culture room was continuously illuminated by full spectrum compact fluorescent lamps (CFL 60W, Fancierstudio, San Francisco, California) with the photosynthetic active radiation at the top surface of the culture at 75 ± 5 µE $m^{-2}$ $s^{-1}$ and the temperature at 22 ± 1°C. The growth of microalgae was monitored through optical density ($OD_{675}$) measurements. Calibration curves were built to correlate the optical density ($OD_{675}$) measurements with dry biomass concentration in unit of gram per liter.

After the cultivation period, the microalgal biomass concentration was assessed through optical density measurements. Following this, the microalgal broth was split between several conical flasks and was either diluted with water or centrifuged (4,800g, 5 min, 20°C) to remove excess water in order to obtain 2 milliliter microalgal suspension samples of 10 g/l, 20 g/l, 30 g/l, 40 g/l, 50 g/l, 100 g/l and 150 g/l. Final optical density measurements were then taken on each of these samples in order to ensure the accuracy of the concentrations of the sample. The volume fraction of microalgal biomass in the medium ($\varphi$) was determined from the dry biomass concentration using the formulae [10]:

$$\varphi = \frac{C}{\rho_w w_D}$$

$$\rho_w = x_w \rho_0 + (1 - x_w)\rho_D$$

Where $\rho_w$ and $\rho_D$ represent respectively the wet and dry density of microalgae expressed in unit of mass per volume and $x_w$ represents the volumetric water content per unit volume of microalgal biomass. $w_D$, representing the mass of dry solid per unit mass of dry microalgae, is computed as follows:

$$w_D = \frac{(1 - x_w)\rho_D}{\rho_w}$$

### 5.2.2. Rheometry and data analysis

The rheological characterization experiments were performed using a Combined Motor and Transducer (CMT) AR-G2 rheometer from TA instruments (New Castle, Delaware). The cone-and-plate geometry with a diameter of 40 mm and cone angle 0° 59' 49" was used for all the measurements. The temperature control was achieved by a Peltier plate using thermoelectric effects that can accurately control the temperature and water circulation for rapid heating and cooling over a temperature range of 0 to 100°C. For these experiments, the temperature was controlled at 20°C. After each run, the Peltier plate was flushed with water to remove the sample and then dried and cleaned using acetone before loading a new sample. Steady shear flow experiments were performed by varying the shear rate from 10 to 800 $s^{-1}$ using logarithmic ramping with 10 intervals per decade of the shear rate. Peak hold experiments were performed by maintaining the shear rate constant at the desired value and the rheological data were collected over a time interval of 1500 seconds.

As described previously, at low biomass concentration a microalgal culture is expected to behave as a Newtonian fluid. In this case, the shear stress is directly proportional with the shear rate with the constant of proportionality being the viscosity. Therefore, at this range of concentration the viscosity obtained through steady shear flow experiments would suffice to describe the flow properties. However, at higher concentrations the culture would most likely exhibit both viscous and elastic behaviors (viscoelasticity) and non-Newtonian behavior. The relationship between the shear stress and the shear rate would no longer be established using the viscosity as the proportionality constant, as the viscosity itself would become a function of the shear rate. The term effective viscosity is used to define the ratio of the shear stress over the shear rate at a specific shear rate. The power law model is among many models used to characterize the shear rate dependence of the effective viscosity. With this model, for a given shear rate $\dot{\gamma}$, the effective viscosity is obtained by [17]:

$$\mu_{eff} = K(\dot{\gamma})^{n-1}$$

Where, $K$ is the consistency index and $n$ is the power law index. For shear thinning non-Newtonian fluids, the power law index is less than one and the viscosity decreases with increasing shear rate and for shear thickening non-Newtonian fluids the power law index is greater than one and the viscosity increases with increasing shear rate.

## 5.3. Results and discussion

### 5.3.1. Rheological characterization of microalgal slurries

The effective viscosity values of microalgal suspensions of 10 g/l, 20 g/l, 30 g/l, 40 g/l, 50 g/l, 100 g/l and 150 g/l dry biomass concentrations were plotted against shear rates ranging from 10 to 800 $s^{-1}$ in figure 5.1 and were compared to a TAP buffer sample with no microalgal cells used as control. Lower shear rates could not be tested because of inaccuracy in the measurements that are most likely the results of surface tension and/or inertia effects. At microalgal concentrations below or equal to 40 g/l, the viscosity did not exhibit any dependence on shear rate which is a strong characteristic of Newtonian fluid. Over this range of concentrations, the viscosity was observed as increasing with increasing microalgal concentrations. It is well documented in literature that the viscosity of most suspensions increases with increasing solute concentrations. Let $\mu_s$ be the solvent viscosity and $\varphi$ the volume fraction of solute, the viscosity of most suspensions can often be modeled as:

$$\mu = \mu_s(1 + \alpha\varphi)$$

While for passive suspensions of rigid spherical particles α is usually reported as being equal to 2.5, for suspensions of active particles α is often reported with higher values potentially due to the effects of swimming.[18,19] After correlating the biomass concentrations from 0 to 40 g/l to match the volume fractions as described previously, linear regression analyses performed with the viscosity results obtained, led to an α value of 6.4 with $R^2$=0.99 for these *C. reinhardtii* suspensions.

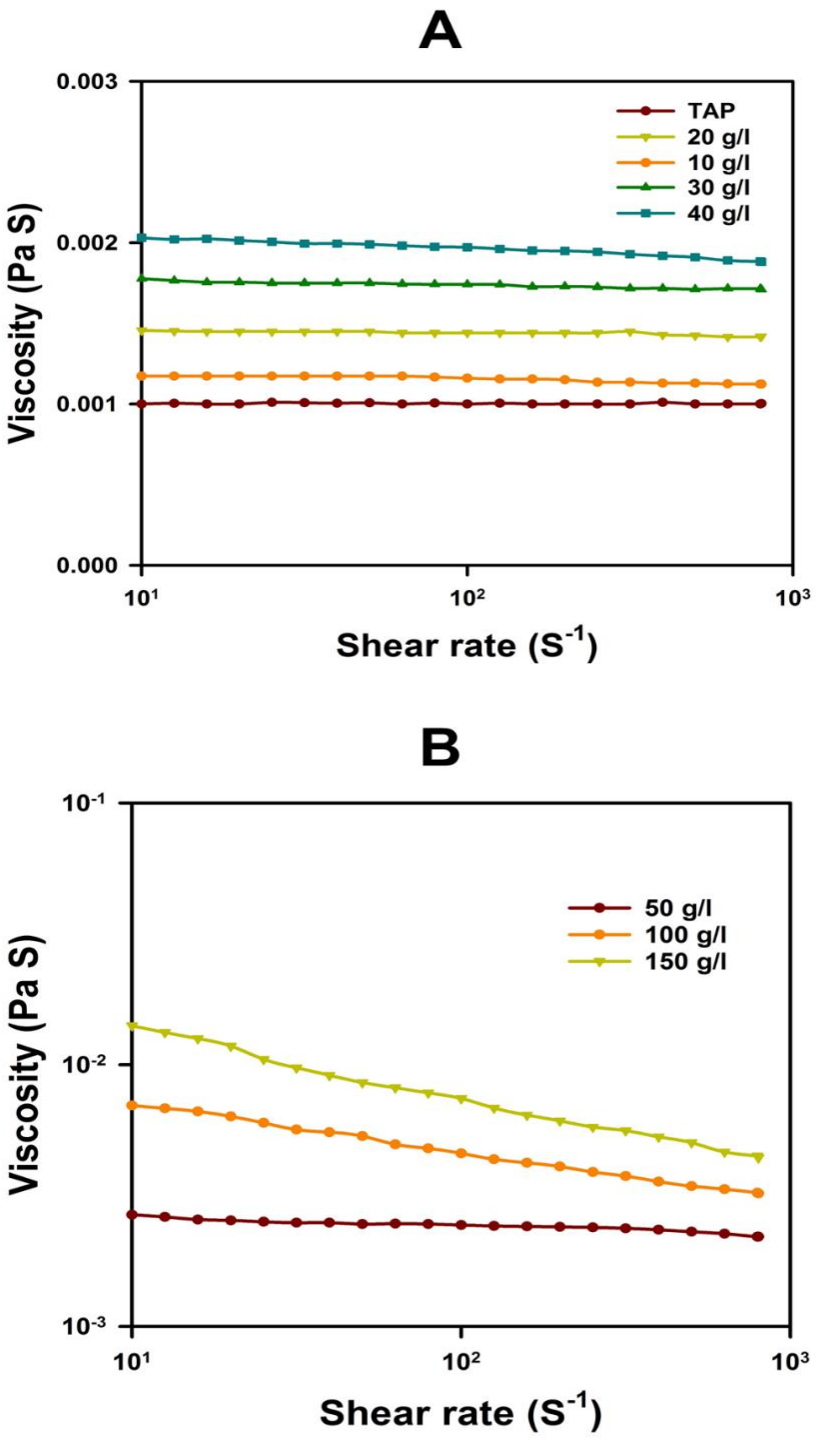


**Figure 5.1: Rheological characterization of microalgal broths.** Viscosity profiles of microalgal suspensions as a function of shear rate at low biomass concentrations (A) and at high biomass concentrations (B).

Three higher microalgal biomass concentrations, namely, 50 g/l, 100 g/l and 150 g/l were also analyzed. For these three concentrations, the effective viscosity exhibited strong dependence on the shear rate value with a shear thinning behavior. Table 5.1 summarizes the results obtained by fitting the effective viscosity results with the power law fluid model. The consistency index K, the power law index n along with $R^2$ values are reported for all the microalgal concentrations considered. For the samples with concentrations equal or less than 40 g/l, the power law index was very close to 1, which corroborates their Newtonian behavior. For the samples with higher concentrations, the power law indices were significantly smaller with values of 0.96, 0.81 and 0.73 respectively for the 50 g/l, 100 g/l and 150 g/l. This shear thinning behavior exhibited by the wild type microalga *C. reinhardtii* at high concentrations has also been reported for other microalgal species although with considerable differences in the values probably due to the large variation in the intrinsic characteristic of the microalgal species [20]. A better detailed rheological characterization of suspensions of microalga *C. reinhardtii* analyzing the normal stress differences under steady flow experiment as well as the viscoelastic properties assessed under small amplitude oscillatory shear flow can be consulted in Appendix III.

**Table 5.1. Rheological characterization of microalgal broths**. Power law fitting parameters for microalgal broths at different biomass concentrations.

| Concentration g/l | Consistency index K | Behavior index n | $R^2$ |
|---|---|---|---|
| **10** | 0.001211 | 1 | 0.999 |
| **20** | 0.001474 | 1 | 0.999 |
| **30** | 0.001803 | 1 | 0.998 |
| **40** | 0.002121 | 1 | 0.999 |
| **50** | 0.002847 | 0.96 | 0.997 |
| **100** | 0.010832 | 0.81 | 0.998 |
| **150** | 0.026056 | 0.73 | 0.993 |

### 5.3.2. Application of the rheological properties for flow manipulation and light absorption optimization in photobioreactors

In the previous sections of this chapter, we clearly documented how the rheological and hydrodynamic parameters can be used to optimize different microalgal photobioreactor operations including mixing, mass transfer etc. In this work, it was a particular interest to use the rheological understanding of microalgal suspensions as a tool to optimize the absorption of light by microalgal cells growing in photobioreactors. Researchers have for long demonstrated that the distribution of light is non-homogeneous inside photobioreactors mainly due to the photobioreactors configuration and shading effects by microalgal cells [21,22]. This becomes a more complex issue over culture time due to microalgal proliferation increasing the shading effects. To palliate this issue some have focused on investigating the effect of flashing light on microalgal metabolism. It has been found that shifting the microalgal cells periodically from high irradiance to low irradiance has beneficial effects on microalgal photosynthetic activities. However, the frequency of such cycle from high light to low light has been deemed very critical for the effects on the biomass productivity [23-26]. We used the rheological and hydrodynamic results obtained for *C. reinhardtii* based microalgal culture to predict and control the cycle at which these microalgal cells are exposed to high light and low light intermittently during their growth within a typical photobioreactor.

While the analysis can be extended to other photobioreactor configurations, we consider the case of a tubular photobioreactor. Considering the case of an airlift tubular photobioreactor with a radius $R$ and an external irradiance $Io$, at any culture time, based on the cells population, the irradiance profile throughout the tube can be determined [27]. Then, based on the critical light requirement for photosynthesis, the photobioreactor volume can be sectioned into light zone ($V_l$)

and dark zone ($V_d$). The motion of the microalgal cells between the light zone and the dark zone can then be controlled using the flow properties to determine the average light/darkness cycle time ($t_c$), *i.e.*, the time it takes the average microalgae cell to travel from the point with the highest light intensity to the one with the lowest light intensity.

The work by Brindley *et. al.*[27] described the mathematical expressions to determine $t_c$ in a typical tubular photobioreactor. Let $\mu$ be the effective viscosity and $\rho$ the density of the culture, for a given axial flow velocity ($v_L$), the velocity ($V_m$) to which the average microalgal cell moves from the center (dark) to the outermost part of the tube (light) is determined using Davies equation [28]:

$$V_m = 0.2(\frac{v_L^7 \mu}{2R\rho})^{1/8}$$

The light/darkness cycle time $t_c$ is then determined using the equation:

$$t_c = \frac{2R}{V_m}$$

The case of an airlift tubular photobioreactor like the one described by Molina *et. al.* [29], with a diameter of 6 cm operating at an axial flow velocity of 50 cm/s first and then 20 cm/s is considered. The light darkness cycle time is determined considering different microalgal biomass concentrations in their impacts on the flow properties. Given the axial velocity, the shear rate was determined as [30]:

$$\dot{\gamma} = \frac{(3n+1)V_L}{nR}$$

The effective viscosity is then determined, based on the shear rate as supported by the rheological results presented in the previous section for each microalgal biomass concentration, in order to find $t_c$. In figure 5.2A values of $t_c$ are plotted for different microalgal concentrations at the two axial flow velocities. In both cases, the effect of microalgal biomass concentration on the flow properties was clearly shown to affect the light darkness cycle time ranging from 4.8s to 3.6s and 2.2s and 1.7s for the high and low axial velocities considered. We also estimated the power requirement for the circulation of the microalgal broths with different concentrations. Figure 5.2B shows the factor by which the power consumption increases to maintain the same operating conditions. The power consumption can, in fact, increase by a factor as high as 2 in order to maintain the same axial flow velocity and the correlating light darkness cycle at different microalgal biomass concentrations.

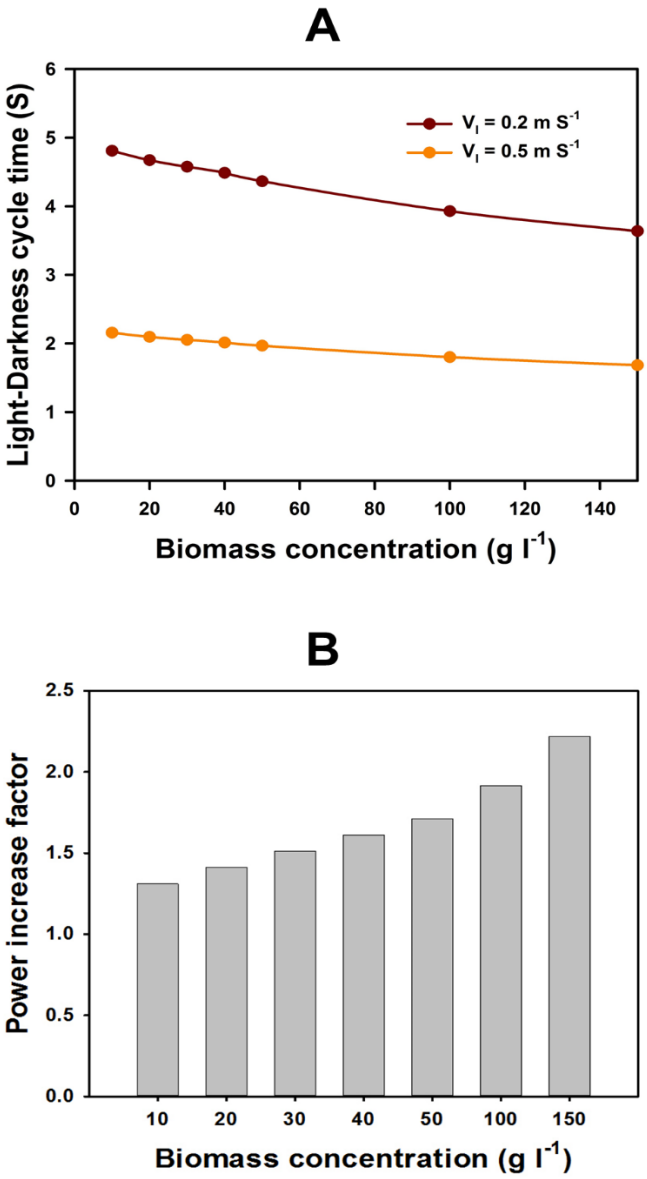


**Figure 5.2: Application of the rheological properties of microalgal broths for flow manipulation.** Light-darkness cycle time *tc* determined at different biomass concentrations using the flow properties (A) and the power consumption with an axial velocity of 0.5 m. $s^{-1}$ at different microalgal concentrations (B).

## 5.4. Conclusions

In summary, the rheological properties of microalgal suspensions were studied using the wild type microalga *C. reinhardtii*. At biomass concentrations below 50 g/l, the microalgal suspensions exhibited Newtonian behavior. The relative viscosity increased with increasing biomass concentration and the relative viscosity could be related to the volume fraction of spherical microalgal cells using Einstein's equation with $\alpha$=6.4. At biomass concentrations above 50 g/l, the microalgal suspensions exhibited shear thinning behavior and was modeled as power law fluids. These hydrodynamic parameters were then used to estimate the time an average microalgal cell would travel from the dark region to a high illumination region of a photobioreactor, often referred to as the light/darkness cycle. While this study assesses the collective behavior of the microalgal suspension, the hydrodynamic properties obtained can also be used for individual particle tracking using mathematical modeling and computer simulations[31]. Further studies can also be performed using the rheological properties of microalgal slurries to optimize pumping and dewatering of microalgal biomass and to assess mass transfer and nutrient concentrations in microalgal culture.

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# CHAPTER 6

# SUMMARY AND RECOMMENDATIONS FOR FUTURE WORK

The issue of distribution and absorption of light in microalgal cultivation systems is a critical factor that affects the microalgal growth rate and the quality of the microalgal biomass produced. Motivated by the need for high throughput microalgal biomass production systems that can sustain the large scale production of biofuels and certain valuable chemicals, this dissertation focused on developing strategies to improve the distribution and absorption of light in microalgal photobioreactors. The strategies included exploiting the plasmon resonance property of metallic nanoparticles for light enhancement, using surface micropatterning to better control the impacts of biofilm and biofouling on light delivery, exploiting the thermoreversible sol-gel transition property of the copolymer pluronic to develop short light path cultivation systems for efficient light absorption and also the use of flow manipulation to control the exposure of microalgal cells to ideal light zones and to create an optimal light/darkness balance for enhanced microalgal growth.

## 6.1 Development of plasmonic film light filters for enhanced microalgal growth and biomass composition

Through plasmon resonance, silver and gold nanoparticles have the ability to selectively backscatter light in different regions of the visible electromagnetic spectrum. This property was used to enhance the irradiance in microalgal cultivation systems not only by increasing the light intensity within the cultivation systems but also by supplementing the specific light wavelengths (blue and red lights) desired by the microorganisms and corollary minimizing the risk of photoinhibition that can be induced by unnecessary wavelengths. The developed plasmonic film technology consists of a tailored synthesis of gold nanorods and silver nanospheres to enhance light at target wavelengths and embedding these plasmonic nanoparticles in a polymeric matrix to fabricate millimeter thin plasmonic films that can be used as light filters in microalgal

photobioreactor design. A first system built by wrapping the base of a conical flask with a silver nanospheres plasmonic film was tested using a *Chlamydomonas reinhardtii* culture. This led to an increase in microalgal biomass production by more than 25% and an increase in total photosynthetic pigment generation by more than 35%, after ten days of cultivation. Light enhancement by plasmon resonance did not affect cellular carbohydrate and lipid accumulation although the increase in cell density resulted in enhanced overall lipid and carbohydrate production. These positive results with selective enhancement of blue light by silver nanoparticles motivated the synthesis of gold nanorods with specific shape and size for the selective enhancement of red light irradiation in microalgal culture. A strong system with dual enhancement of red and blue lights necessary for microalgal photosynthetic activities proved that biomass production and photosynthetic pigments accumulation can be increased by up to 50% and 78% respectively, through light enhancement induced by the use of the plasmonic film light filters.

## 6.2 Development of a short light path energy efficient microalgal cultivation and harvesting system

The length of the light path refers to the depth the incident light needs to penetrate in order to reach the last layer of microalgal cells in a microalgal cultivation system. For an open pond system, as an example, the bottom of the pond is often subject to low light penetration due to accumulating shading effects over the length of the light path. Short light path microalgal cultivation systems are therefore beneficial for better distribution and absorption of light. Aiming at developing a short light path cultivation system for enhanced microalgal biomass production, we described in this dissertation an energy efficient microalgal cultivation and harvesting system that we have developed using the thermoreversible sol-gel transition properties of pluronic. With this system that we proposed, after seeding microalgae in the novel TAPP medium in a solution

phase at 15°C, the temperature was increased by 7°C to induce gelation. Within the gel, microalgae were observed to grow in large clusters rather than as isolated cells. The settling velocity of the microalgal clusters was approximately ten times larger than that of individual cells cultured in typical solution media. Such clusters were easily harvested gravimetrically by decreasing the temperature to bring the medium to a solution phase. The microalgal biomass production as well as the composition of the biomass was not affected by such entrapped cultivation mode, as compared to typical suspension based cultures. This novel system developed, along with addressing the energy consumption issue with constant mixing in typical suspended culture and that related to microalgal biomass harvesting, may also help solving several issues related to the distribution and absorption of light in microalgal cultivation systems. First, because this medium would allow microalgae to grow in a confined environment, light delivery to these photosynthetic microorganisms can better be controlled. Second, the very confinement would prevent microalgae from sticking to the wall of photobioreactors which often hinders light penetration. Ultimately, the gel structure of the medium during the growth period offers a lot of flexibility to divide the systems into short light path layers and also would offer a lot of flexibility when it comes to the orientation and inclination of the cultivation systems to receive the optimal irradiance.

## 6.3 Controlling light delivery through manipulation of cells attachment on the surface of photobioreactors

In the context of large scale microalgal biomass production, adhesion of microalgae to surfaces can be perceived as desirable or undesirable based on the cultivation mode. In enclosed and suspended microalgal cultivation systems, biofilm development hinders light penetration and therefore decreases the growth rate and the production of valuable photosynthetic compounds [1]. It can further lead to mechanical functioning issues to the microalgal biomass production

installations. On the other hand, microalgal biofilm has recently been largely investigated as a good cultivation method for largescale microalgal biomass production. With the biofilm cultivation systems, the light path is much shorter, a higher biomass concentration is often obtained, less volume of water is used and the harvesting of the microalgal biomass produced is less complicated and more energy efficient [2,3]. In this dissertation, we studied the adhesion ability of microalgae on the surface of photobioreactors with the goal of acquiring the ability to control the enhancement or the inhibition of bioadhesion as desired based on the cultivation mode. Specifically, based on the current theories and reported experimental findings available, we designed and performed experiments to provide some microtopographic cues on how to control the adhesion of certain microalgal strains on the surface of photobioreactors. Based on the results of our experiments, we inferred that the adhesion of such microalgae was largely decreased on surfaces that were patterned with protruding squares of length smaller than the diameter of the cells and a valley distance largely smaller than the diameter of the cells, as compared to a smooth surface. It was also proven that microalgal adhesion was greatly increased when the size of the protruding squares and the valley length were slightly bigger than the diameter of the cells. An adhesion enhancement factor was computed to specify how surface micropatterning affects the total microalgal adhesion and it was found that for valley distances larger than the cells diameter, this adhesion enhancement factor decreased with increasing valley distances between protruding squares and the effects of surface micropatterning became almost non-significant when the valley distance approaches 100 µm. Further, no critical protruding square size for maximal adhesion over the top of such protruding squares was found, like it has been reported for rod-like shaped *E. coli* cells. These adhesion trends observed with the wild

type *C. reinhardtii* CC-124 were also observed for the motility impaired mutant *C. reinhardtii* CC-1036 and the cell wall deficient mutant *C. reinhardtii* CC-503.

## 6.4 Rheological characterization and flow manipulation to improve light absorption in microalgal photobioreactors

For microalgal cultivation systems with a light path length not favorable for optimal distribution and absorption of light, mixing of microalgal broths is often used as a means to help with the periodical shuttle motion of microalgal cells to the photobioreactor sections with optimal irradiance. However, mixing is an energy intensive process and some microalgal species are also sensitive to shear. Therefore, there has been a need to optimize the mixing operation in microalgal cultivation. Further, it has been known that specifically designed light/darkness cycle exposure for microalgal cells can increase the productivity [4]. To optimize the mixing and create the necessary light/darkness cycle for better light absorption in microalgal cultivation systems, an understanding of the hydrodynamic properties of typical microalgal broths was necessary. In this dissertation, we characterized the rheological properties of suspensions of microalga *Chlamydomonas reinhardtii* and showed that such suspensions exhibit Newtonian behavior at low microalgal concentration and non-Newtonian shear thinning behavior at concentrations above 50 g/l. The rheological insights obtained were then used as tools to control the cycle to which an average microalgal cell would travel from high light to low light zones within a typical photobioreactor.

## 6.5 Recommendations for future work

This dissertation has provided some clear and proven strategies to improve the distribution and absorption of light in microalgal photobioreactors. In this section, some ideas on how to implement these developed technologies for large scale microalgal cultivation and also

on their applications to address other issues or to provide further insights on certain pertinent scientific questions are presented.

One issue to consider during the application of the plasmonic film light filter technology is the fact that the light enhancement through plasmon resonance is only effective over a few centimeters of light path length. We clearly demonstrated how other strategies such as the use of short light path systems and optimal light darkness balance can help palliating this fact. A systematic approach to implement the plasmonic film light filter technology may require computer modeling to describe the light distribution profile throughout the adopted photobioreactor configurations. Mie's theory can be used to characterize the enhancement of irradiance through light backscattering based on the shape, size and nature of the plasmonic nanoparticles in use [5]. Then, the photobioreactor configuration can be manipulated for optimal light distribution [6]. Further, while we focused on enhancing blue and red lights that were necessary to enhance microalgal biomass production and photosynthetic pigments accumulation using microalga *C. reinhardtii*, other species or the production of other compounds may favor other wavelengths [7]. The use of plasmonic nanoparticles with backscattering properties at other wavelengths may be a good option to address such needs.

The Tris-Acetate-Phosphate-Pluronic (TAPP) system with interesting microalgal cultivation and harvesting as well as light distribution potentials has introduced a wide range of new insights that can lead to further studies and applications. We have discussed how the copolymer pluronic has largely been investigated for its potential in controlling biofouling and its effectiveness in producing stable surface patterns that can be useful in long term single-cell culture. We have also noted that single-cell cultivation of microalgae can be a good method for preparing colonies of promising microalgal strains for large scale cultivation[8]. It is therefore

envisioned that thermoreversible TAPP-like systems will find further applications in microalgal biomass production for biofouling control and single-cell cultivation[9,10,11]. Further, future studies to continue this work may include potential functionalization of the copolymer in order to minimize the concentration necessary to confer the required thermorheological properties. Other studies may also be undertaken to elucidate the interactions of different microalgal species and strains with the pluronic matrix and to further characterize the microalgal clusters obtained through confinement in the TAPP matrix. In addition, the scale up of the TAPP technology for large scale microalgal biomass production will necessitate further design consideration to maintain the required thermoreversible properties while also minimizing the cost associated with such method of production.

The idea consisting of manipulating microalgal adhesion to the surfaces of photobioreactor via surface micropatterning should also be extended to address other scenario. This can be done through the use of different protruding feature shapes and also the use of microalgal species with non-spherical shape or with different surface properties [12].

The use of the hydrodynamic properties to control the shuttle motion of microalgal cells and coordinate their periodic exposure to low and high light sections of photobioreactors necessitates further studies as well. This can include the study of different microalgal species and the impacts of their specific properties (shape, size, motion, Exopolymeric secretions) on the real time rheological behavior of microalgal culture [13]. Other rheological properties of microalgal broths such as viscoelasticity can also be studied. In addition, the rheological insights can be used for computer simulations and particle tracking to understand the motion of microalgal cells which may help further optimize their light/darkness exposure.

In summary, in this dissertation we have proposed several strategies to improve the distribution and absorption of light in microalgal photobioreactors. There are other strategies to approach such issues that were not explored in this work and some studies by other research groups have proposed other ideas to approach this problem [14]. It is envisioned that these technologies whose performance has been demonstrated in bench scale systems will be subject to scale up studies for imminent large scale application. Solving the issue of poor distribution and absorption of light in photobioreactors, along with the other issues mentioned in this dissertation, will make microalgal biorefining a viable enterprise for the production of biofuels and valuable chemicals.

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# APPENDICES

## APPENDIX I Protocol for TAP medium preparation

*From* Gorman, D.S., and R.P. Levine (1965) *Proc. Natl. Acad. Sci. USA* **54**, 1665-1669.

Make the following stock solutions:

**#1 TAP Salts**

| | |
|---|---|
| $NH_4Cl$ | 15.0 g |
| $MgSO_4 \cdot 7H_2O$ | 4.0 g |
| $CaCl_2 \cdot 2H_2O$ | 2.0 g |
| water to 1 liter | |

**#2 phosphate solution**

| | |
|---|---|
| $K_2HPO_4$ | 28.8 g |
| $KH_2PO_4$ | 14.4 g |
| water to 100 ml | |

#3 **Hutner's trace elements** (see next page)

To make the final medium, mix the following:

2.42 g Tris

25 ml solution #1 (salts)

0.375 ml solution #2 (phosphate)

1.0 ml solution #3 (trace elements)

1.0 ml glacial acetic acid water to 1 liter

For solid medium, add 15 g agar per liter Autoclave.

For 'Tris-minimal' medium: omit acetic acid, titrate final solution to pH 7.0 with HCl.

**#3 Hutner's trace elements**

Hutner et al. (1950) *Proc. Am. Philos. Soc.* **94**, 152-170

For 1 liter final mix, dissolve each compound in the volume of water indicated. The EDTA should be dissolved in boiling water, and the $FeSO_4$ should be prepared last to avoid oxidation.

| **compound** | **amount** | **water** |
|---|---|---|
| EDTA disodium salt | 50 g | 250 ml |
| $ZnSO_4 \cdot 7 H_2O$ | 22 g | 100 ml |
| $H_3BO_3$ | 11.4 g | 200 ml |
| $MnCl_2 \cdot 4 H_2O$ | 5.06 g | 50 ml |
| $CoCl_2 \cdot 6 H_2O$ | 1.61 g | 50 ml |
| $CuSO_4 \cdot 5 H_2O$ | 1.57 g | 50 ml |
| $(NH_4)_6Mo_7O_{24} \cdot 4 H_2O$ | 1.10 g | 50 ml |
| $FeSO_4 \cdot 7 H_2O$ | 4.99 g | 50 ml |

Mix all solutions except EDTA. Bring to boil, then add EDTA solution. The mixture should turn green. When everything is dissolved, cool to 70 degrees C. Keeping temperature at 70, add 85 ml hot 20% KOH solution (20 grams / 100 ml final volume). Do NOT use NaOH to adjust the pH.

Bring the final solution to 1liter total volume. It should be clear green initially. Stopper the flask with a cotton plug and let it stand for 1-2 weeks, shaking it once a day. The solution should eventually turn purp and leave a rust-brown precipitate, which can be removed by filtering through two layers of Whatman#1 filter paper, repeating the filtration if necessary until the solution is clear. Store refrigerated or frozen convenient aliquots. Some people shorten the time for formation of the precipiate by bubbling the solutio with filtered air.

If no precipitate forms, the solution is still usable. However, you might want to check the pH in this case and adjust it to around 7.0 using either KOH or HCl as needed. To prepare sulfur-free trace elements for hydrogen generation, the sulfate salts can be replaced with equimolar chloride salts ($ZnCl_2$ 10.0 g; $CuCl_2 \cdot 2 H_2O$ 1.00 g; $FeCl_2 \cdot 4 H_2O$, 3.60 g).

**APPENDIX II Protocol for preparation of plasmonic film filters**

**Gold Nanorod synthesis**

Initial solutions

- 5 mL of 0.5 mM HAuCl4
- 5 ml of 1mM HAucl4
- 5 ml of 1mM HAucl4
- 5 mL of 0.20 M CTAB
- 10 mM NaBH4 solution
- 4 mM AgNO3 solution
- 0.0788 M ascorbic acid

Seed solution preparation

- Mix 5 ml of 0.5 mM HAuCl4 with 5 ml CTAB (0.2 M)
- Add 0.6 ml of cold ice NaBH4 to the stirred mixture (this will lead to brownish sol)
- Stir vigorously for 2 minutes
- After that keep the solution at 25°C.

Nanorods growth

- Add 5 ml of 0.2M CTAB to 0.06 ml of 0.004 M AgNo3 at 25°C
- Then, add 5 mL of 1 mM HAucl4 and mix gently
- After that add 70 µL of 0.0788 M of Ascorbic acid
- Add 12 µL of seed solution at 30°C.
- Finally, allow 30 minutes for the gradual growth of the nanorods by incubating the medium at 30°C.

**Synthesis of silver nanospheres**

- Prepare a 30 ml solution of 2 mM sodium borohydride ($NaBH_4$) in water.
- Prepare a 10 ml solution of 1 mM silver nitrate ($AgNO_3$) in water.
- Put the sodium borohydride in an ice bath until it reaches 4°C.
- Then gradually add (1 drop/second) the silver nitrate solution to the sodium borohydride solution under constant mixing.
- Stop the mixing immediately after the addition of the silver nitrate.

To prepare the plasmonic film, add a 5% weight of polyvinyl alcohol (PVA) powder to the silver nanoparticles suspension and/or centrifuge the gold nanorods suspension and redisolve the gold nanorods ink in a 5% weight PVA in water solution. Finally, pour the mixture into petri-dish to be dried in darkness (1-2 days).

## APPENDIX III Rheological characterization of microalga *C. reinhardtii* suspensions


### Abstract

The rheological characterization of microalgal broths can serve to several purposes related to microalgal biomass production and microalgal biomass biorefining. For instance, the periodical motion of microalgal cells from well illuminated to poorly illuminated sections of a photobioreactor is a good way to optimize light absorption by the microalgal cells that first requires a deep understanding of the hydrodynamic properties of typical microalgal culture. In addition, an understanding of the rheological properties of microalgal broths can help optimizing mixing, oxygen removal, mass transfer and nutrient delivery and can also help minimizing the energy requirement for pumping. In this study, we characterized the rheological properties of suspensions of microalga *Chlamydomonas reinhardtii* and found, among other properties, that such suspensions exhibited Newtonian behavior at low microalgal concentration and non-Newtonian shear thinning behavior at concentrations above 50 g/l. The normal stress difference under steady shear flow experiment was also evaluated and the viscoelastic properties of concentrated microalgal broths were analyzed and discussed based on the data obtained through small amplitude oscillatory shear experiment.

## Introduction

The optimization of microalgal biomass production and microalgal biorefining requires a thorough understanding of some inherent parameters and their impacts on the biomass productivity and/or on the energy consumption by such processes. While some of the parameters such as nutrient concentration, salinity, photobioreactor configuration etc. have for long been extensively studied, it is only recently that the hydrodynamic properties of microalgal broths and microalgal slurries have received great consideration as an optimization parameter for enhanced microalgal biomass production[1-4].

The hydrodynamic characteristics of microalgal broths have direct impacts not only on the microalgal cultivation in typical microalgal photobioreactors but also on the harvesting of the biomass generated. In fact, constant mixing in photobioreactor operations is often deemed necessary to keep the microalgal cells in suspension, to help nutrient distribution, to facilitate gas exchange and mass transfer etc.[5] On the other hand, excessive mixing can have bad effects on microalgal culture that can even lead to cell damage [6]. Some experimental studies using hydrodynamic manipulation for proper mixing of microalgal culture have known great success [7]. Further, the liquid velocity affecting, among others, the residence time and the rate of oxygen removal in most photobioreactors can easily be controlled using the hydrodynamic properties [8]. Adding to these, the harvesting and dewatering of microalgal biomass is known to be an energy intensive process. Some studies have focused on characterizing the hydrodynamic properties of microalgal slurries for an efficient design of harvesting units and for the minimization of the energy requirement [9]. The need for hydrodynamic optimization in microalgal cultivation systems is becoming more and more crucial as higher biomass concentrations are becoming more and more achievable and practical in the microalgal biorefining industry.

The nature of microalgal slurries makes the study of the hydrodynamic properties of such systems very tedious. Although water is the main constituent, the presence of micronutrients, dissolved salts, polymeric substances and microalgal cells makes microalgal broths complex fluids [10]. The characterization of the hydrodynamic properties of microalgal broths is even more difficult because the metabolism of microalgal cells has real time impacts on the nature of the fluids not only in terms of increased biomass concentrations due to proliferation of microalgal cells but also through motion of the microorganisms and potential bio-secretions in the medium [11]. Moreover, the large heterogeneity of microalgal species and strains used in microalgal biomass production and the largely reported morphological and physiological differences make it impractical to define broad hydrodynamic characteristics of microalgal broths. For instance, the work by Zhang *et. al.* reported the influence of different cell properties on the rheological characterization of microalgal suspensions [12].

In this work, we focus on the characterization of suspensions and culture of microalga *C. reinhardtii*. *C. reinhardtii* has been largely investigated for microalgal biomass production, among other reasons, because of its large potential for genetic engineering manipulations[13]. *C. reinhardtii* is a unicellular microorganism mainly spherical with diameter around ten micrometers. As microswimmers, they are classified among the pullers. As opposed to the micropushers such as *E. coli* that use a rotating flagellar bundle to push the fluid behind them, micropullers propel themselves through breaststroke-like motion with a pair of flagella attached at the front of their body. While it has been very challenging to characterize the macroscopic behaviors of micropushers, a few rheological studies on the collective properties of micropullers have been very promising [14]. Some of these studies have reported certain similarities between the behavior of micropullers in water to that of passive suspensions comprising of rigid spheres at

low concentration while others have reported shear thinning behavior over larger concentrations [9,10].

We aimed at studying the collective rheological behavior of broths and slurries of the microalga *C. reinhardtii.* A clear understanding of such behavior may serve to different applications including effective mass transfer and optimal mixing and light/darkness cycle. Herein, a summary of the major findings on the rheological behavior of microalga *C. reinhardtii* suspensions under steady shear flow are characterized along with the viscoelastic properties under small amplitude oscillatory shear experiments is presented.

## Materials and methods

### Microalgal culture and sample preparation

The wild type microalga *C. reinhardtii* CC-124, the motility impaired mutant CC-1036 and the cell wall deficient mutant CC-503 obtained from the Chlamydomonas Resource Center (University of Minnesota, St. Paul, Minnesota) were used in this study. Prior to the rheological characterization experiments, these strains were inoculated with a Tris-Acetate-Phosphate (TAP) medium in conical flasks placed on a rotary shaker (150 rpm). The microalgal culture duration was varied between 7 to 22 days in order to monitor any influence of culture time on the rheological properties. The culture room was continuously illuminated by full spectrum compact fluorescent lamps (CFL 60W, Fancierstudio, San Francisco, California) with the photosynthetic active radiation at the top surface of the culture at 75 ± 5 μE $m^{-2}$ $s^{-1}$ and the temperature at 22 ± 1°C. The growth of microalgae was monitored through optical density ($OD_{675}$) measurements. Calibration curves were built to correlate the optical density ($OD_{675}$) measurements with dry biomass concentration in unit of gram per liter.

After the cultivation period, the microalgal biomass concentration was assessed through optical density measurements. Following this, the microalgal broth was split between several conical flasks and was either diluted with water or centrifuged (4,800g, 5 min, 20°C) to remove excess water in order to obtain 2 milliliter microalgal suspension samples of 10 g/l, 20 g/l, 30 g/l, 40 g/l, 50 g/l, 100 g/l and 150 g/l. Final optical density measurements were then taken on each of these samples in order to ensure the accuracy of the concentrations of the sample. The volume fraction of microalgal biomass in the medium ($\varphi$) was determined from the dry biomass concentration using the formulae [10]:

$$\varphi = \frac{C}{\rho_w w_D}$$

$$\rho_w = x_w \rho_0 + (1 - x_w)\rho_D$$

Where $\rho_w$ and $\rho_D$ represent respectively the wet and dry density of microalgae expressed in unit of mass per volume and $x_w$ represents the volumetric water content per unit volume of microalgal biomass. $w_D$ representing the mass of dry solid per unit mass of dry microalgae is computed as follows:

$$w_D = \frac{(1 - x_w)\rho_D}{\rho_w}$$

### 5.5.1. Rheometry and data analysis

The rheological characterization experiments were performed using a Combined Motor and Transducer (CMT) AR-G2 rheometer from TA instruments (New Castle, Delaware). The cone-and-plate geometry with a diameter of 40 mm and cone angle 0° 59' 49" was used for all the measurements. The temperature control was achieved by a Peltier plate using thermoelectric effects that can accurately control the temperature and water circulation for rapid heating and cooling over a temperature range of 0 to 100°C. For these experiments, the temperature was controlled at 20°C. After each run, the Peltier plate was flushed with water to remove the sample and then dried and cleaned using acetone before loading a new sample. Steady shear flow experiments were performed by varying the shear rate from 10 to 800 $s^{-1}$ using logarithmic ramping with 10 intervals per decade of the shear rate. Peak hold experiments were performed by maintaining the shear rate constant at the desired value and the rheological data were collected

over a time interval of 1500 seconds. The viscoelastic properties were assessed under small amplitude oscillatory shear experiments.

## Results and discussion

### Rheological characterization of microalgal slurries under steady shear flow experiments

The effective viscosity values of microalgal suspensions of 10 g/l, 20 g/l, 30 g/l, 40 g/l, 50 g/l, 100 g/l and 150 g/l dry biomass concentrations were plotted against shear rates ranging from 10 to 800 $s^{-1}$ in figure I and were compared to a TAP buffer sample with no microalgal cells used as control. Lower shear rates could not be tested because of inaccuracy in the measurements that are most likely the results of surface tension and/or inertia effects. At microalgal concentrations below or equal to 40 g/l, the viscosity did not exhibit any dependence on shear rate which is a strong characteristic of Newtonian fluid. Over this range of concentrations, the viscosity was observed as increasing with increasing microalgal concentrations. It is well documented in literature that the viscosity of most suspensions increases with increasing solute concentrations. Let $\mu_s$ be the solvent viscosity and $\varphi$ the volume fraction of solute, the viscosity of most suspensions can often be modeled as:

$$\mu = \mu_s(1 + \alpha\varphi)$$

While for passive suspensions of rigid spherical particles α is usually reported as being equal to 2.5, for suspensions of active particles α is often reported with higher values potentially due to the effects of swimming.[18,19] After correlating the biomass concentrations from 0 to 40 g/l to match the volume fractions as described previously, linear regression analyses performed with the viscosity results obtained, led to an α value of 6.4 with $R^2$=0.99 for these *C. reinhardtii* suspensions.

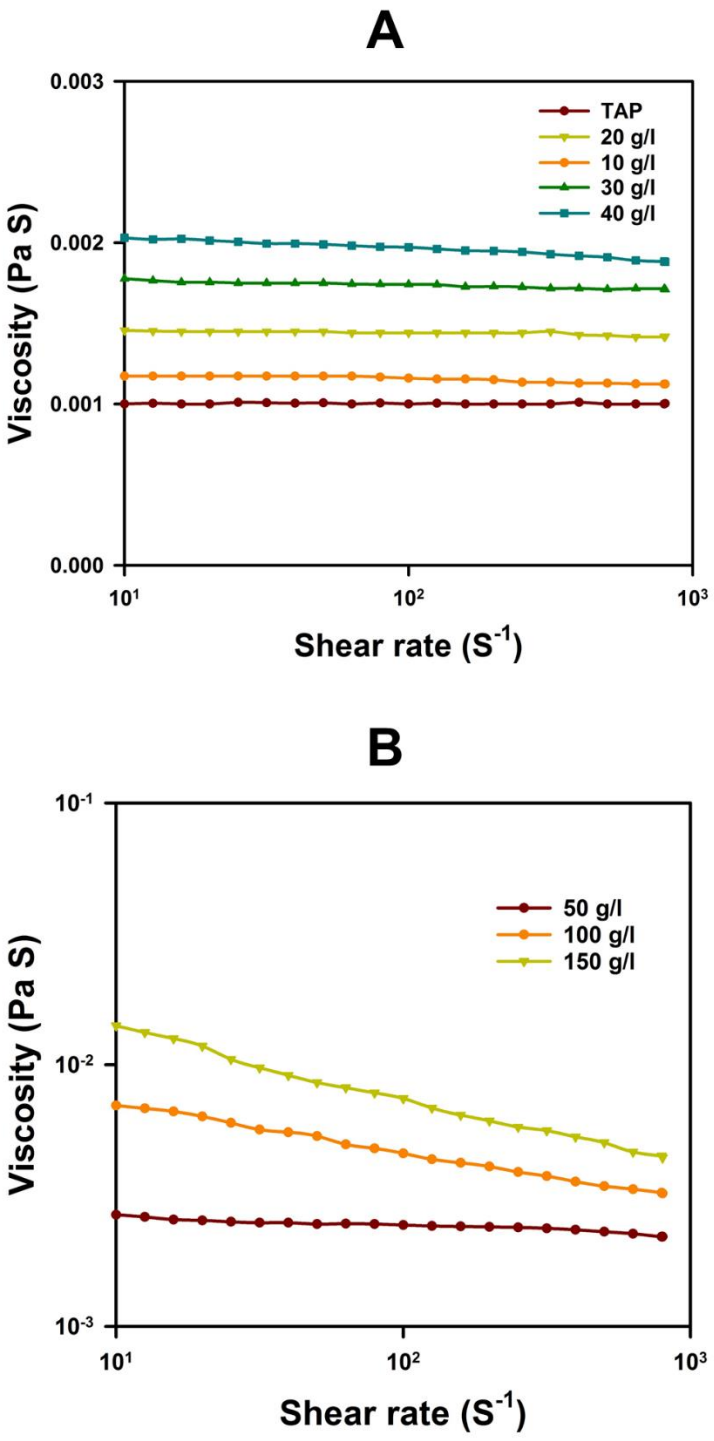


**Figure I: Rheological characterization of microalgal broths.** Viscosity profiles of microalgal suspensions as a function of shear rate at low biomass concentrations (A) and at high biomass concentrations (B).

Three higher microalgal biomass concentrations, namely, 50 g/l, 100 g/l and 150 g/l were also analyzed. For these three concentrations, the effective viscosity exhibited strong dependence on the shear rate value with a shear thinning behavior.

The power law model is among many models used to characterize the shear rate dependence of the effective viscosity. With this model, for a given shear rate $\dot{\gamma}$, the effective viscosity is obtained by [17]:

$$\mu_{eff} = K(\dot{\gamma})^{n-1}$$

Where, $K$ is the consistency index and $n$ is the power law index. For shear thinning non-Newtonian fluids, the power law index is less than one and the viscosity decreases with increasing shear rate and for shear thickening non-Newtonian fluids the power law index is greater than one and the viscosity increases with increasing shear rate.

Table I summarizes the results obtained by fitting the effective viscosity results with the power law fluid model. The consistency index K, the power law index n along with $R^2$ values are reported for all the microalgal concentrations considered. For the samples with concentrations equal or less than 40 g/l, the power law index was very close to 1, which corroborates their Newtonian behavior. For the samples with higher concentrations, the power law indices were significantly smaller with values of 0.96, 0.81 and 0.73 respectively for the 50 g/l, 100 g/l and 150 g/l. This shear thinning behavior exhibited by the wild type microalga *C. reinhardtii* at high concentrations has also been reported for other microalgal species although with considerable differences in the values probably due to the large variation in the intrinsic characteristic of the microalgal species [20].

**Table I. Rheological characterization of microalgal broths.** Power law fitting parameters for microalgal broths at different biomass concentrations.

| Concentration g/l | Consistency index K | Behavior index n | $R^2$ |
|---|---|---|---|
| **10** | 0.001211 | 1 | 0.999 |
| **20** | 0.001474 | 1 | 0.999 |
| **30** | 0.001803 | 1 | 0.998 |
| **40** | 0.002121 | 1 | 0.999 |
| **50** | 0.002847 | 0.96 | 0.997 |
| **100** | 0.010832 | 0.81 | 0.998 |
| **150** | 0.026056 | 0.73 | 0.993 |

To characterize the response of a fluid to steady shear flow, the viscosity, the first normal stress coefficient and the second normal stress coefficient are the material functions often evaluated. While the viscosity characterizes the resistance to shear and the tangential friction component of the stress tensor, the normal stress differences, arising mainly from particle interactions in most suspensions, characterize the non-isotropic nature of the diagonal elements of the stress tensor [21]. In the previous section, we characterized the viscosity profile of the microalga *C. reinhardtii* suspensions subjected to steady shear flow experiments. While it was not possible to evaluate the second normal stress difference with the cone and plane geometry on the AR-G2 rheometer, the first normal stress differences were measured at the different steady shear rates. In figure IIA, the first normal stress difference (N1) data are plotted for different steady shear rates and the first normal stress coefficient data are plotted in figure IIB for the 100 g/l and the 150 g/l microalgal suspensions. The suspensions at lower concentrations led to either zero or low and unrepetitive normal stress responses most likely due to the quasi-Newtonian behavior and/or the very low and random interactions between the microalgal cells. The first normal stress values were positive and decreased slightly with increasing shear rates whereas the first normal stress coefficient decreased almost proportionally to the square of the steady shear rate.

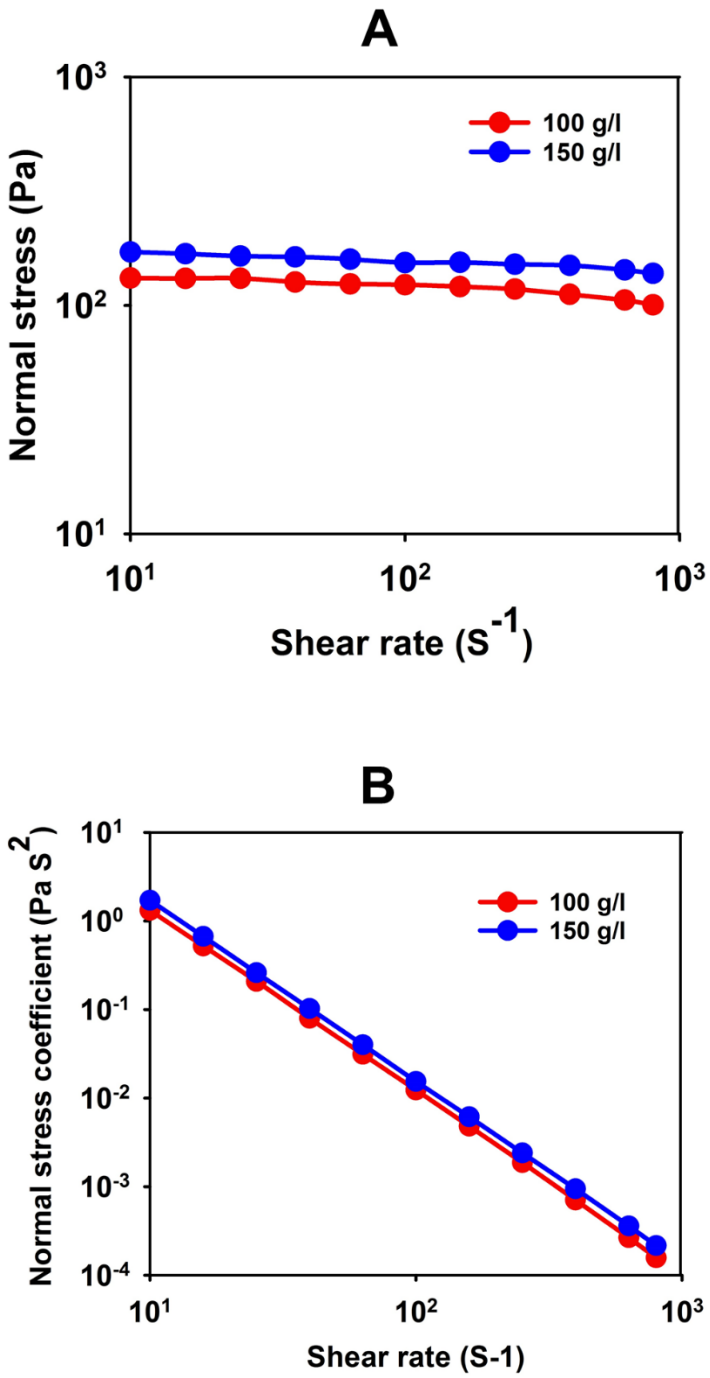


**Figure II: Normal stress and normal stress coefficient profiles under steady shear flow experiments.** (A) Normal stress as a function of shear rate and (B) Normal stress coefficient as a function of the shear rate.

**Rheological characterization of microalgal slurries under small amplitude oscillatory shear experiments.**

Following the steady shear flow analyses, the viscoelastic properties of microalga *C. reinhardtii* suspensions were assessed using the same stress-controlled AR-G2 rheometer. Suspensions with microalgal concentrations of 100 g/l and 150 g/l were prepared for such experiment. The few measurements from lower concentrations of microalgal suspensions resulted in weak viscoelastic responses and easily led to inaccurate results. First, dynamic strain sweep measurements were performed at a constant frequency of 1Hertz to determine the linear viscoelastic region (LVR) in which the loss modulus and the storage modulus are independent of the shear strain to which they are subjected. The storage modulus (G') and the loss modulus (G") are material functions characterizing the response from a small amplitude oscillatory shear flow. The storage modulus relating the elastic property of the material represents the portion of the stress wave that is in phase with the strain wave whereas the loss modulus relating the viscous property of the material represents the portion of the stress wave that is out of phase with the strain wave. The dynamic strain sweep results for the microalgal concentrations considered are presented in figure II. The loss modulus and storage modulus were found to be constant for shear strain of up to 10% irrespective of the concentration of the microalgal suspension. Above the 10% strain, the moduli were observed as decreasing with increasing strain with a steeper slope for the storage modulus as compared to the loss modulus.

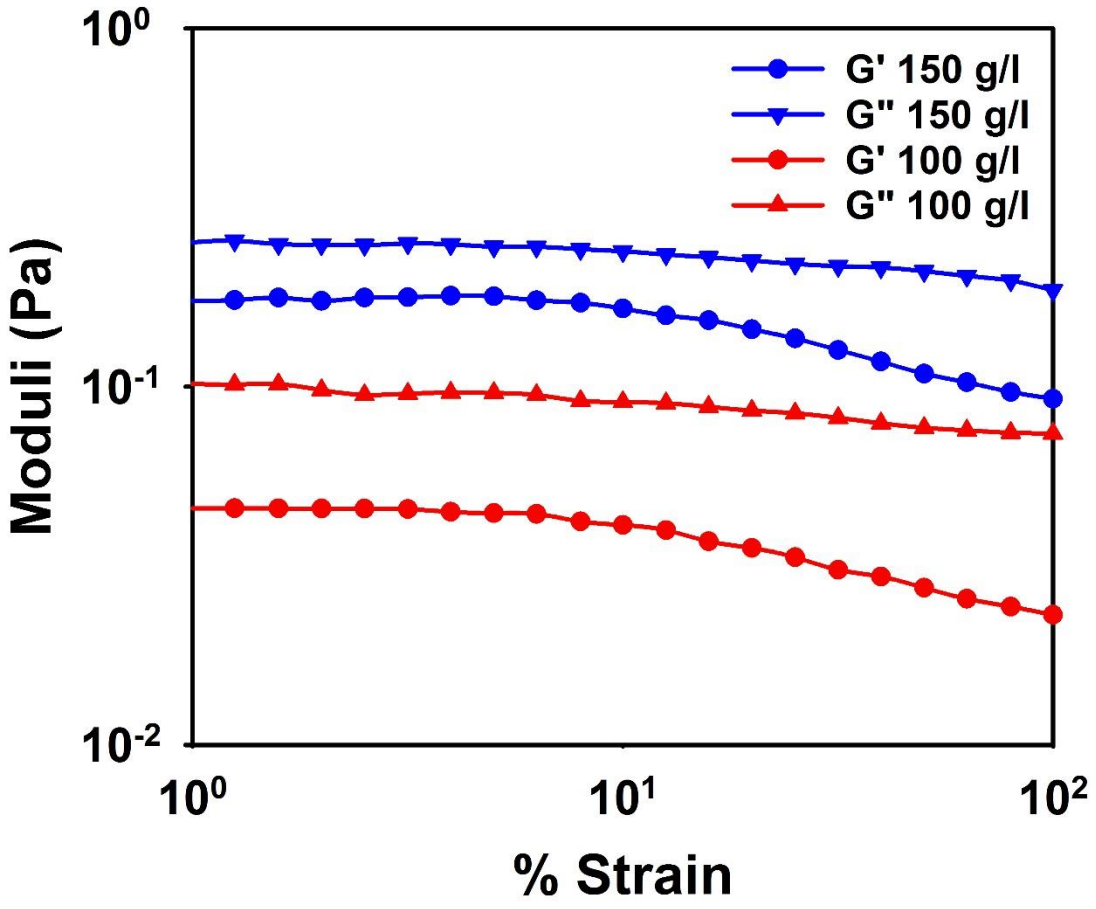


**Figure III: Dynamic strain sweep measurements of microalgal broths of *C. reinhardtii* at 100 g/l and 150 g/l microalgal concentrations.** Loss modulus and storage modulus as a function of % strain at a constant frequency of 1Hz.

To further characterize the rheological behavior of the microalgal broths, the 100 g/l and 150 g/l microalgal suspension samples were subjected to oscillatory frequency sweep analyses in the linear viscoelastic region. Frequency sweep measurement has been an effective strategy to assess materials performance that is also often used to identify the structuring mechanisms within a suspension or a polymer dispersion. Usually, small amplitude oscillatory shear deformations are applied at a constant % strain and over a range of frequencies and the structural responses are evaluated using the material functions such as the loss modulus, the storage modulus and the complex viscosity. The suspensions of microalga *C. reinhardtii* were evaluated over frequencies ranging from 1Hz to 100 Hz and the resulting storage and loss moduli are presented in figure III. Both the loss modulus and the storage modulus were found to increase with increasing frequencies. For the 150 g/l and the 100 g/l microalgal suspensions considered, the loss modulus values were always higher than the corresponding storage modulus over the range of frequencies considered which means that the viscous fluid effect was dominant in the mild viscoelastic behavior of the microalgal suspensions. The frequency sweep results also deviated from the common Maxwellian behavior of fluids where the slope of the loss and the storage moduli over the frequency axis have values of 1 and 2 respectively [22]. This deviation from the Maxwellian response has also been reported for other similar active suspensions [20]. The complex viscosity was also measured as a function of frequency through the frequency sweep experiments and the data are plotted in figure IV. The viscosity profile exhibited a trend of decreasing viscosities for increasing frequencies. On another note, at very high concentration of microalgal biomass (350 g/l), the storage modulus was observed to be higher than the loss modulus and crossover was observed under frequency sweep experiments. However, such high

microalgal concentrations are not realistic for microalgal cultivation although they may be reachable during certain downstream biomass processing.

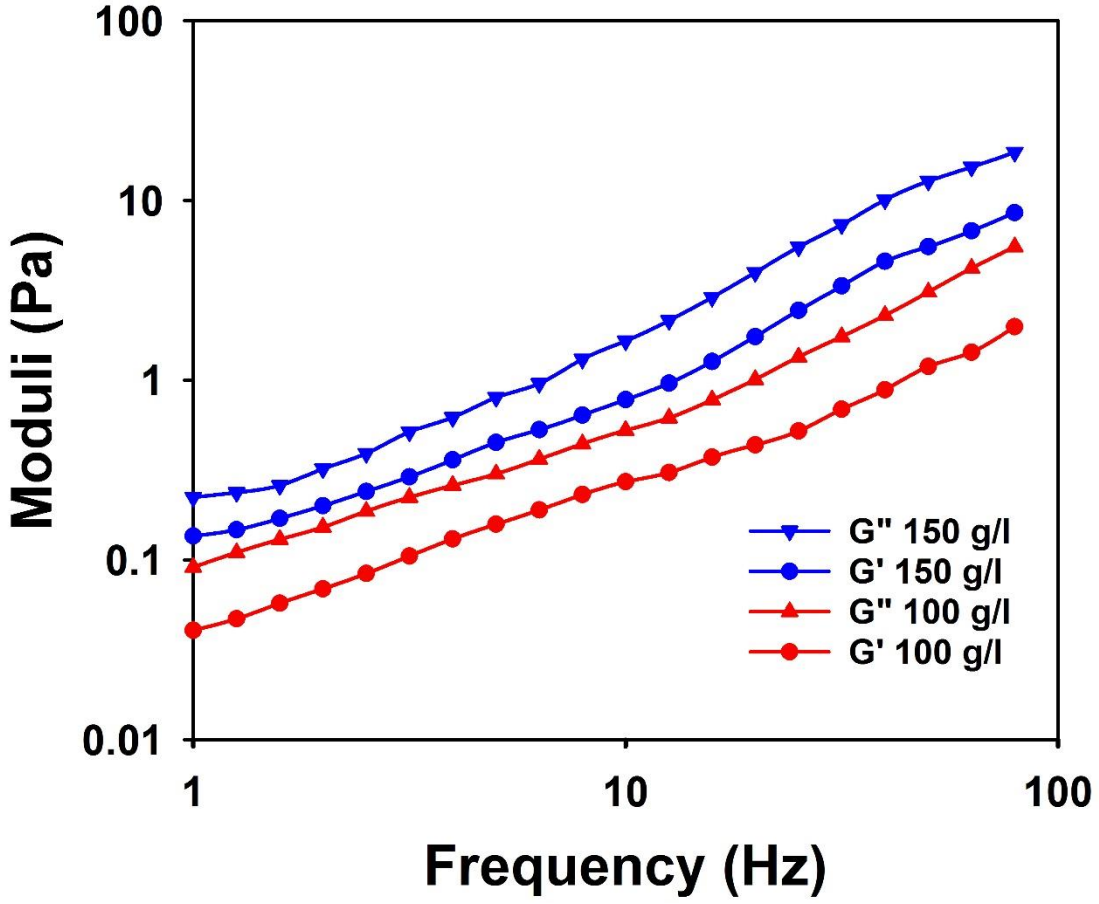


**Figure IV: Frequency sweep measurements of microalgal broths of *C. reinhardtii* at 100 g/l and 150 g/l microalgal concentrations.** Loss modulus and storage modulus as a function of the frequency.

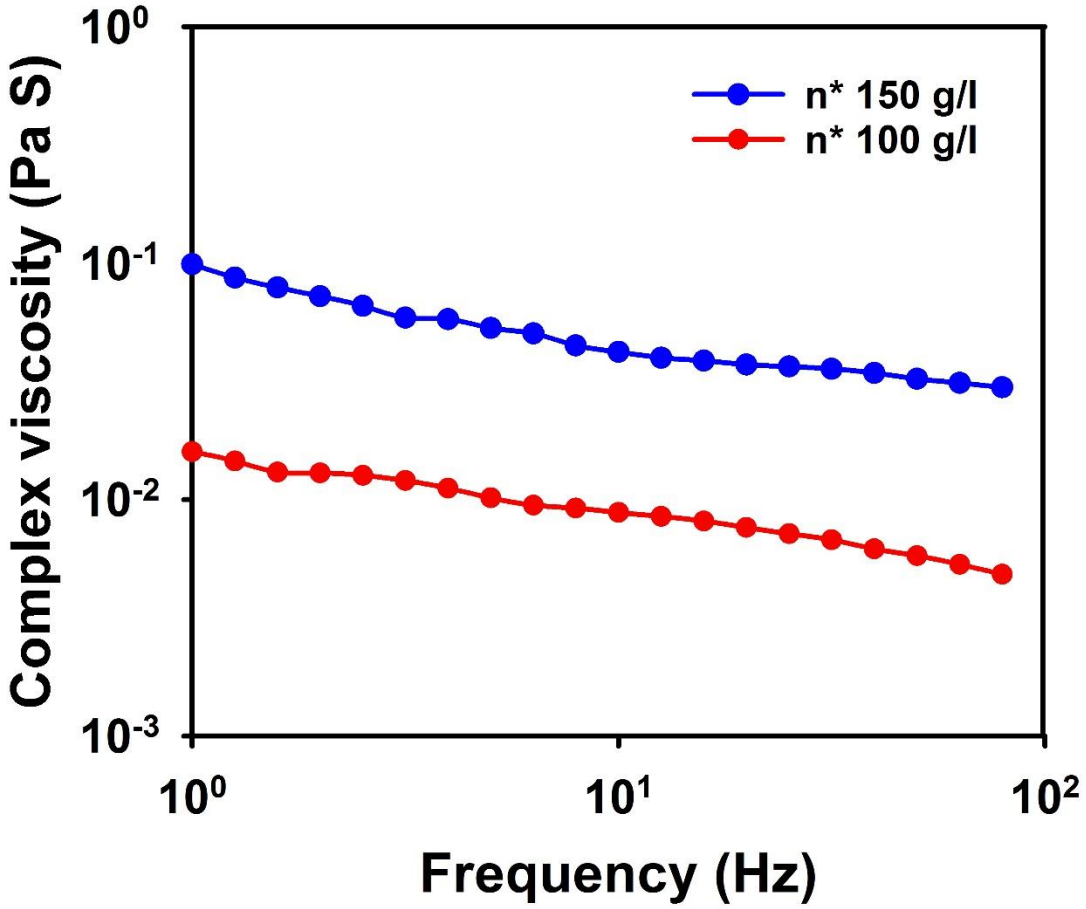


**Figure V: Complex viscosity profile of microalgal broths of *C. reinhardtii* at 100 g/l and 150 g/l microalgal concentrations under frequency sweep experiment.** Complex viscosity at different frequency values.

## Conclusions

In summary, the rheological properties of microalgal suspensions were studied using the wild type microalga *C. reinhardtii*. At biomass concentrations below 50 g/l, the microalgal suspensions exhibited Newtonian behavior and the relative viscosity increased with increasing biomass concentration. The relative viscosity could be related to the volume fraction of spherical microalgal cells using Einstein's equation with $\alpha$=6.4. At biomass concentrations above 50 g/l, the microalgal suspensions exhibited shear thinning behavior and was modeled as power law fluids. The first normal stress difference and the first normal stress coefficient was also evaluated at different steady shear rates. Further, the viscoelastic properties of the microalgal suspensions were characterized through small amplitude oscillatory shear experiment. These rheological findings are important and can serve to several purposes related to microalgal biomass production and biorefining. The hydrodynamic parameters can be used to estimate the time an average microalgal cell would travel from the dark region to a high illumination region of a photobioreactor, often referred to as the light/darkness cycle. They can also be used for individual particle tracking using mathematical modeling and computer simulations[23]. Further studies can also be performed using the rheological properties of microalgal slurries to optimize pumping and dewatering of microalgal biomass and to assess mass transfer and nutrients distribution in microalgal culture.

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# CURRICULUM VITAE

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## EDUCATION

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- **U.S. Patent No. 62/347,282:** Cultivation and energy efficient harvesting of microalgae using thermoreversible sol-gel transition. By **Estime, Bendy**, Dacheng Ren, and Radhakrishna Sureshkumar (Provisional patent)
- **Estime, Bendy**, Dacheng Ren, and Radhakrishna Sureshkumar. "Cultivation and energy efficient harvesting of microalgae using thermoreversible sol-gel transition." Sci. Rep. 7, 40725; doi: 10.1038/srep40725 (2017). http://rdcu.be/oEw9
- **Estime, Bendy**, Dacheng Ren, and Radhakrishna Sureshkumar. "Effects of plasmonic film filters on microalgal growth and biomass composition." ***Algal Research*** 11 (2015): 85-89.

- **Estime, Bendy,** Dacheng Ren, and Radhakrishna Sureshkumar. "Development of plasmonics-based technology for microalgal biorefinery." ***Sustainable Engineering Forum*** *- AICHE 2015.*
- **Estime, Bendy,** Dacheng Ren, and Radhakrishna Sureshkumar. "Rheological characterization of a novel thermoreversible microalgal cultivation system." ***Institute of Biological Engineering*** *2016.*
- **Estime, Bendy,** Dacheng Ren, and Radhakrishna Sureshkumar. "Novel approaches to improve distribution and absorption of light in microalgal photobioreactors" BMCE seminar series, Syracuse University, Fall 2016.
- **Estime, Bendy,** Dacheng Ren, and Radhakrishna Sureshkumar. "Development of a Thermoreversible Medium to Culture and Efficiently Harvest Microalgae." ***Sustainable Engineering Forum*** *- AICHE 2016.*

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