Investigating the effect of substrate properties on attached algal cultivation

by

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Abstract

There is growing interest in attached algae cultivation systems because they could provide a more cost and energy-efficient alternative to suspended (planktonic) microalgae cultivation systems for many applications. However, attached growth systems have been far less studied than planktonic systems and have largely emphasized algae strains of most interest for biofuel production. Currently, through the introduction and exploration of new algal biorefinery pathways, commercial potentials of algal biomass are being assessed beyond biofuel production. More emphasis is being placed on the production of value-added products from the biomass while coupled with water remediation. Therefore, algal strain selection criteria and biomass cultivation methods need to be updated for improved efficiency. One possible way of improving attached cultivation systems is through engineering substrate surface characteristics to boost algal adhesion and enable strain selective algal colonization and growth. This work has explored the effect of substrate chemical and topographical characteristics on the attached cultivation of algae with a focus on periphytic filamentous algae. There is a need to understand the practical implications of the substrate surface characteristics and algae-substrate interactions on attached cultivation, especially in the case of less explored groups such as periphytic filamentous algae. Periphyton-based systems and the filamentous algae that often dominate them are known for their superior wastewater treatment capabilities and have shown promise as a source of biomass. These algae can form extensive three-dimensional networks of filament growing attached to a substrate (often through specialized attachment mechanisms), differentiating their attached growth from prostrate microalgal biofilms. Yet, lack of knowledge about their substrate preferences and lack of controlled studies tailored for the attached cultivation of these species in a scale appropriate to their community structure is an obstacle to utilizing these algae for commercial applications. This

work aimed to fill the knowledge gaps about periphyton-based algae systems by designing an intermediate scale framework for studying the attached cultivation and substrate effects of periphytic filamentous algae. This was done through the design and optimization of a flow way photobioreactor for the cultivation of filamentous algae *Stigeoclonium tenue* on polylactic acid (PLA) substrate surfaces. The photobioreactor design and the cultivation protocols developed were employed to study the effect of substrate chemical and topographical characteristics on the attached growth of *Stigeoclonium tenue*. The results of this study showed the effectiveness of simple millimeter-scale surface topographical features in the shape of concave hemispheres in significantly increasing the amount of biomass cultivated in a given period of time without increasing the footprint of the cultivation area. Additionally, investigating the material-related effects revealed that substrate chemical composition effects are more prominent at the early stages of adhesion. This work has also attempted to develop and modify surface characterization methods for finding zeta potential and surface energy of green periphytic filamentous algae like *Stigeoclonium tenue* through contact angle and streaming potential measurements, respectively. Characterization of physicochemical characteristics of the surface enabled modeling of the algae-substrate interactions for this algae through the extended Derjaguin-Landau-Verwey-Overbeek (XDLVO) approach. Overall, this work provides fundamental insights into understanding and engineering attached cultivation systems for periphytic algae, which are naturally advantageous for attached growth and remediation applications. In addition to the primary research topic, this work also presented results on the development and implementation of engineering activities focused on water quality and water quality assessment for K-12 students and college freshmen and assessed and showed the positive impact of the activities on engaging the students and introducing engineering as an altruistic career path.

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List of Abbreviations

AB	Acid-Base
ABS	Acrylonitrile butadiene styrene
ANOVA	Analysis of variance
ATS	Algal Turf Scrubber
BBM	Bold's Basal Medium
CI	Confidence interval
CFD	Computational fluid dynamics
CNC	Cellulose nanocrystal
DI	Deionized
EL	Electrostatic
ELS	Electrophoretic light scattering
EPS	Extracellular polymeric substance
FANS	Filamentous algae nutrient scrubbers
FFF	Fused filament fabrication
HSD	Honestly significant difference
HS	Helmholtz-Smoluchowski
L-CNC	Lignin coated CNC
LSD	Least significant difference
LW	Lifshitz van der Waals
PBR	Photobioreactor
PET-G	Polyethylene terephthalate glycol
PTFE	Polytetrafluoroethylene, also known commercially as Teflon

PFD	Photon flux density
PLA	Polylactic Acid
PMMA	Polymethylmethacrylate
PNRS	Periphyton nutrient removal systems
SA-CNC	Sulfated cellulose nanocrystal
STEM	Science, technology, engineering, and mathematics
TGA	Thermogravimetric analysis
UTEX	University of Texas at Austin
XDLVO	Extended Derjaguin-Landau-Verwey-Overbeek

Chapter 1: Introduction

The overall goal of this research was to understand the role of substrate surface physicochemical characteristics such as surface energy and surface charge and topographical features on the attachment and growth of algae. Algae are a diverse group of organisms that are mostly photosynthetic and found in aquatic habitats [1]. In addition to their crucial ecological role in the environment, algae have several technological applications. Attached algal cultivation systems come with the promise of improving the yield, efficiency, and consistency of algae-based/algal biomass products and processes [2, 3]. Immobilized biomass is inherently more concentrated than the biomass obtained from planktonic cultivation. This can make harvesting and downstream processing more feasible. Additionally, the wastewater treatment capabilities of the attached systems are well established.

In attached algae cultivation, cells grow adhered to a physical surface (the substrate). Physicochemical and topographical characteristics of the substrate are known to influence initial and long-term algal attachment and growth [2-5]. Therefore, there is an opportunity to use substrate characteristics as control parameters in designing attached algae cultivation systems. The efficiency and applications of the attached systems could be enhanced by developing the fundamental understanding of algae-substrate interactions needed for the rational design of cultivation systems that maximize pollutant removal, biomass product production, or both. However, much of the available information about the effects of substrate on attached algae growth is either from observations and studies in natural systems/habitats, biofouling studies, or from studies on a select number of microalgae species with high lipid content. In addition, the possible impacts of diverse algae morphologies, community structures, and attachment mechanisms on the

interaction with the substrate and overall performance of the attached system are usually overlooked [5]. Periphyton-based systems such as algal turf scrubbers (ATS) are a subcategory of attached systems with proven application capabilities, yet they are not well explored with regards to the influence of substrate effects on improving the design, control, and efficiency. The algae community in periphyton-based systems is often dominated by filamentous algae. Many of these algae species, often including typical species from *Oedogonium* and *Stigeoclonium* taxa, have been studied for their wastewater treatment capabilities and potentials as sources of biomass feedstock. These algae have preference for attached growth in nature and have complex life cycles involving morphological changes of the cells throughout different stages of their lifecycle. Additionally, they have specialized attachment adaptations, often growing in the form of multicellular filaments. Hence, it is difficult to describe and understand the attachment and substrate interactions that influence the growth of these algae. Moreover, it is difficult to reconcile this knowledge with what is known about the extensively studied microalgae such as *Chlorella* and *Scenedesmus* that lack similar life cycle stages and morphological attributes.

Therefore, this research was designed to explore the effects of substrate characteristics on attached algae growth through the following approaches:

1- Highlighting the potentials of substrate effects as engineering parameters in attached algae cultivation systems (Chapter 2);
2- Identifying the effective time window of the substrate chemical effects and its practical implications (Chapter 4);
3- Designing and developing an intermediate scale flow-way photobioreactor that enables controlled studies on substrate effects in the cultivation of periphytic filamentous algae (Chapter 5);

4- Understanding the effect of practical macroscale topographical features on biomass cultivation for turf-forming periphytic algae with 3D printed substrates (Chapter 6);

5- Modeling algae cell-substrate interaction while considering cell morphological diversity within a species using XDLVO theory (Chapter 7);.

6- Exploring the obtained outcomes and how they can inspire future research or be used in the field of attached algae cultivation (Chapter 9);

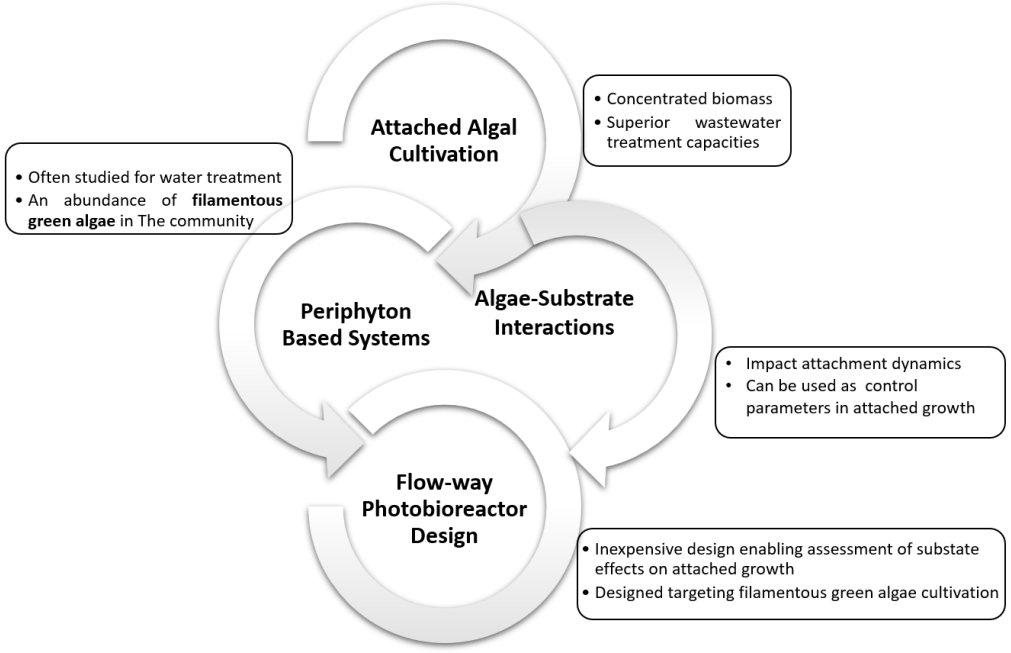


Figure 1.1 A visual summary of the main research scope

In addition to the main research topics concerning attached algae cultivation, a chapter of this dissertation (Chapter 8) is devoted to some of the STEM education research conducted by the author over the course of her studies focusing on development and implementation of water related activities to introduce engineering and engineering challenges to K-12 students and college freshman.

Chapter 2: Background

This chapter is written based on a manuscript by Karimi et al. (2021) titled "Substrate properties as controlling parameters in attached algal cultivation" published in the Journal of Applied Microbiology and Biotechnology [5].

Algae are a polyphyletic group of mostly photosynthetic organisms with substantial ecological significance and numerous application potentials in the human economy. High growth rate, high bio-compound concentration, ability to use wastewater as a nutrient source, and lower land area requirements for cultivation compared to conventional crops are among the characteristics which make algae compelling for commercial applications [6, 7]. Rich in high-value bio-compounds including carbohydrates, lipids, proteins and pigments, algal biomass is a promising feedstock in many industries including biofuels, pharmaceuticals, cosmetics, animal feed, human food, fertilizer, industrial enzymes, bioplastics, and composites [8-10]. In addition, algal cultivation has been considered for environmental and bioremediation (i.e., phycoremediation) applications, including nutrient recovery, heavy metals adsorption, and carbon dioxide sequestration [11-14]. Aligned with the scope of this research work, the focus of the background review is on literature related to planktonic and periphytic algae and cyanobacteria and excludes the research on seaweeds (marine macroalgae).

Since the early 1950s, various systems and cultivation methods have been developed and investigated with the goal of using algae for different applications [15]. Much of the research on algal cultivation systems was spurred by the goals of the Aquatic Species Program (ASP), which was funded by the US Department of Energy between 1978 and 1996 [16]. The main focus of the

ASP was to develop renewable transportation fuels/biofuels from algae. Initially, many different photosynthetic aquatic species including microalgae, macroalgae, and emergent aquatic plants were investigated for their potential as a source of natural oils, but then the focus of the program shifted to producing biodiesel from high-lipid planktonic microalgae [10, 16]. Therefore, most of the algal cultivation systems and methods developed in conjunction with ASP, including raceway ponds and photobioreactors (PBRs), were explicitly designed for a few species of microalgae, most of which are high in lipid content and naturally prefer growing in suspension [17]. This approach omitted a variety of algae of different morphologies and ecologies, and thus other cultivation methods with potential applications beyond biofuel production.

Development of novel algal biorefinery pathways that aim for the simultaneous production of high-value or value-added products alongside biofuel is frequently suggested as a strategy for improving the economic viability of algae-based products and processes [9, 18-21]. This requires innovation in the cultivation methods and moving beyond the current understanding of how to best cultivate high-lipid planktonic algae. Assessing algal strains based on their prospective performance in a multifunctional system targeting a range of applications including, for example, bioremediation, carbon dioxide (CO_2) sequestration, biofuels, and biochemical compound production may require reevaluating the conventional cultivation methods and criteria for strain selection.

To date, major challenges for the commercialization of many algae-based products and processes include the cost associated with harvesting and dewatering the algal biomass and the low reliability and yield consistency of the cultivation systems [4, 22]. It is estimated that up to 30% of the production costs of algae cultivated in suspension (planktonic algae) is due to the harvesting and dewatering process [4]. One potential way of reducing the harvesting and

dewatering cost is through attached cultivation, which consistently produces harvested biomass with a solids content higher by roughly an order of magnitude. In attached algae cultivation systems, algae grow attached to a physical substrate and are harvested by mechanical scraping, typically resulting in solid biomass content of 10-20% of the wet harvest [15, 23]. This solids content is comparable to that obtained from planktonic (suspended) cultivation systems after multiple steps of concentration processing, including sedimentation, flocculation/flotation and centrifugation [22, 24]. In addition to the potential for easier harvesting processes and greater energy efficiency, attached algal cultivation systems can be advantageous because of smaller space requirements, higher productivity rates, lower water consumption, enhanced water treatment performance, and flexible lipid accumulation strategy (i.e. easier process of nutrition depletion for targeting higher lipid accumulation) [25-28].

There has been an increase in attention towards designing and studying attached algal cultivation systems. Attached algal systems vary based on several operational parameters, including the substrate material, algal species present, water flow regime, and the chemical composition of the growth medium [2, 3]. In these systems, the attachment substrate could be stationary or moving. Based on the orientation of the substrate (configuration), attached systems can be classified into four categories; horizontal, vertical, radial, and rotating [3]. The green microalgae, *Scenedesmus* and *Chlorella*, are the most common taxa chosen for cultivation in attached systems [2, 4, 25]. The popularity of these species might be due to the residual influences of the ASP on the field in encouraging the use of high lipid producing microalgae and despite their natural preference for unattached planktonic growth. There are also a small number of studies that try to evaluate attached algal biomass (biofilm based and periphytic cultivation) in biorefinery frameworks [20, 29-31].

Substrate selection is a critical component of attached cultivation systems. Substrate characteristics influence initial algal cell adhesion, adhesion strength, growth dynamics and algal community composition in non-axenic and mixed culture conditions [2, 3, 26, 32-35]. Both the intrinsic properties of the substrate material and its topography have a significant effect on the attachment [36-41], and these parameters have been the subject of many different studies involving a range of materials and application of textured substrate surfaces. However, there is limited fundamental understanding and synthesis of the effects of physical, chemical, and topographical properties of the substrate on selection for colonizing species of algae, and this remains a significant challenge in engineering substrata optimized for accelerated and selective algae cultivation. Moreover, current knowledge on the effect of substrate characteristics on algal attachment is spread across different fields and disciplines which muddles the process of acquiring a comprehensive outlook on the subject. In addition, there is limited understanding of the effect of substrate properties on the various stages of algal attachment and growth. Advances in these areas of understanding may enable improved economic feasibility of the algal cultivation processes through the ability to design systems selective for specialized periphytic algae, which are often less studied.

While many of the attached algal cultivation systems developed and studied to date have been at the lab or pilot scale, a notable exception are algal turf scrubbers (ATS) [3, 42] (note that algal turf scrubber and ATS are trademarks of HydroMentia Technologies LLC, Ocala, Florida). ATS systems and other similar periphyton-based systems such as filamentous algae nutrient scrubbers (FANS) and periphyton nutrient removal systems (PNRS) [43, 44] are engineered mini-ecosystems that can be used for managing water quality through cultivation of three dimensional mats of attached algae (‘algal turfs’) in a shallow flow environment [45]. As the most widely

recognized variation of the mentioned periphyton-based systems, algal turf scrubbers have been built at scale for use in removal of excess nutrients from agriculture and municipal wastewaters, and recovery of non-point source waste nutrients from natural waters [14, 27]. Algal communities in ATS systems typically have complex ecological structure, affording a unique ability to be competitive under low and variable environmental nutrient conditions [46]. A combination of their effectiveness in uptake of excess pollutant nutrients such as phosphorus and nitrogen from water [43, 45, 47-49] and three-dimensional multi-story periphytic community structure, makes ATS a good candidate for further investigation in attached growth systems. The lack of knowledge regarding the interaction between the algal community in the ATS and the substrate surface, however, makes understanding the colonizing dynamics of algae and performance of the system in many applications challenging.

The next two sections summarize the current knowledge on the effect of physicochemical and topographic characteristics of the substrate on attached algae cultivation systems. The goal is to assess the potential of these parameters to control and optimize attached algal systems through algal colonization and attachment and community structural assembly. In addition, the example of periphytic filamentous algal species with specialized attachment adaptations that are often found in ATS systems is used to highlight the importance of understanding the dynamics of biofilm formation and structure in controlling attached algal cultivation systems, especially in the case of algae with complex life cycle and morphology. Moreover, efforts have been made to clarify ambiguities in the terminology that are common to attached algal systems because of the multidisciplinary nature of the field.

2.1 Algal attachment (from biofilms to periphytic algae in ATS)

Attached algal systems rely on growth of algae immobilized on surfaces (substrates). Depending on the cultivation conditions, including the algal species and/or strains present and the substrate characteristics, the structure and life cycle of the attached algal biomass can vary significantly, impacting the algae from the initial attachment stage all the way to maturation/harvesting [50, 51]. Algal biofilm, attached algae, and periphytic algal community are among the terms which have been used in the literature for referring to microbial assemblages dominated by algae attached to a substrate. Despite the similarities between the three, these terms cannot necessarily be used interchangeably and can often refer to different entities. While understanding algal-substrate interactions offers promise for improving attached cultivation systems, the challenge arises from the provincial nature of relevant fundamental knowledge about algal colonization and growth derived from in situ phycological studies on a limited number of species discordant from application-based needs of cultivation systems. The next section is devoted to defining algal biofilms and periphyton community in the context of attached algal and cyanobacterial systems and to discussing the knowledge gaps in understanding the interaction of attached algae with the attachment substrate.

In nature, biofilms are oftentimes mixed communities consisting of microbial organisms including algae and bacteria living within a matrix of extracellular polymeric substance (EPS) while attached to a surface [50, 51]. The focus of this background review and work is on oxygenic aquatic phototrophic biofilms--otherwise stated, biofilms that are colonized mainly by chlorophyll-bearing algae and cyanobacteria, or more succinctly, algal biofilms. In algal biofilms, photosynthesis by algae and cyanobacteria produces organic compounds (by carbon dioxide reduction) and molecular oxygen which contributes to the metabolic processes of the entire

community including the heterotrophs (mainly bacteria and protists) [52, 53]. Green algae, cyanobacteria and diatoms are often the major primary producers in algal biofilm communities [54].

Algal biofilm formation and development is a dynamic process consisting of several steps including cell attachment, colony formation, maturation and sloughing [55]. Each microbial species present in the biofilm has a specific response to the environmental limitations and opportunities which can also change throughout the course of biofilm development, but there are general principles which guide the succession within the biofilm community [56]. It is believed that in non-axenic/mixed communities, bacteria are the pioneering species that attach to and condition the surface initially by secreting EPS, although pre-conditioning of the surface happens when organic and inorganic molecules are brought to the solid-liquid interface from the bulk flow [57]. Therefore, in the early stages of algal biofilm development, there is a high proportion of EPS and bacteria attached to the surface relative to algae and cyanobacteria [4]. Several studies have shown that the bacterial conditioning in the early stage promotes algal cell adhesion [15, 55, 58]. The early stages are followed by colonization of algae and cyanobacteria in the biofilm (Fig. 2.1).

Attachment, growth, and reproduction of algae and cyanobacteria can majorly influence the structure of the biofilm [59]. Depending on the characteristics of the species of cyanobacteria and algae present in the algal biofilm including lifecycle, morphology and attachment adaptations, many biofilm characteristics such as thickness, interaction with the flow, interaction with the substrate (attachment strength), nutrient transport and light penetration can potentially change. These changes have not been comprehensively studied before and there are significant knowledge gaps, particularly with respect to ATS systems.

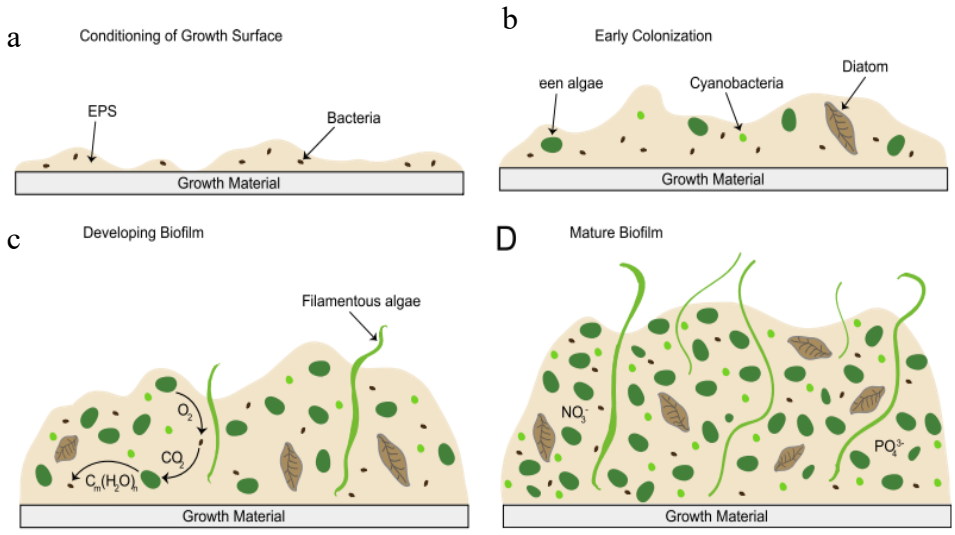


Figure. 2.1 Development sequence of a mixed community algal biofilm on a substrate: (a) bacterial conditioning of the substrate through EPS exudation; (b) early colonization of the EPS matrix by various species of algae from the overlying medium; (c) growth and reproduction of algal cells and formation of a algal-bacteria symbioses in the EPS matrix; (d) mature biofilm matrix, densely populated with algal cells, that retains nutrients in the EPS matrix [4].

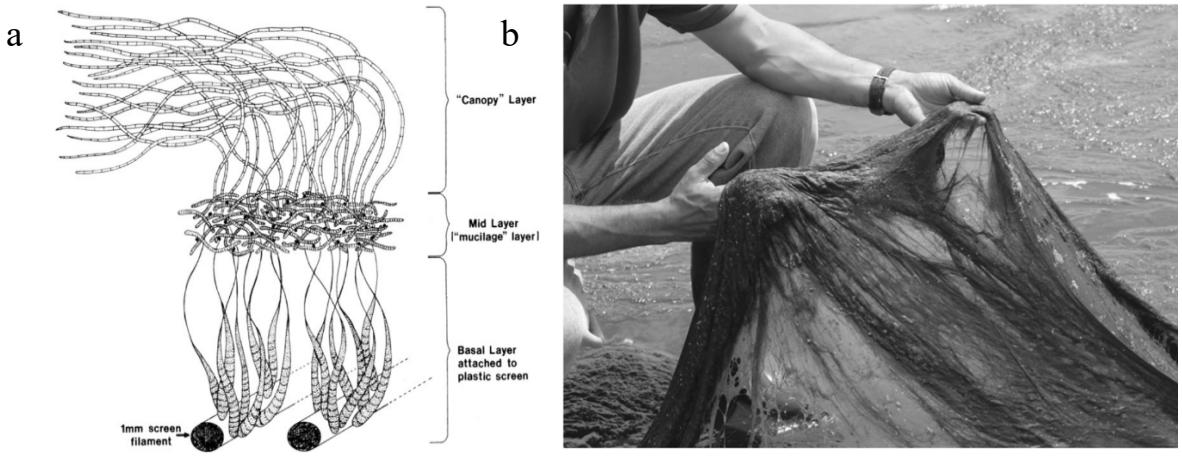


Figure. 2.2 (a) Concept of a multi-layered algal community on a freshwater algal turf scrubber screen; (b) mat of filamentous algae from an algal turf scrubber. [46]

Although the early stages of algae colonization in ATS systems are similar to other algal biofilms, the mature community includes turf-like three-dimensional structures, often formed by filamentous algae that grow as a canopy outside the EPS matrix (Fig. 2.2). In freshwater systems, periphyton is a more accurate term for referring to attached algae found in ATS. By definition, periphytic communities are the aquatic biota including photosynthetic organisms attached to a submerged substrate. Similar in concept to the algal biofilm, periphyton typically includes sessile organisms attached to the substrate and mobile forms that live associated to the attached community [60].

Periphytic algal communities in the ATS can have high growth rates in low nutrient environments (~5-50 g m^{-2} day^{-1} dry weight) [35, 46]. High surface area-to-volume ratio of the cells in the web of mostly filamentous algae growing outward from the substrate into the overlying flow might be the factor that allows periphytic algal communities to overcome nutrient transport limitations typically experienced by more prostrate biofilm structures. Studies on freshwater periphytic communities suggest that the attachment strength and dominant algal species are influenced by substrate type [60, 61]. However, not much is known about the effect of the interactions between the substrate and periphytic algae on the performance of the ATS systems. While various substrate materials, including nylon, polypropylene mesh, rock and concrete, have been used in ATS systems [46, 62], materials effects on algal community structure and function in ATS systems are poorly understood. A study by Kangas et al. (2017) found that the periphyton communities cultivated in an ATS system can be highly diverse in both algal species and attachment adaptations, with attached filamentous species dominating the total biomass. The diverse set of attachment adaptations found within periphytic algal communities include mucilage, mucilage pads, holdfasts and rhizoids [60, 63, 64], and each of these are expected to have different

interaction characteristics with any given substrate material [65]. The set of adaptations for attachment within the algal community is often an overlooked subject of study for designing systems for cultivation of attached algae, despite its potential importance in understanding the algae-substrate interactions and inter-specific competition within the community.

There is generally a lack of understanding of colonization dynamics for many algal species present in ATS attached communities, which often have complex morphologies and life cycles. For example, the filamentous green algal genera *Oedogonium* and *Stigeoclonium* have been reported to be present in several ATS systems [48, 49, 66], and each of these taxa has been individually studied for potential applications in wastewater treatment and as biomass feedstock [12, 25, 67]. *Oedogonium* is an unbranched filamentous green alga known to attach to substrates via mucilage basal pads, and *Stigeoclonium* is a branched filamentous green alga that attaches via basal rhizoids [68-72]. Based on current understanding, it is challenging to describe the potential impacts of these specific attachment adaptations or even morphological features of the mentioned algae on the performance or operation of an ATS system or any other system targeting attached cultivation of these species. Attachment adaptations are an often-neglected aspect of interaction of algae with a substrate. The differences in the attachment strategy between the two suggests different colonization dynamics for any given substrate material. Knowledge about colonization dynamics differences between the two might be useful in designing species-specific substrate materials; however, the effects of substrate chemistry and texture to these, and most other periphytic filamentous algae are generally unknown. Attachment adaptations and complex responses to surface are not exclusive to periphytic filamentous algae and can be found in different groups of algae including green microalgae that have been widely studied in biofilm-based

attached systems. Therefore, consideration of attachment adaptations in studying the algae-substrate interaction needs to be further explored.

2.2 Substrate effects on algal attachment

The main component that separates attached algal cultivation methods from other algal biomass cultivation systems is the presence of an attachment surface that allows for immobilization of algal cells and their growth. Therefore, investigating the initial contact and growth interactions of algae with the attachment substrate is a key factor in better understanding attached systems. Since the early 1900s, glass slides and other artificial substrates have been used for sampling and studying periphyton communities in freshwater inland water systems [61], which led to observations that the taxonomic composition of the attached assemblages can be affected by substrate [73]. Certain species were oftentimes under or overestimated depending on the characteristics of the substrate used for sampling, environmental factors (e.g., temperature and flow regime) and colonization period [61, 74, 75]. Therefore, a better understanding of the effect of substrate characteristics on attached algal growth can lay the foundations for designing strain selective substrates. This is important since difficulties of downstream processing of mixed algal biomass are among the obstacles to yield consistent algae products and fulfilling the potential of attached systems in providing a more cost and energy efficient alternative over planktonic cultivation systems [2, 20, 76, 77]. While there have been a number of studies on the role of the substrate in algal growth, the majority of these studies have focused on microalgae which can generate a biofilm, and there are only a few studies that include filamentous algae which have evolved attachment adaptations and are able to form long three dimensional networks [78-82]. Despite the need for more in-depth understanding, it is generally accepted that two factors that

have a significant impact on attachment are the substrate's intrinsic chemical properties and topography.

2.3 Effects of intrinsic chemical properties

The attachment of algae to a solid surface is a complicated process involving the living cell, the substrate, and the surrounding liquid [36]. Studies on the effect of material chemistry on algal attachment and growth fall into three general categories: those that do not quantify surface properties of the substrate and only test for adhesion on different material; those that quantify and relate chemical surface properties to the attachment/adhesion behavior; and those that quantify the physicochemical properties of both algae and substrate to explain the attachment and adhesion [4]. Artificial substrates made from many different materials, including glass, ceramics, cotton, cellulose acetate, stainless steel, brass, aluminum, polycarbonate, polystyrene, polyethylene, polytetrafluorethylene and nylon have been used for investigating the effect of substrate material on algal attachment [2, 15, 39, 83]. The effect of material physicochemical surface properties on cell-substrate adhesion and attachment has been described using a range of explanations; the effect of surface hydrophobicity/hydrophilicity and surface energy on the attachment [51, 84-87], measuring the work of adhesion [88], and more detailed measurements of interaction energy between a single algal cell and the substrate using thermodynamic theories such as extended Derjaguin-Landau-Verwey-Overbeek (XDLVO) theory [80, 89-91].

The XDLVO thermodynamic modeling approach measures the favorability of bio-adhesion by treating the algae and the substrate as colloidal entities and ultimately measuring the total interaction energy between an algal cell and the substrate as a function of distance. The total interaction energy G^{TOT} (Equation 2.1) is the linear sum of three interaction energies that are functions of distance (Fig. 2.3), comprising the following: Lifshitz-van der Waals (LW)

interactions (G^{LW}), which originate from instantaneous asymmetrical distribution of electrons in molecules; electric double layer/electrostatic (EL) interactions (G^{EL}) from the electrostatic interactions between the cell and the substrate; and acid-base (AB) interactions (G^{AB}), which originate from polar interactions in the aqueous media [92]. Negative G^{TOT} values indicate attraction, while positive values indicate repulsion between the cell and the substrate.

$$G^{TOT}(d)= G^{LW}(d)+ G^{AB}(d) + G^{EL}(d) \quad (2.1)$$

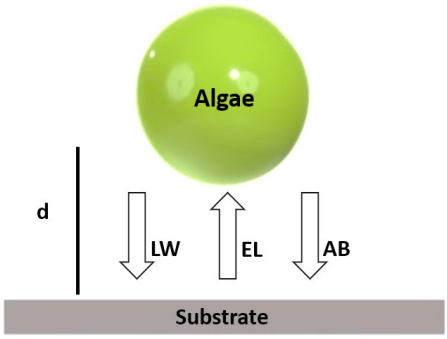


Figure. 2.3 Algae-substrate interactions, where Lifshitz-van der Waals (LW) interactions are usually attractive, electric double layer/electrostatic (EL) interactions are usually repulsive due to negative charge of both surface and algae, and acid-base (AB) interactions are usually attractive. All are defined as a function of the distance (d) between the substrate and the algae cell [93].

Ozkan and Berberoglu (2013b) found that acid-base interactions were the dominant component in cell-substrate interaction for several microalgae species including, *Tetradesmus* (=*Scenedesmus) dimorphus* (Turpin) M.J.Wynne and *Chlorella vulgaris* Beyerinck. A later study by Yuan et al. (2019) on several microbial adhesion data obtained via experimentation and literature for microalgae, bacteria, and fungi showed that either acid-base or electrostatic interaction can be dominant in the microbial adhesion to an abiotic substrate. In electrostatic-interaction dominated systems, the increase or decrease in the adhesion strength can be predicted

using the surface zeta potential of algae and substrate, whereas in acid-base interaction dominated systems, the electron donor surface characteristics of both the algae and substrate are better indicators of the adhesion behavior [90]. A key limitation of most studies is that they do not consider the dynamic nature of the algal attachment. Physicochemical properties of the algal cell and the substrate can change throughout the colonization process as a result of environmental conditions, ultimately influencing the number of algal cells attached and the strength of attachment. A study by Zeriouh et al. (2017) on biofouling of marine microalgae showed that the surface energy (described by contact angle measurements) and zeta potential of algae and the attachment substrate surface change over time and with biofilm development. Therefore, the XDLVO measurements based on initial algae and substrate physicochemical properties are inaccurate in describing the long-term adhesion behavior [89, 94].

Despite the overall convenience of XDLVO theory in describing the early-stage attachment behavior of mostly monocultures of algae, its applications have only been explored for a limited number of algal species neglecting morphological complexities. Lack of species diversity and not considering specialized features of algae is not exclusive to studies that have used XDLVO and is a major limitation of most studies trying to explain the effect of chemical surface properties on attachment. To the authors' knowledge, there are no studies available which thermodynamically model and explain the adhesion behavior and cell-substrate interaction of freshwater algae with attachment adaptations such as rhizoids (root-like structures common in some filamentous algae) and mucilage pads (common in many algae including diatoms and cyanobacteria), or address the possible effects of morphological changes that several algal species undergo from the initial to mature phases of growth on algae-substrate interactions. Thermodynamic models and explanations generally neglect the possible effects of other microbial components present in the system, such

as bacteria, on adhesion and attachment. Bacteria are believed to be the early colonizers of the algal biofilm and periphyton communities [4, 60]; consequently, presence of bacteria and EPS exudation on the substrate prior to algal attachment can shift the physicochemical properties of the substrate. This introduces possible errors into modeling and/or quantification of algae-substrate interactions that rely on measuring the substrate properties at pristine conditions. Changes in the properties of both algae and substrate during different stages of colonization and biofilm formation, limiting the interaction measurement to a single algal cell and the substrate, are also among other factors often neglected in the modeling.

It has been speculated that, like many other microorganisms, attachment of microalgal cells is preceded by the transport of cells to the proximity of the substrate surface and within the range of interaction forces. The attachment process starts with an instantaneous reversible phase of attachment, followed by an irreversible molecular and cellular phase, which is time-dependent [78]. Substrate properties can affect all the mentioned stages of the attachment process, but the magnitude of the effect of substrate chemical properties on each stage is not well understood. When it comes to physicochemical properties of the substrate material and their effects on the interaction energy, it is not clear whether the number of attached cells or strength of the attachment would be affected the most. Therefore, more effort on designing experiments with the goal of identifying and investigating the effect of physicochemical substrate properties on the adhesion and attached growth is warranted. Additionally, any material intended to be used in algal attachment systems should be able to withstand the environmental and harvesting conditions [2]. Properties such as mechanical strength and degradability of the substrate are also important to assess.

2.4 Effect of substrate topography

Alongside the physicochemical properties, topography of a substrate is another crucial factor that influences algal attachment and growth. Substrate topography impacts the flow characteristics including at the surface boundary layer, which can ultimately affect algal attachment and growth [33, 85, 95]. Many of the studies on the effects of substrate texture on algal attachment have been conducted in the field of marine biofouling with the goal of understanding the fouling behavior of marine microorganisms such as bacteria and algae [96, 97]. These studies suggest that in addition to substrate material properties and environmental factors, cell attachment is influenced by the scale of surface micro-texture (feature size), size of cells and settlement behavior of the cells [36, 98-100]. Biofouling is a broad field of study which includes algal biofouling in both marine and freshwater environments. Chlorophytes and diatoms are amongst the most intensively studied microorganisms for determining the effect of surface texture and interrelated properties in marine biofouling. The point-attachment-theory is a common explanation in the marine biofouling literature for describing the effect of texture features on algal adhesion, associating a greater contact point between the algae and substrate surface with increased chance of successful attachment [96]. Despite the relationship between the fields of biofouling and attached algal cultivation, there seems to be an academic disconnection between these two areas of knowledge. Surface modification methods, modeling approaches, and the available knowledge from algal biofouling literature have rarely been used for promoting substrate adhesion properties in the design of systems for the cultivation of attached algae.

While studying the microalgal species *T. dimorphus* and *Nannochloropsis oculate* (Droop) D.J.Hibberd, Cui et al. (2013), reported that rates of attachment are greatest on surfaces with feature sizes close to algal cell diameter, when compared to larger or smaller sizes. In that study

and others, surface wettability was used to explain the effect of substrate texture on algal attachment [36, 85, 101, 102]. Although wettability is not solely a function of texture, and material properties of a substrate can vastly affect it, it can be used for comparing the effects of texture across surfaces made of the same material, or it can be considered as summative for both material properties and texture. The effect of topography on surface wetting can be explained using Wenzel or Cassie-Baxter models [103, 104]. Although algal adhesion is a complex process to predict, it is speculated that textured substrate surfaces falling into Wenzel wetting behavior are more favorable for attachment since cells can theoretically fully penetrate into the texture (Fig. 2.4) [36]. A study by Gross et al. (2016) investigated the co-effect of substrate physicochemical properties and texture on initial colonization and long-term growth of microalgal biofilms. Results from this study suggested that co-effect of these parameters on cell attachment could be quantitatively described using a second order polynomial regression. A recent study on the adhesion of *C. vulgaris* to different rough natural surfaces (i.e., rice husk and pine sawdust) with root mean square roughness (S_q) values between 13 to 45.2 µm found increased algal cell retainment and adhesion rates on rougher substrates at early stages followed by long term effects on the cultivation [95].

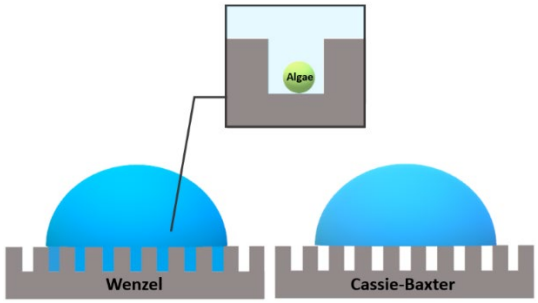


Figure. 2.4 Wenzel and Cassie-Baxter wetting mode. In Wenzel mode the liquid can fully penetrate the rough surface, theoretically enabling algae with cell sizes smaller than the roughness features to reside between the surface features. In contrast, Cassie-Baxter mode prevents full penetration of liquid into the features.

In recent years, additive manufacturing has provided researchers the ability to easily create substrates with controlled surface topographical features for algal attachment [33, 41, 79, 81]. There have been several recent studies on the effects of topographies produced by additive manufacturing on attached filamentous algae. In one of these studies, 3D-printed acrylic surfaces with feature sizes ranging from 100 - 2000 μm were exposed to a natural freshwater stream for 33 days. Results showed that surface feature size influenced the overall species diversity of a periphyton community grown on the substrates, indicating the preference of some species for specific feature sizes [33]. Khoshkhoo et al. (2019) showed that by mimicking the surface characteristics found on periphyton-covered natural rocks, early colonization of filamentous algae on substrata can be enhanced through control of three interrelated descriptor parameters describing the microscale topography. Surface texture can also impact the local hydrodynamic conditions which are important in determining whether or not the algal cell would be able to settle on a substrate and stay attached in flow environments [85]. It has been reported that v-shaped grooves are more favorable than u-shaped grooves for attachment of *Tetradesmus* (=*Scenedesmus*) *obliquus* (Turpin) M.J.Wynne [81]. Zhang et al. (2020) used CFD and particle tracing simulation for demonstrating the effect of surface roughness on adhesion behavior of *C. vulgaris* finding v-shaped grooves important for algal cell retention in the flow environment. This study indicated the formation of low shear regions and particle accumulation in the v-grooves.

A small number of studies have explored the possible benefits of employing three-dimensional substrates (woven fabrics with rather large feature sizes) in ATS systems and cultivation of periphytic filamentous algae as an alternative for highly common plastic screens [35, 105]. In one study, flat HDPE screens (mesh size 3×5 mm) were compared to three dimensional screens which had 1-2 cm thick and loose braided fibers in ATS flow ways [35]. The study

concluded that utilizing three-dimensional screens as substrate significantly increased the overall algal biomass production from the system. Ekong et al. (2019) also reported the advantage of three-dimensional woven fabrics over the commonly used nylon mesh in attached cultivation of a periphytic algal community dominated by filamentous algae *Microspora floccosa* (Vaucher) Thuret and *Mougeotia scalaris* Hassall. Topographical features can impact the liquid flow pattern around the substrate and create low or high shear stress zones. The consequences of the creation of such zones and changes in the hydrodynamics of the systems can have different implications for different algae and at different stages of periphytic algal community/biofilm formation [44, 106]. Low shear zones can promote a sheltered space for algal cell accumulation but might also induce limitations in nutrient advection from the bulk flow. One may consider the example of periphytic algae mats found in the ATS systems, where there are often species in the community that have specialized adaptations for attachment [60, 63]. With the exception of some algae found in ATS systems such as species of *Ulothrix* and *Ulva* [99, 107, 108] the details of interaction of these adaptation features (such as rhizoids) with the substrate topographical features are not generally well understood. In addition, because mature ATS mats have macroscale web structures, a combination of micro- and macro- scale topographical features can be assessed for efficiency optimization. More experiments and studies (especially simulations and modeling work) are required for understanding the optimized feature size for long-term promotion of algal biomass production. In addition, substrate texture (depending on feature size, shape, and other parameters) can promote or demote bacterial attachment [96, 109]. Since bacteria are often the species pre-conditioning the substrate for the attachment, the effect of the substrate topographical features on the pioneer bacteria might be a worthwhile topic to investigate. From a practical aspect, topographical substrate features intended for use in attached algal cultivations systems should not

introduce complications to the harvesting process and need to be assessed for their performance over several cycles of growth and harvest.

2.5 Current research needs

Many of the research efforts in designing and optimizing of attached algal systems build upon the knowledge available on planktonic (suspended cultivation) systems and target a limited number of algae species and/or strains that often have high lipid content. With the recent efforts in utilizing algae for applications beyond biofuel and in a biorefinery context, optimization and understanding of the attached systems will require a thorough understanding of substrate-algae interaction that is inclusive of different lifecycles and morphologies. Substrate chemical and topographical characteristics have been shown to have an important effect on the favorability of algal attachment, especially during the early stages of development. Therefore, there is potential for designing substrates to target attached algae cultivation through altering the surface topographical features (texture) and chemical properties such as surface energy and zeta potential. But there are limitations in the application of the conclusions obtained about the effect of topography and chemistry of the substrate on the attached algal cultivation systems due to the following reasons:

a) Most of the conclusions are drawn based on studies undertaken on only a few algal species (mostly green microalgae), resulting in a lack of knowledge about algae with specialized mechanisms for attachment.
b) The effect of other microbial components presents in the system, including bacteria, is often neglected despite evidence of their interaction with the substrate. For example, in the case of the application of XDLVO theory for predicting algal attachment

favorability, surface conditioning by bacteria can vastly change the properties of the substrate that are used for measuring the total interaction energy.

c) The effect of biofilm/periphyton architecture and size (thickness) on the algae-substrate interaction is not well explored. Depending on the size and properties of the algal community, the short- and long-term interactions with the substrate may be affected.

d) There is a disconnection between the fundamental phycological knowledge of attached algal communities and what is known about attachment adaptations, and how these communities are described in studies involving engineered attached systems. Attached algal systems are part of a broad interdisciplinary field that relies on information from several fields of study, including phycology, ecology, engineering, and chemistry. Therefore, the terminology for referring to attached algal communities and the associated phenomena and processes is different across fields, creating difficulty in finding available background information. Literature available on algal biofilms, periphyton communities attached to the artificial substrates for water quality assessment and monitoring and, marine and freshwater biofouling can be good sources for acquiring fundamental knowledge about algae-substrate interactions that can help in improving attached cultivation systems.

e) Only a limited number of algal species and/or strains have been studied in attached cultivation systems under very specific environmental conditions, but results obtained from these studies are overgeneralized to all attached cultivation systems. However, differences in the morphology, lifecycle, and environmental preferences of different algae and cyanobacteria are substantial.

f) Many of the algal species targeted for cultivation in attached systems are found mostly in planktonic habitats in nature. One might argue that these species are less compatible with attached cultivation in comparison to benthic algae that are often found attached to substrates. As a result, there is a need for assessing the potentials of species that naturally thrive while growing attached.

Chapter 3: Experimental methods

In this chapter, a list of the methods and tools that have been repeatedly used throughout this work in experimentation and data analysis are provided alongside some general information about them. Given the evolvement and modification of the protocols and introduction of new tools throughout the course of this research, the detailed materials and methods are described thoroughly in each of the corresponding chapters.

3.1 Cultivation substrate preparation

3.1.2 Additive manufacturing of flat and textured polymeric substrates

Additive manufacturing has been used throughout this work for creating custom polymeric substrate surfaces with different topographical features. Polylactic acid (PLA) substrate surfaces used in Section 4.1 were created using MakerBot white PLA filament on a MakerBot Replicator Plus 3D printer (MakerBot, New York City, NY). Additionally, Stratasys Objet 30 PolyJet printer (Stratasys, Eden Prairie, MN) was used for printing the acrylic tiles in Chapter 4. On this printer, Stratasys Verowhite Plus, a polymethylmethacrylate (PMMA) / acrylic based photopolymer blend was used as the feed material. Although the exact composition of Verowhite Plus is not disclosed by the manufacturing company, the following composition table can be found in the MSDS sheet of the product [110]:

Table 3.1 Verowhite Plus approximate material composition [110]

Component	Percent
Acrylic monomer	<30
Isobornyl acrylate	<25
Phenol, 4,4'-(1-methylethylidene) bis-, polymer with (chloromethyl)oxirane, 2-propenoate	<15
Diphenyl-2,4,6-trimethylbenzoyl phosphine oxide	<2
Titanium dioxide	<0.8
Acrylic acid ester	<0.3
Propylene glycol monomethyl ether acetate	[0.1, 0.125]
Phosphoric acid	[0.002, 0.015]

PLA and acrylonitrile butadiene styrene (ABS) substrate surfaces used in Sections 6.1 and 6.2 were created using a Monoprice Maker Ultimate 3D printer. White Monoprice PLA plus filaments and white Hatchbox ABS filaments were used for printing each substrate material type. In Section 6.3 Prusa i3 MK3S printer was used for creating PLA, ABS and polyethylene terephthalate glycol (PET-G) polymeric substrate surfaces with desired features. Among the printers used, Stratasys Objet 30 was a PolyJet 3D printer relying on UV curing of the feed material while the other three printers used were fused filament fabrication (FFF) based printers. For the FFF based printers, the used nozzle and filament diameters were 0.4 mm and 1.75 mm respectively.

3.2 Algae and substrate surface characterization methods

The methods employed in determining physicochemical properties of the algae cells and substrate surfaces used in the cultivation experiments are described in this section.

3.2.1 Thermogravimetric analysis

Thermogravimetric analysis (TGA) is a thermal method which involves measuring changes of the mass in response to changes in temperature. TGA can provide information about many properties of a sample including thermal decomposition and adsorption. In this research, TGA in nitrogen was carried on the prepared PLA composites and PLA substrate surfaces (used in Section 7.1) using a TGA-Q5000 apparatus (TA instruments, New Castle, DE) to determine the exact mass ratio of the additives in the composites and deviation of decomposition temperature from the original PLA base material.

3.2.2 Contact angle measurements

Contact angle of a liquid with a solid at the liquid-vapor interface is a parameter which can be used for describing the thermodynamic equilibrium between the three phases [111]. Additionally, the wettability of a surface with a liquid can be quantified using contact angle values.

Throughout the course of this work two different goniometer instruments were used for obtaining the contact angle values: Data physics OCA 50 and ramé-hart Instrument Model 200-00-115 goniometers that both rely on optical imaging of the solid-liquid or gas/liquid interface and image processing for analyzing the contact angles created. In this work, the interface of interest for measurement was the liquid-solid interface, and liquid droplets sized 2-5 microns were utilized for contact angle measurements. Under ideal conditions, the resolution of the contact angle measurements with both instruments is advertised to be 0.1°. Throughout this work modified Young's equation (Equation 3.1) was used to find the surface energy and surface energy components based on the acid-base method [92, 112].

$$(1+\cos\theta)\gamma_l = 2\left(\sqrt{\gamma_s^{LW}\gamma_l^{LW}} + \sqrt{\gamma_s^{+}\gamma_l^{-}} + \sqrt{\gamma_s^{-}\gamma_l^{+}}\right) \tag{3.1}$$

Where the subscripts s and l refer to surface and the probe liquid and surface energy components of each are shown as the following: liquid surface tension (γ^{l}), electron donor (γ^{+}), electron acceptor (γ^{-}), and Lifshitz van der Waals (γ^{LW}) surface energy components. Obtaining the contact angle between the surface and at least three liquids with known surface energy components (two polar and one non-polar liquid) and solving Equation (3.1) enables obtaining surface energy components for the surface. In this work liquid surface energy components were obtained from the following reference:[92]. Additionally, the total surface energy value can be obtained using equation (3.2).

$$\gamma = \gamma LW + 2\sqrt{\gamma - \gamma +} \tag{3.2}$$

3.2.3 Zeta potential measurement

Zeta potential (**ζ**) is a measure of the electrostatic potential at or very near the beginning of the diffuse double layer created by surface charges at the solid/liquid interface in a polar medium and can be used for making interpretations about charge and adsorption characteristics of a surface

[113, 114]. The polymeric and glass substrates and algae cells were analyzed for their zeta potential using SurPASS electrokinetic analyzer (Anton Paar,Graz, Austria) for solids. This instrument operates on the basis of measuring a streaming potential or streaming current value over the solid sample surface under controlled pressure. Standard monovalent electrolyte recommended for carrying zeta potential measurements with this instrument is 0.001-0.01 M potassium chloride (KCl) solution. However, from the theoretical standpoint, any electrolyte can be used for the measurement if the electrokinetic response is detectable by the instrument.

3.3 Statistical analysis

In processing the results, Analysis of Variance (ANOVA) was performed on data obtained at a significance level of $p < 0.05$ after normality test. Linear regression analysis was performed to correlate the parameters and determine the effect sizes using readily available tools in Minitab (v 18.1) and RStudio (build 351). Additionally, Tukey's honestly significant difference (HSD) and Fisher's least significant difference (LSD) post hoc analysis were performed in some cases for finding the significantly different data pairs. In interpretation of Tukey's HSD and Fisher's LSD tests, 95 % confidence intervals (CI) for differences of means were presented in graph format were inclusion of zero in an interval was associated with a non-significant pair. Throughout this work, in the graphs representing data results, error bars are based on the standard deviation unless indicated otherwise.

3.4 Algae culture initiation and maintenance

Throughout the course of this work, flask cultures of three different algae species were initiated and maintained for experimentation. Green alga *Stigeoclonium tenue*, *Oedogonium foveolatom* and *Scenedesmus dimorphous* were obtained from the UTEX (the University of Texas at Austin) algae culture collection (corresponding catalog numbers: UTEX LB 1572, LB 933 and

B 746 respectively). The obtained algae were moved to a flask under aseptic conditions. Bold's Basal Medium (BBM) [115] (Table 3.2) ,was used as the growth medium for the cultures. The cultures were kept at room temperature, constantly aerated, and under an 8:16 h dark-light cycle to remain metabolically active. These flask cultures were the inoculum source for photobioreactor cultivation experiments. Transmitted light microscopy was performed regularly using a Nikon (Melville, NY) Eclipse 80i microscope to monitor for contamination and cell viability.

Table 3.2 Composition of Bold's Basal standard algae growth medium [115].

Components	**Stock solution (g/L)**	**Used quantity (ml)**	**Final molar concentration (M)**
Macronutrients			
$NaNO_3$	25	10	2.94 mM
$CaCl_2 \cdot 2H_2O$	2.5	10	0.17 mM
$MgSO_4 \cdot 7H_2O$	7.5	10	0.30 mM
K_2HPO_4	7.5	10	0.43 mM
KH_2PO_4	17.5	10	1.29 mM
NaCl	2.5	10	0.43 mM
Alkaline EDTA solution			
EDTA	50	1	0.17 mM
KOH	31		0.53 mM
Acidified iron solution			
$FeSO_4 \cdot 7H_2O$	4.98	1	17.9 µM
H_2SO_4	(1 ml)		
Boron solution			
H_3BO_3	11.42	1	0.18 mM
Trace metal solution			
$ZnSO_4 \cdot 7H_2O$	8.82	1	30.7 µM
$MnCl_2 \cdot 4H_2O$	1.44		7.28 µM
MoO_3	0.71		4.93 µM
$CuSO_4 \cdot 5H_2O$	1.57		6.29 µM
$Co(NO_3)_2 \cdot 6H_2O$	0.49		1.68 µM

Concentration of *Scenedesmus dimorphus* was measured and monitored by counting the cells under the microscope and obtaining a working curve relating absorbance of the algae culture

to the cell population. In the case of filamentous algae *Oedogonium* and *Stigeoclonium*, directly counting the algae cell population was not a viable option because of the chain-like structure of the cells, growing in multicellular filaments. Therefore, dry biomass content of the culture was determined as an indirect measure of population or cell numbers. Dry biomass was measured by taking multiple samples from the cultures after homogenization by shaking and vortex mixing. The samples were then dried in a convection oven at temperatures 100-106 °C overnight.

3.4.1 Algae harvesting method

The substrate samples were removed from the flow way channels at the end of the incubation period of the experiment. The attached biomass was harvested from the top surface of each substrate sample using a hollow plastic tube connected to a filtering flask and placed under vacuum. The biomass collected from each sample in the flask was then transferred to aluminum weighing boats and dried overnight at 160°C in a convection oven. The dry biomass content per sample was then measured by weighing the samples and deducing the weight of the boats.

Chapter 4: The effect of material properties on attached algae cultivation in flow environments

4.1 Preliminary photobioreactor algal cultivation

This section presents the results of a preliminary investigation conducted with the objective of monitoring the effects of chemical substrate surface properties such as surface energy and zeta potential on the attached growth of a periphytic algae community dominated by filamentous algae in a lab-scale flow way photobioreactor. Zeta potential and surface energy (based on acid-base method) were selected as characteristics of interest because they are crucial in finding the interaction energies between the substrate surface and algae (G^{TOT}), as will be discussed further in this chapter and Chapter 7. The three main areas of emphasis in this cultivation experiment were as follows:

1- Incorporation of polymeric material commonly used in 3D printing as the attachment substrate. This was because 3D printable material enabled feasible manufacturing of substrate surfaces with desired geometry and topographical features, which was aimed to be benefited from in this study and prospective studies of this work/dissertation.

2- Assessment of suitability of the existing periphytic algae cultivation protocols and a flow-way photobioreactor, designed and used previously in Blersch's research group [113, 114] for answering the specific needs of this work. This included assessment of adequacy of sample replicates for producing significant results and observing the material effects on attachment.

3- Cultivation of an attached algae community dominated by periphytic filamentous algae. As described previously in Chapters 1 and 2, one of the main objectives of this work is to

fill some of the knowledge gaps in the literature about the influence of substrate characteristics on attachment and growth of turf-forming filamentous algae. This is because turf-forming filamentous algae are adapted for attached growth in nature, have been studied extensively for their wastewater treatment capabilities as dominating species of periphytic communities in systems like ATS, and have the potential as a source of biomass.

4.1.2 Photobioreactor set up and cultivation conditions

Polylactic acid (PLA) and polymethyl methacrylate (PMMA) also referred to as acrylic surfaces/tiles were tested for algal attachment and growth in a re-circulating flow way photobioreactor, previously designed for experimentation with benthic algae [116]. The reactor was made from a PVC sheet and had five 10 cm wide by 100 cm long lanes (four lanes used for experimentation). The water was pumped into each of the lanes through an adjustable flow meter (Hydronix, Greenville, SC) by a Pondmaster 4500 liter per hour magnetic drive pump (Danner Manufacturing, Inc., Islandia, NY) placed in the 75.7-liter reservoir. Five T5 6500K fluorescent grow lamps were placed perpendicular to the flow lanes to cover up the whole lane area and provide algae with the required density of light flux for cultivation and growth. The average photon flux density (PFD) was measured to be 300 µmol/m^2s.

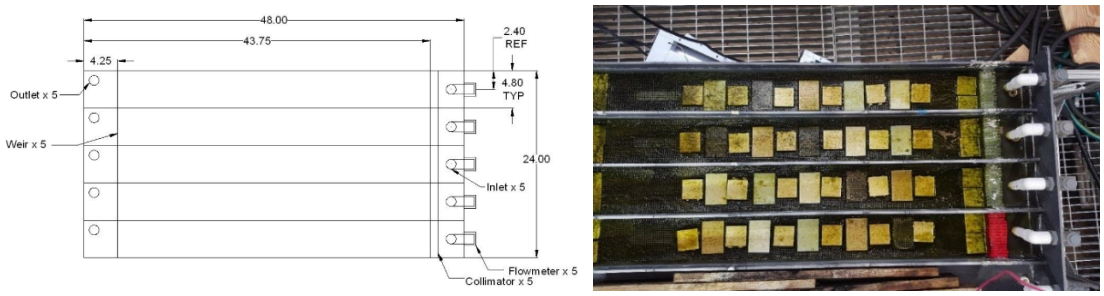


Figure 4.1 Overall layout and dimensions (in inches)[116] of the photobioreactor

Opaque polylactic acid sheets with a smooth finish (Smallparts Logansport, IN) and clear Plexiglass sheets were used as commercially available counterparts of the 3D printed tiles. The

contact angle of water, ethylene glycol and hexadecane with the tile surfaces were measured following the method presented in Chapter 3. Prior to placement of the tiles in the reactor, polypropylene screen mats with a 3 mm mesh gap (XV1672, Industrial Netting) cultivated with filamentous algae *Oedogonium* were placed at the bottom of the reactor and inoculated with modified F/2 media as the nutrient source (Pentair Co., Apopka, FL) and subjected to 24 hr light from the lamps to pre-inoculate the polypropylene screens for 7 days. The pre-inoculation step was carried to ensure presence of enough algae cells in the system to initiate biofilm/turf formation. After the seeding period, 5 tiles per material type sized 7 by 5 cm total number of 20 tiles were randomly placed in 4 separate lanes of the reactor. 56.8 L of water enriched with nutrients (F2 media) [115] was pumped from the reservoir into the lanes at a total flow rate of 31 ml/s.

The evaporation water loss was replaced every day. Water quality parameters such as pH, temperature, conductivity, and total dissolved solids content were monitored daily using a HANNA instruments HI98130 Combo tester. Digital images were taken of the tiles every other day by temporarily turning off the pump and discharging the water in the lanes into the reservoir. After 15 days, the biomass accumulated on the tiles were harvested by using a vacuum apparatus in combination with mechanical scraping. The obtained biomass from each tile was dried overnight at 100 ºC. The dry weight of biomass was then measured and recorded for each tile sample.

4.1.3 Material surface energy and zeta potential quantification

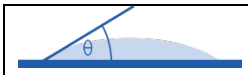
The sessile drop contact angle of three probe fluids (water, ethylene glycol, and hexadecane) was measured on PLA and PMMA surfaces, using a goniometer (ramé-hart Instrument Co., Mountain Lakes, New Jersey). The contact angle values were measured using 2 µL droplets every 0.5 seconds over the course of first 20 seconds of contact and averaged to result a single value. The measurements were repeated 10 times. The obtained contact angle values were

later used to calculate the surface energy and surface energy components of the PLA and PMMA substrate surfaces using Equations (3.1 and 3.2) [117] (also see Section 3.2.2). The streaming zeta potential of the PMMA and PLA substrates were measured via streaming current measurements on a SurPASS 3 instrument (the measurements were done by Anton Paar team). The 0.001M standard KCl solution was used as the electrolyte and the measurement was conducted over the pH range of 3 to 9 (Figure 4.2).

4.1.4 Results and discussion

Table 4.1 summarizes the physicochemical characteristics of the PMMA and PLA, obtained through contact angle measurement and streaming potential measurement.

Table 4.1. Surface characteristics of PMMA and PLA

	**Contact angle ◦**			**ζ (pH=7)**	**γ^{LW}**	**γ-**	**γ+**	**γ**
Material	Water	Ethylene Glycol	Hexadecane	(mV)	(mJ/m^2)			
PMMA (Acrylic)	86	58	14	-14	26.7	3.4	1.1	30.6
PLA	78	56	19	-45	26.1	9.3	0.7	31.2

(Zeta potential (ζ**), **total** surface energy (**γ**), surface energy components:* Lifshitz-van der Waal *(γ^{LW}), electron acceptor (γ-) and electron acceptor (γ-))*

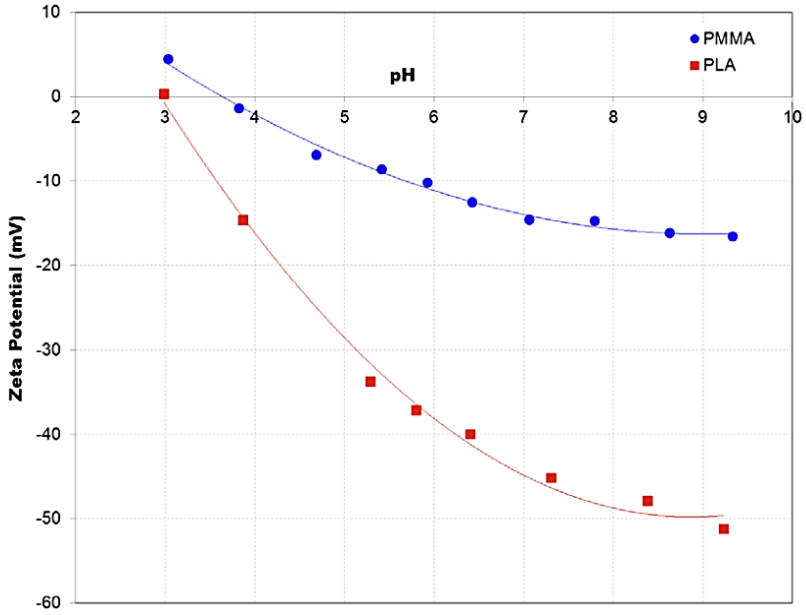


Figure 4.2. Zeta potential vs pH values for PMMA and PLA susbstrate surface (solid lines are guide to the eye**)**

The average amount of biomass per unit area harvested from each 3D-printed and commercially acquired sheet sample is as depicted in Figure 4.3 a. Statistical analysis reveals that the average of obtained biomass values from PMMA and PLA substrates were significantly different from one another (p= 0.014), Given the high standard deviation of the obtained results, especially in the case of printed PMMA surfaces, Fisher's least significant difference and Tukey's honest significant difference post hoc tests were performed on the data to compare each of the material pairs based on the 95 % (approximately 2 standard deviations) confidence intervals (CI) for the means (Figure 4.3 b). The results indicate presence of significant biomass difference between printed PMMA and PLA pair and printed PMMA and PLA pair (based on Fisher's test). Looking at the Table 4.1 and from the water contact angle values it can be concluded that PMMA is more hydrophobic than the PLA with significantly different zeta potential value which might explain the differences observed in the attached biomass value. Generally, algae are known to have moderately ranged negative surface charges [118]. When a negatively charged substrate is used

for attachment, the electrostatic interactions will be often repulsive with larger absolute charge values often associated with more repulsion.

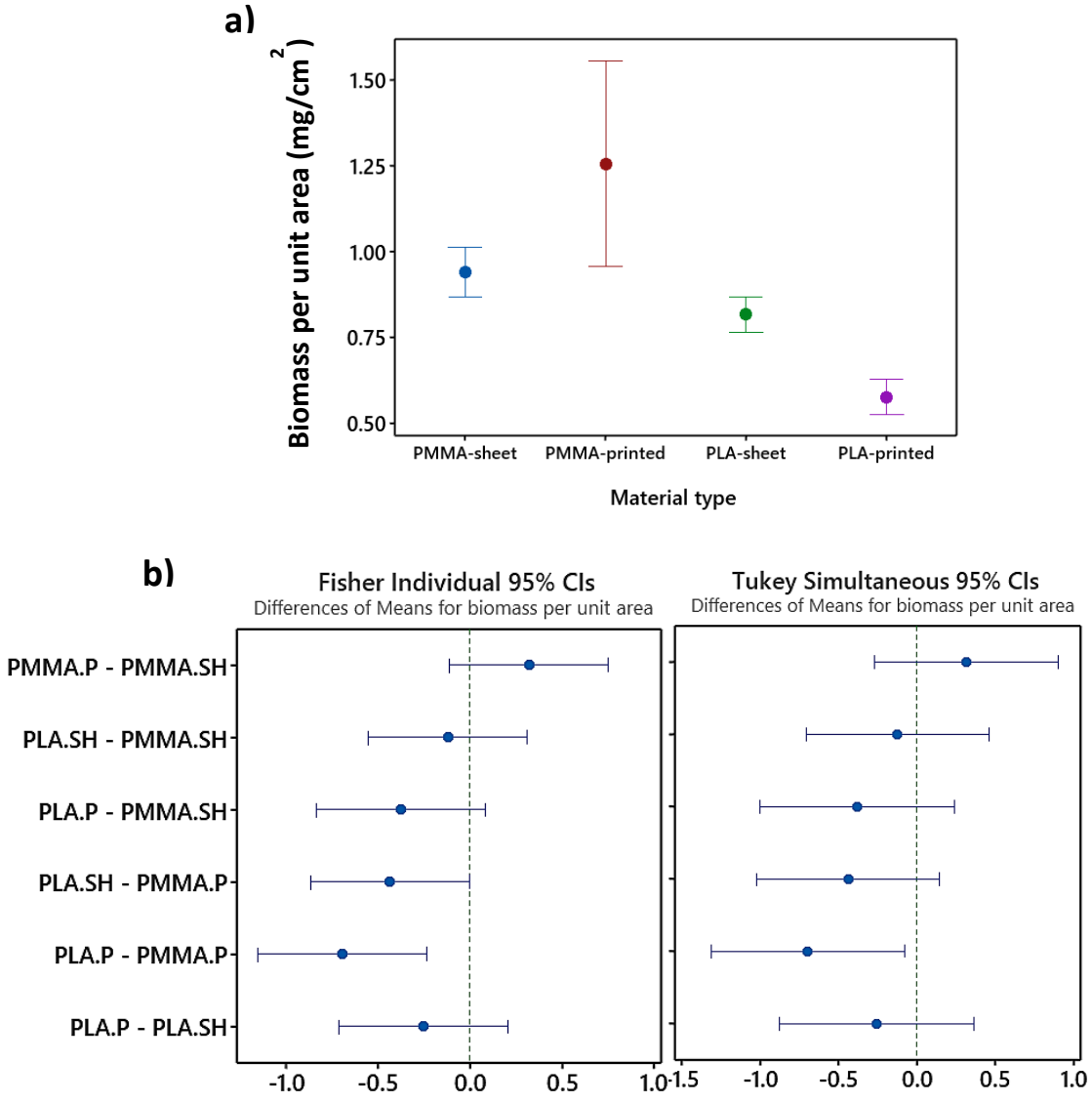


Figure 4.3 a) Dry biomass obtained from PLA and Acrylic (PMMA) per unit area b) Tukey's and Fisher's post hoc analysis for pairwise comparison of means between harvested biomass per area from each material type (the intervals are representing the differences in mean values and intervals not containing zero, indicate significantly different means).

4.1.5 Conclusions from preliminary photobioreactor cultivation

The initial experiment described here served as preliminary step for observing material related influences on algal attachment in a flow environment. This experiment helped in building the foundations required for setup of a more controlled flow environment, described in the next section. The experiment also served as a proof of concept to the use of harvested biomass as an indication of favorability of a substrate for attached growth. Meanwhile, spatial limitations of this reactor have resulted in small coverage area by the samples and as a result low collected biomass which might have contributed to the high standard deviation of the biomass harvested per sample.

4.2 The effect of material chemical properties on early stage and long-term cultivation

In this section results of a series of experiments performed to explore the effects of material properties on early stage and long-term algae cultivation are discussed. This experiment focused on targeted cultivation of periphytic filamentous algae *Stigeoclonium tenue*.

4.2.1 Photobioreactor set up and cultivation conditions

The flow way photobioreactor was comprised of two 12:165:7 cm (W:L:H) PVC channels built from residential rain gutters and a reservoir (Sterilite, 38 L Tote Box). The media was continuously cycled in the systems by two Pondmaster pumps (Danner Pondmaster Magnetic Drive Pump Model 7 (700 gph). The pumps were submerged in the reservoir and fed the inlet into the channels. The pipes connecting the pumps to the channel was comprised of 0.5-inch PVC pipes (600-PSI Schedule 40 PVC Plain End Pipe) and a PVC ball valve was used at the inlet for flow adjustment. Furthermore, each channel was equipped with an individual light fixture (Lithonia Lighting 2-Light White T8 Fluorescent Residential Shop Light), placed 20 cm above the channels. The average photon flux density (PFD) in the channels was measured to be 250 ± 30 μmol/m^2s.

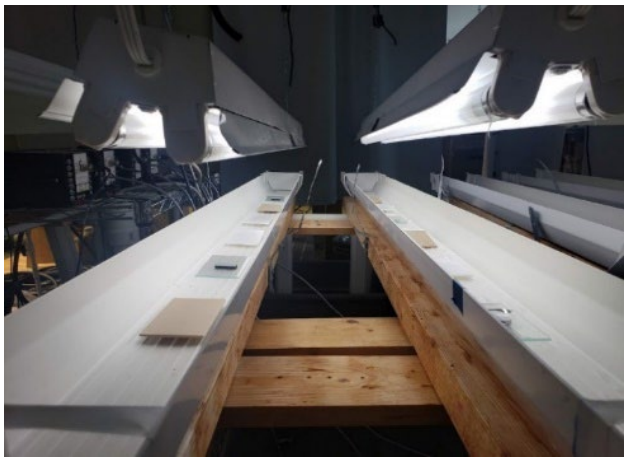


Figure 4.4 Substrate surfaces (glass, PTFE, and PLA) in the photobioreactor prior to cultivation.

Stigeoclonium tenue obtained from UTEX (University of Texas at Austin) algae culture collection (catalog number UTEX LB 1572) was moved to a flask under aseptic conditions. Bold's Basal Medium (BBM) [115] was used as the growth medium for the flask culture. The culture was at room temperature, constantly aerated, and under the 8:16 h dark-light cycle to reach dry biomass content of 10 mg/liter for photobioreactor inoculation. The inoculum was triple washed using DI water and strained using a steel fine mesh tea strainer before adding to the photobioreactor. The dry biomass content was determined by sacrificially drying three individual 15 ml samples of the washed and homogenized inoculum overnight. Microscopy was performed to ensure viability of the inoculum cells post washing and straining and before adding to the photobioreactor.

Twelve substrate samples (tiles) (5×9×0.02 cm) made from PTFE, soda-lime glass, and PLA were placed in the flow channels through random permutation. The average flow rate in lanes was 150 ml/s and the used nutrient media was BBM. The cultivation period was 10 and 18 days in each of the short- and long-term experiments, respectively.

4.2.3 Results and discussion

The amount of dry biomass harvested from each of the material categories in the long term (18 days) and short-term (10 days) experiment are plotted in Figure 4.5. From the obtained results, PLA was the most favorable substrate (Figure 4.5, short-term) for the attachment at the short term. But as the cultivation continued for longer, the substrate-induced biomass differences disappeared. ANOVA of different sample materials resulted in p values equal to 0.01 and 0.59 for the short- and long-term cultivation respectively suggesting significant biomass differences between material types for the short-term cultivation. The observed results might be due to the differences in the composition and structure of the settled community. Even though algae-substrate interaction is important at the early stages, as the biofilm/turf becomes more mature, newly depositing algae are no longer in direct contact with the substrate and are rather adhering to the algae-EPS matrix already settled onto the surface.

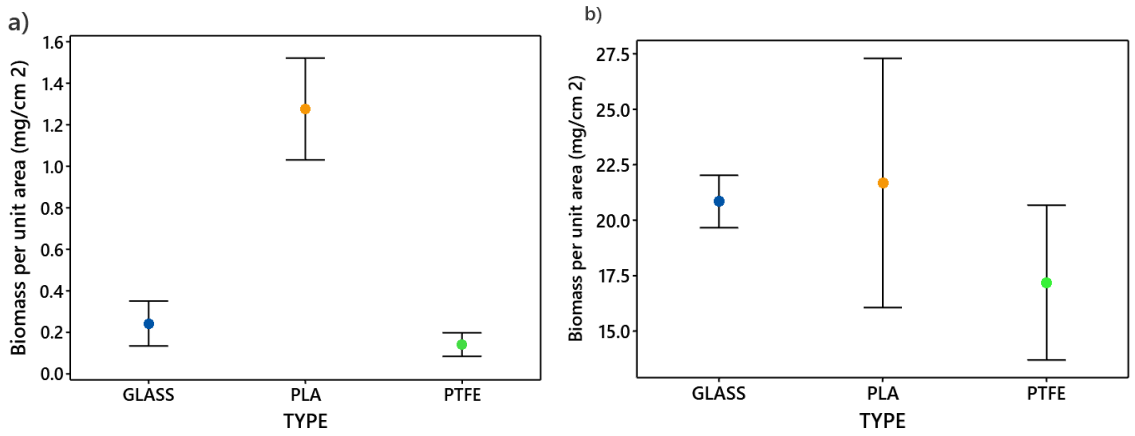


Figure 4.5 Harvested biomass per unit area for PLA, glass, and PTFE substrates at a) short-term (10 days) (b) and long-term (18 days) cultivation periods.

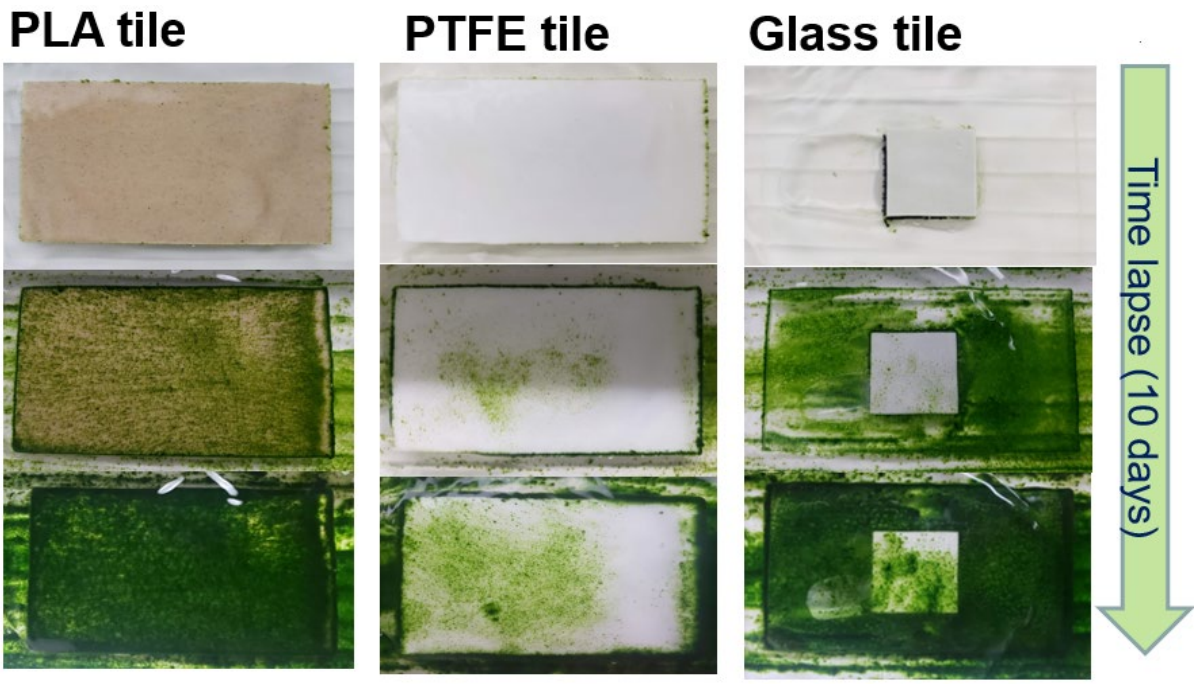


Figure 4.6 Time sequence of algae colonization on PTFE, glass, and PLA substrate during the early stage of attachment and growth.

4.2.4 Conclusions

Results from experiments described in Section 4.2 are important from the application perspective because they give more insight into the time window of influence of the substrate surface intrinsic chemical characteristics. It appears that change of the substrate material is an effective tool in increasing early-stage colonization but might not be as important in long term cultivation. Additionally, the need for a better controlled environments for monitoring the material effects may be necessary. This topic is discussed further in Chapter 5.

Chapter 5: Design and analysis of a flow way photobioreactor for substrate assessment in attached cultivation of filamentous green algae

This chapter is based on a manuscript by Karimi et al., titled "Design and analysis of a flow way photobioreactor for substrate assessment in attached cultivation of filamentous green algae", submitted to the journal of Algal Research in March 2022.

The lack of application-focused knowledge for substrate effects in periphyton based systems is a limiting factor in using these systems' potentials for nutrient recovery and biomass production. Therefore, there is a need for investigation (on effects of the substrate) at an intermediate scale that bridges fundamental periphyton studies (in natural systems) and large-scale application-focused studies on systems like ATS. In this chapter, design of an intermediate-scale laboratory flow way photobioreactor intended for studying substrate effects on attached cultivation is introduced. In addition to filling the gaps in knowledge regarding substrate effects on periphyton based systems, this photobioreactor was designed for overcoming some of the challenges identified in previous photobioreactor experiments (Chapters 4). These challenges included the limited number of repetitions for the substrate samples, flow complexities caused by the grooves on the bottom of the channels and limited channel length.

The photobioreactor described in this chapter is used as a set-up to cultivate the periphytic filamentous green alga *Stigeoclonium tenue* on polylactic acid (PLA) polymeric substrates. The results of the cultivation experiment are used as an example for reviewing and assessing the influence of some of the critical design and growths factors such as light exposure, flow conditions, inoculation method, growth media, and more. Ultimately, the presented design aimed to create a low-cost and controlled environment that enables and improves intermediate scale lab studies on

filamentous green algae. This could lead to understanding the combination of growth factors and operational parameters required for species selective cultivation.

5.1 Photobioreactor set up and *Stigeoclonium* cultivation

5.1.1 Flow way photobioreactor's external structure and water circulation system setup

Four open polyvinyl chloride (PVC) channels (243 cm x 10 cm x 6 cm) were used as the flow channels. The channels were mounted on a fixed wooden frame (Figure 5.1). A 55 Gallon heavy-duty plastic tub (HDX-tough storage tote) was used as the reservoir and ½ inch opaque schedule 40 PVC pipes were used to circulate the water in the reactor. Four submersible magnetic drive pumps (Pond Master-Pond Mag 700 GPH) were placed inside the reservoir and connected to the PVC piping to feed the inlet from the reservoir into each channel. The water flow rate into each channel was controlled using a ball valve. The water depth during the experiments was measured at the beginning, middle, and end of the flow channel to ensure uniformity and was 1.4 cm throughout the channels.

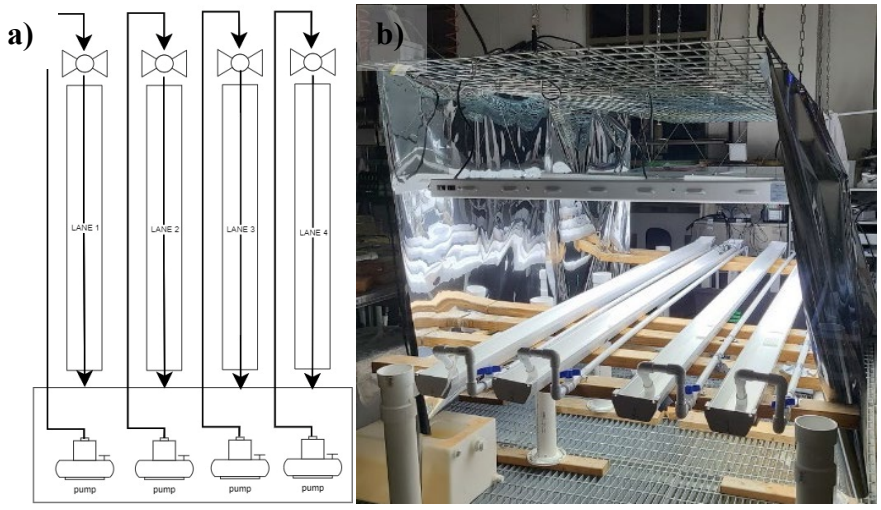


Figure 5.1 a) A schematic diagram of the photobioreactor circulation system b) An image of the photobioreactor channels.

5.1.2 Light conditions

Four Sun Blaze model T5HO-48 light fixtures (127×61×10 cm) were mounted above the flow ways to cover the test section area. Each fixture contained eight fluorescent 6500K T5 growth light bulbs. The operating photon flux density was adjusted by changing the distance between the channel and the light fixtures. Reflective Mylar sheets were hung around the reactor to help with irradiance in the channels. The average photon flux density in the flow ways was 330 ± 56 $\mu mol/m^2s$. The light intensity distribution inside the four flow ways (lanes) was measured over 36 points in the test section corresponding to the assigned placement positions for the substrate samples (refer to Figure 5.1 for details), using an Apogee MQ-200 Quantum Flux meter.

5.1.3 Growth medium and inoculum

Stigeoclonium tenue obtained from UTEX (the University of Texas at Austin) algae culture collection (catalog number: UTEX LB 1572) was moved to a flask under aseptic conditions. Bold's Basal Medium (BBM) [115] was used as the growth medium for the flask culture. The culture was kept at room temperature, constantly aerated, and under an 8:16 h dark-light cycle to reach a dry biomass content of 15 mg/liter for photobioreactor inoculation. The inoculum was triple washed using DI water and strained using a tea strainer before adding to the photobioreactor. The dry biomass content was determined by sacrificially drying three individual 15 ml samples of the washed and homogenized inoculum overnight. Transmitted light microscopy was performed using a Nikon (Melville, NY) Eclipse 80i microscope with a 20X/0.45 LU Plan Fluor Nikon objective lens to ensure the integrity of the inoculum post washing and straining and before adding to the photobioreactor. The concentration of inoculum algae in the 50 L cycling media was 15 mg/L (dry biomass basis).

5.1.4 Substrate sample and placement

PLA sheets with a thickness of 25 mm were cut into 9×5 cm rectangular pieces. The samples were placed at nine equally distanced spaces in the channels (corresponding to placement positions 1-9) along their long edge, starting at 70 cm from the inlet through 223 cm downstream.

5.1.5 Operation, maintenance, and monitoring the media parameters

Upon placing the PLA substrate samples, the BBM media was circulated in the flow way channels as described in Section 2.1. The inoculum algae were added to the media as described in Section 2.3. The photobioreactor was operated in batch mode under an 8:16 h dark-light cycle for 8 days. The water loss due to evaporation was replaced with deionized water daily. Water quality parameters such as pH, temperature, conductivity, and total dissolved solids content were monitored daily using a HANNA instruments HI98130 Combo tester. In addition, visual monitoring of the attached growth was done by digital imaging the PLA samples daily (while submerged in the water) from above (90-degree angle, at 8cm distance).

5.1.6 Biomass harvest and analysis

The PLA substrate samples were removed from the flow way channels at the end of the 8-day inoculation. The attached biomass was harvested from the top surface of each substrate sample using a hollow plastic tube connected to a filtering flask and placed under vacuum. The biomass collected from each sample in the flask was then transferred to pre-weighed aluminum weighing boats and dried overnight at 160°C in a convection oven. The dry biomass content per sample was then measured by weighing the samples and subtracting the weight of the weigh boats.

5.2 Results and discussion of design parameters

Interpreting the substrate effects on attached growth requires careful consideration of the impact of several design and growth factors [119], particularly inoculation method, community

structure, light regime, flow characteristics, nutrient concentrations, temperature, and pH. To obtain practical data that aids interpretation of substrate effects, either adequate control or dynamic monitoring of the mentioned factors is needed. Considerations in reactor setup and operation and design decisions for the presented photobioreactor and their impact on the attached cultivation experiment on PLA substrates are described in the following alongside their general significance.

5.2.1 Inoculation and community structure

From the application standpoint, targeted inoculation of specific algae species (monocultures) presents an opportunity to understand growth conditions and growth dynamics that enables designing cultivation systems with enhanced control over the algal community composition. Currently, controlling the algal community composition is often hard to achieve when increasing scale from the lab to larger scales [10], and open systems are especially prone to contamination issues [120, 121]. In the case of many periphytic filamentous algae, lack of information regarding growth dynamics, optimal growth conditions and substrate preferences of specific species has resulted in limited exploration of potential applications of that species.

In this work, we report successful attached cultivation of an algal community heavily dominated by filamentous green algae, *Stigeoclonium tenue*. *Stigeoclonium* was chosen as the target genus because it has been reported to be found in several periphyton-based systems and has significant potential as biomass feedstock and in wastewater treatment [12, 25, 48, 49, 66, 67]. Additionally, *Stigeoclonium* has specialized attachment adaptations (differentiated basal cells) [68] that could aid in attached growth. *Stigeoclonium tenue* was initially adapted to a bubbling flask growth environment and brought to a dry biomass concentration of 15 mg/liter using the standard BBM media as the nutrient source before being added to the photobioreactor as inoculum. Even though perpetual aeration in a bubbling flask culture may result in entanglement and excess

shear stress on the filaments and pathogenic contamination over time, pre-inoculation of *Stigeoclonium tenue* resulted in a highly populated, viable culture of the target species. This may be due to physiological advantages of *Stigeoclonium tenue* such as its branched structure. Microscopy on the biomass harvested from the PLA substrates at the end of the cultivation experiment confirms that *Stigeoclonium tenue* remains dominant in the community (Figure 5.2) with the rare presence of some diatom, microalgae, and cyanobacteria species.

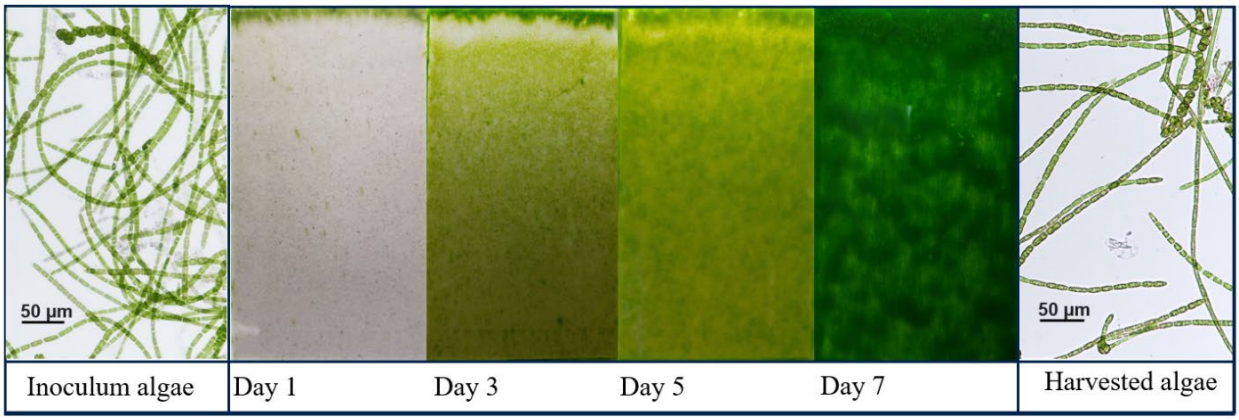


Figure 5.2 Microscopic image of inoculum and harvested algae alongside images of chronological sequence of attached growth on a PLA substrate sample throughout the cultivation period

5.2.2 Flow characteristics and water depth

The flow characteristics of the growth environment have both direct and indirect effects on algae attachment and growth. Current velocity and pattern directly affect the transport of nutrients from the bulk flow to the attached community and the mixing of the overlaying layers. They can also affect the shear stress on the attached algae and, ultimately, the drag forces. Light and shading conditions can be indirectly affected by the flow as well. Understanding the effect of flow on attached algal communities and drawing direct conclusions about optimal flow conditions is often complicated. This is due to several factors including, but not limited to, the dynamic process of

algae attachment and growth, differences in physiological and morphological characteristics of different algae, properties of the substrate such as texture, and presence of grazers [122]. Nonetheless, based on the studies conducted in natural systems (inland freshwater ecosystems), it is believed that many benthic algae, including filamentous green algae, will benefit from moderate currents with velocities in the range of 10 - 50 cm/s [38]. Fast currents may limit the initial deposition of algae cells on the substrate and disrupt the community's growth in later stages due to high shear stress. Slow currents are also not optimal due to nutrient convection and diffusion limitations.

Ideally, for targeted studies on the effect of substrate surface characteristics on attached growth, the hydrodynamic characteristics of the algae cultivation environment should be suitable for attached growth. They must be both known and well-controlled to avoid further complexity in the system. The goal of the current design was to create a flow environment that enables the placement of multiple substrate samples for attached cultivation while ensuring minimal variabilities among the substrate samples caused by non-uniformity in the flow. The choice of channel geometry, circulation inlet type, flow rate and the distance of testing substrate samples from the inlet can all affect the flow patterns around the substrate samples and contribute to non-uniformity and/or creation of flow-related variabilities among the samples. A rectangular open channel was used to minimize flow pattern complexities induced by the channel geometry and a 70 cm distance between the inlet flow and testing section was placed to mitigate the disruptive inlet effects. A ball valve was used before the inlet to adjust flow rate into each channel. The flow rate at the inlet for each channel was measured for channels 1-4 and an average flow rate of 105 ± 8 ml/s and estimated average velocity of 7.5 cm/s was calculated based on the channel dimensions and the reported flow rate and water depth (1.4 cm). A linear regression model relating the effect

of channel and placement position to the harvested dry biomass per substrate sample results in p-values larger than 0.05, indicating no significant difference between the average biomass harvested from substrate samples placed in different channels. This shows that the variations in the flow rate in each channel and other possible hydrodynamic differences between the channels and placement positions downstream are not large enough to create variations in the amount of harvested biomass among substrate samples.

5.2.3 Light conditions

Light conditions such as the light spectrum, light intensity, and dark-light cycles all play a crucial role in the growth of attached algae [123]. Light conditions can influence physiology, growth dynamics, and community structure. In attached systems, water depth combined with the opacity of the cycling media is also able to influence the amount of light that algal cells obtain [38, 122]. A wide range of operating photon flux densities (PFDs) have been reported for attached cultivation systems (~20-650 μmol/m^2s) [4]. In addition to light intensity, the dark-light cycles are also known to influence the algal growth rate and chemical composition (pigments, lipids, carbohydrates, etc.) [124].

The current design aimed to achieve a lighting set-up that assures homogeneous irradiance of the incubation area, enables changing the PFD easily (by changing the light fixture height) and provides adequate light intensity to support the growth. In this work, an 8:16 dark-light cycle was chosen to mimic a natural environment. The PFD inside the channels at substrate sample placement positions ranged from 200-412 μmol/m^2s with an average of 330 ± 56 μmol/m^2s, which is within the reported range for other systems. Despite efforts to create homogeneous light intensity throughout the test section, there was some variability in the light intensities (Fig. 5.3a and Fig. 5.3b). The effect of light variability on the attached biomass (visualized in Fig 5.3d) was

investigated through statistical analysis of the light-biomass relationship in each channel and at each placement position. Based on the results of linear regression analysis on the effect of light intensity on the biomass, the variations in the light intensity at each of the placement positions (Fig. 5.3c) do not significantly affect the amount of harvested biomass implying adequate control over the parameter ($p = 0.7$).

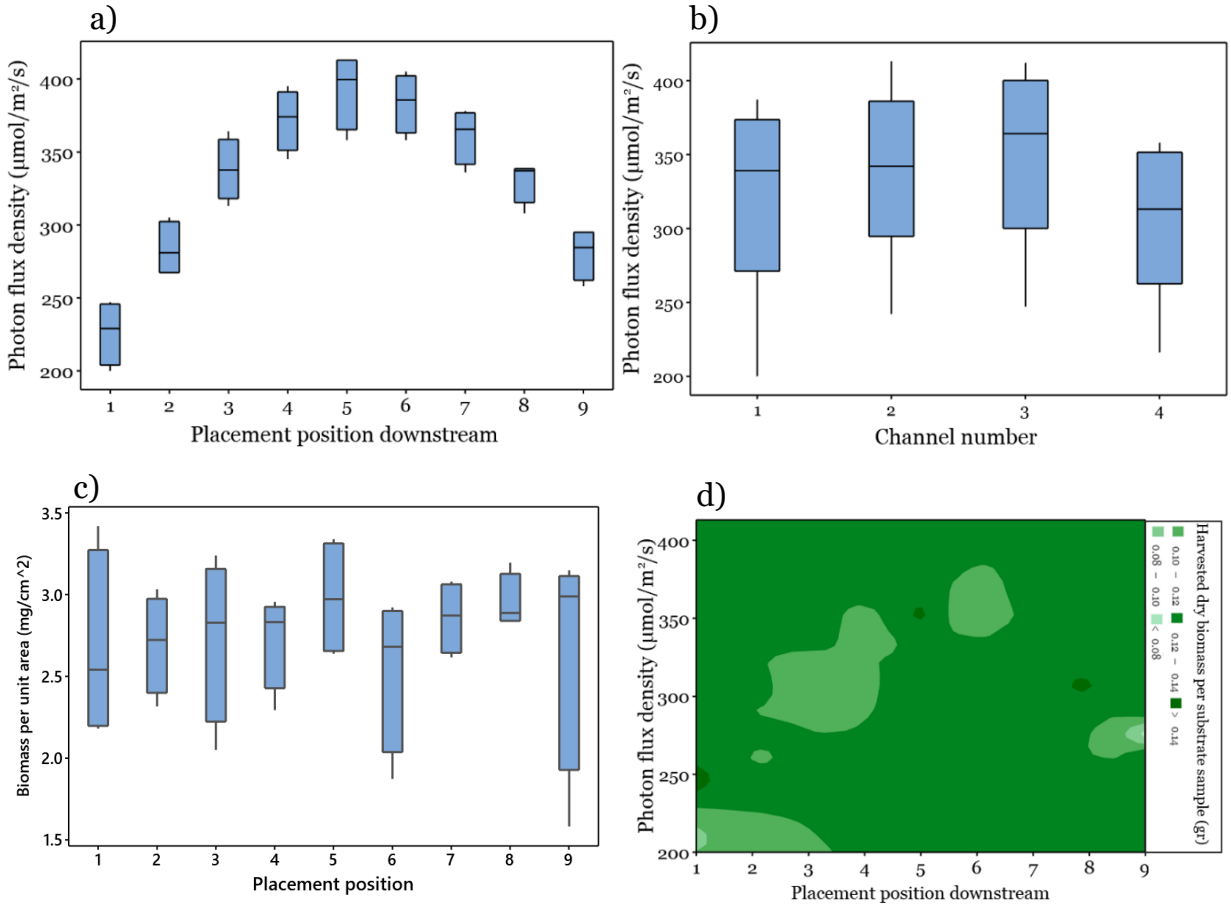


Figure 5.3 a) PFD vs substrate placement downstream the channels (measured for each placement at each channel); b) Average PFD in each channel over the 9 placement positions; c) The amount of harvested dry biomass per PLA substrate sample at each placement position (total of 4 samples per placement); d) Counter map of the amount harvested biomass per PLA sample at each placement position and at the corresponding PFD (created with Minitab through Inverse distance weighting interpolation (distance power = 2).

5.2.4 Other parameters

In addition to the previously mentioned parameters, abiotic factors such as temperature, pH, and carbon dioxide concentration also have an effect on algal growth in both attached and planktonic systems. Optimum cultivation temperature highly depends on the algae species present (algal community composition) [125-127]. For outdoor periphyton-based systems, community composition and productivity changes are often observed because of the seasonal light and temperature variations [35]. Light intensity and temperature are usually a set of correlated parameters, whether in outdoor or indoor cultivation systems, with higher light levels being associated with increased temperatures. Changes in the ambient temperature can affect the population, community composition, growth rate, metabolic activity, and biomass production of periphyton algae [38, 128, 129].

Carbon dioxide concentration and pH are another set of correlated parameters that are known to affect algal growth. In general, in a batch operation mode, algae's uptake of carbon dioxide will shift the system towards the abundance of bicarbonates and increase the pH. Changes in pH will affect the solubility of different nutrients in the water and can change the nutrient concentrations. In attached algal growth, physicochemical characteristics of the substrate and algae are also believed to influence the algae-substrate interactions and impact the initial adhesion [85, 88, 99, 118]. Some of these characteristics such as surface charge are highly influenced by pH.

In this experiment, daily monitoring of total dissolved solids (TDS) content and conductivity of the recycling media in the photobioreactor showed stable trends with an average of 0.27 ± 0.1 ppm for the TDS, and 0.54 ± 0.04 mS for the conductivity throughout the cultivation period. This could be an indirect indication of a constant concentration of nutrients in the recycling media. The recorded temperatures were 25 ± 1°C, which were both stable and suitable for

cultivations of *Stigeoclonium tenue*. On the other hand, the pH increased from 5.3 at the beginning to 9.0 at the end of the cultivation period; this is an expected outcome of algae population increase (growth) in the batch operation mode.

5.2.5 Overall results and combined evaluation

The average amount of biomass cultivated on the PLA substrate samples (total of 36 samples across 4 channels) over the course of 8 days was 124 ± 18 mg (2.8 ± 0.4 mg/cm^2). As discussed above, statistical analysis on the biomass data reveals that placement position, light intensity, and flow rate and channel do not have a significant effect on the harvested biomass value. Linear regression model relating the effect of placement on the harvested dry biomass results in a p-value larger than 0.05, and further analysis of each possible placement position pairs downstream using Tukey's method indicates no significant differences between any placement pairs across the 4 channels. This shows that variabilities in light conditions, channel, and flow conditions experienced by each of these 36 sample placements do not significantly affect biomass uniformity in the test sections.

Overall, the analysis of the harvested biomass per substrate shows that the designed flow way photobioreactor, despite its simplicity, provides adequate control over parameters such as light intensity and flow rate in the channels to create an environment suitable for testing attached growth on multiple substrate surfaces/samples at once. Further improvement in the uniformity of the biomass obtained across the placements may be enabled by limiting the test section to sample placements positions 2-8 and assigning sacrificial/placeholder substrate surfaces to placements at the beginning and end of the photobioreactor. As evident by the box plot in Figure 5.3 c, placements 1 and 9 (beginning and end of the test section) have the most variability in the amount

of harvested biomass. This can potentially be because of the flow non-uniformities created at the inlet and outlet and the lower PFD values.

5.3 Conclusions

Attached algal cultivation systems remain an interesting avenue for further exploration of commercial algae-based products and processes. Understanding key parameters in controlling attached growth and using them to design cultivation systems with improved efficiency and inclusive of diverse algal species and community types remains a challenge. One of the crucial parameters in attached growth that can be leveraged for controlling and improving the cultivation is the substrate. Gaps in the body of knowledge about substrate effects on attached algal cultivation, especially for periphyton-based systems, call for the design of experiments and systems suitable for studying these effects and translating them to a larger scale. The results indicate that the presented flow way photobioreactor design provided an intermediate scale environment for testing multiple substrate samples that is also well controlled with regards to growth parameters such as light intensity, flow rate, and nutrient levels. In addition, the attached growth experimentation on *Stigeoclonium tenue* provides insight into designing and operating attached systems heavily dominated by monocultures of periphytic filamentous green algae and further exploring the effect of algae-substrate interactions on the growth. The design and methods introduced in this chapter were used to conduct studies on the effect of material topographical features and physicochemical properties on attached growth of *Stigeoclonium tenue.*

Chapter 6: Exploring the effect of topography and material properties on algal attachment using 3D printed polymeric substrates

In this chapter the photobioreactor design and experimental setup analyzed in Chapter 5 is utilized to explore the effect of topography and material chemical properties on attached cultivation of algae on polymeric substrates. The chapter consists of a series of three experiments that were conducted with the goal of understanding the combined effect of macro-scale topographical features and material properties on the attached growth of turf-forming algae. As discussed in Chapter 2, there are several studies that suggest substrate topographical features within the size range of algae cells (diameter in the case of most microalgae) promote algae adhesion and biofouling [36, 82]. Additionally, there are studies that have shown the influence of substrate feature sizes in the range of 100-2000 microns on the diversity of the species in the periphyton community growing attached to substrates in natural systems [33].

The filamentous periphytic algae that are the subject of interest in this work such as *Stigeoclonium*, can form macroscale networks of filaments that grow attached to the substrate. These macroscale three-dimensional networks that can grow up to meters create an algae-substrate interaction dynamic different from the thin microalgae biofilms. Additionally, existence of specialized attachment mechanisms such as root-like structures add to the complexity of the algae-substrate interactions for these algae. Therefore, this chapter focuses on the effect of macro scale substrate topographical features on attached growth. In Section 6.1, flat PLA substrates are compared to substrates with concave hemispherical features in terms of favorability of attached growth. In Section 6.2, the experiment described in Section 6.1 is repeated with ABS and PLA substrate surfaces. Ultimately, in Section 6.3 polymeric substrates made from polyethylene

terephthalate glycol (PET-G), PLA and ABS with concave hemispheres of different features sizes are used for attached cultivation. The goal was to find the optimized material and feature size for attached cultivation of filamentous algae *Stigeoclonium tenue.*

6.1 Concave hemisphere topographical features for attachment enhancement

6.1.1 Substrate surface preparation

PLA substrate surfaces of 5 × 9 × 0.7 cm were 3D printed with two different topographical features using a Monoprice Maker Ultimate printer. Monoprice white PLA Plus 1.75 mm filament was used as the feed and printed at 190 °C with a layer thickness of 0.1 mm. A total of 21 tiles were printed and used for cultivation, seven of which were flat and fourteen were textured with an array of forty-five equally distanced concave hemispheres with diameter of 9.2 mm (Figure 6.1).

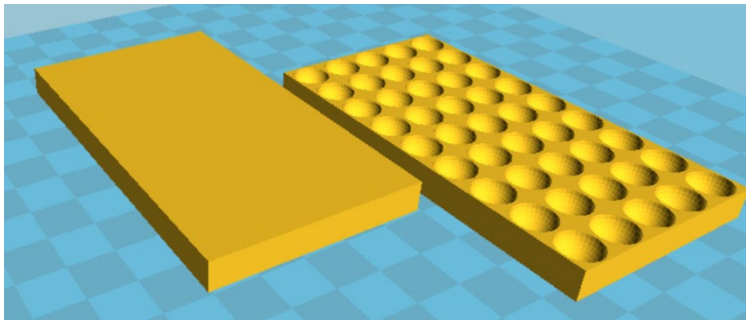


Figure 6.1 A three-dimensional sketch of the flat (left) and textured (right) substrate surfaces.

6.1.2 Cultivation experiment

The photobioreactor set up and algae inoculation method described in Chapter 5 was used for this experiment. Substrate surfaces were placed in the photobioreactor lanes by assigning one lane to flat surfaces and two lanes to textured surfaces. The concentration of added *Stigeclonium tenue* was 0.01 g/L (dry biomass basis) in the 50 liters of the BBM after addition of the inoculum. Average flow rate in the lanes was 96 ± 4 mL/s. The cultivation period was 11 days. Algae grown

attached to substrates were collected from each sample and dry biomass content was determined using the method described in Chapter 3.4.1.

6.1.3 Results

Textured PLA tiles with concave hemispheres had more algae biomass attached with an average of 0.08 ± 0.02 g per sample in comparison to the 0.05 ± 0.01 g per sample for the flat substrate samples. This shows that the hemisphere features have effectively created a favorable environment for attached growth. The concave hemispheres create more surface area for algae to attach with the textured surfaces having a total surface area of 105 cm^2 in comparison to 45 cm^2 for the flat surface. Figure 6.2 (a) compares the amount of biomass harvested from flat and textured substrate samples per both actual total surface area (45 cm^2 for flat tiles and 105 for textured tiles) and nominal surface area (45 cm^2 for both flat and textures substrates). Even though, the biomass harvested per total area of the substrates which includes the surface area of the hemispheres for the textured substrates did not improve by the presence of texture, the harvested biomass per nominal unit area of the substrates improved significantly. This could be deemed favorable from the practical standpoint, given the concave hemispheres do not add to the footprint area of the substrates but enable more biomass cultivation.

6.2 Combined effect of topography and material properties on attached growth

An experiment with the same cultivation protocols described in Section 6.1 was performed this time by introducing ABS substrate surfaces alongside the PLA substrates to observe the combined effect of material type and substrate topographical features on attached growth.

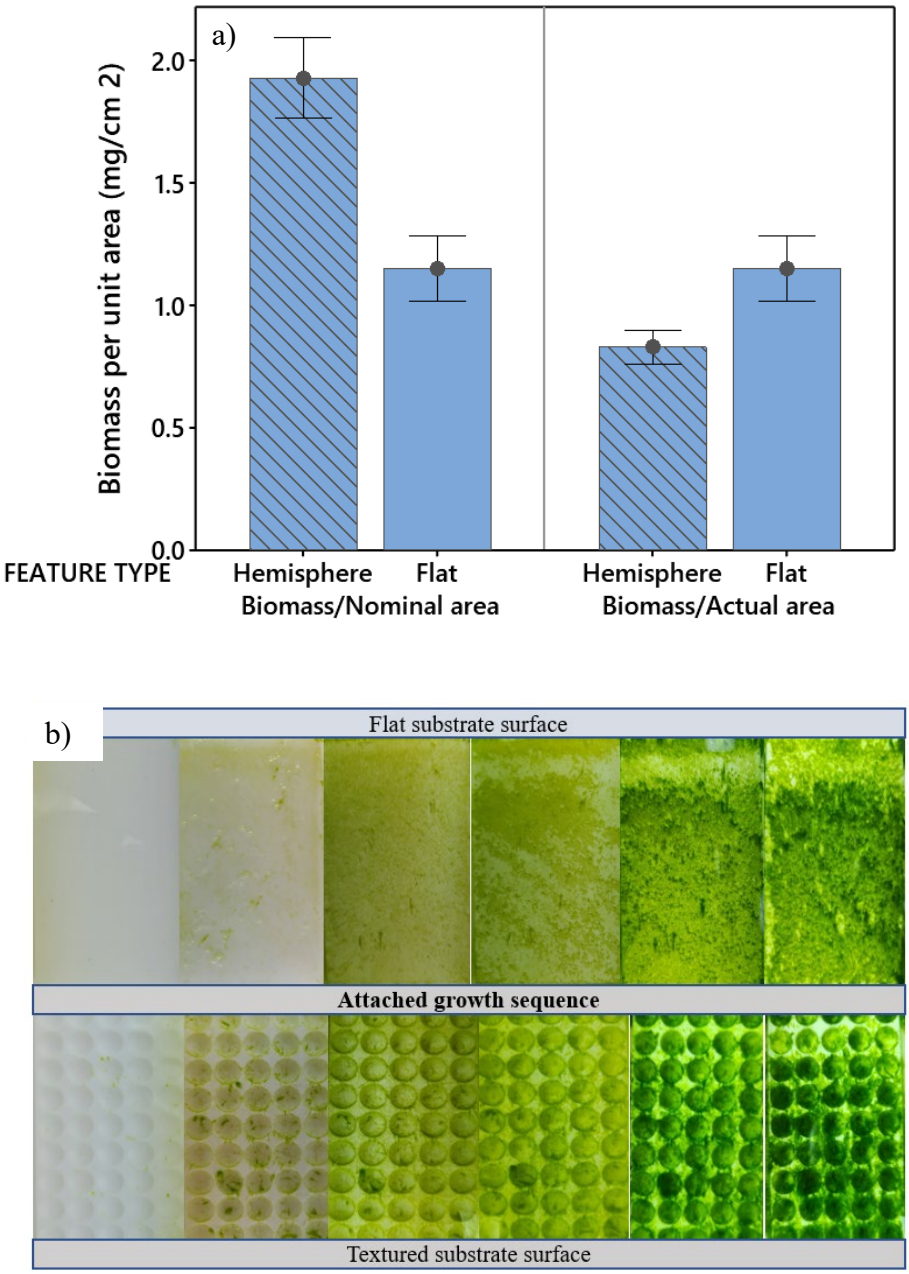


Figure 6.2 a) Biomass harvested from flat and textured PLA substrate surfaces per nominal surface area (left) and actual surface area (right) b) Cultivation sequence throughout the incubation period for a flat and textured sample

6.2.1 Substrate surface preparation

PLA and ABS substrate surfaces of 5 by 9 by 0.7 cm were 3D printed with two different topographical features using a Monoprice Maker Ultimate printer. Monoprice white PLA Plus and Hatchbox white ABS, 1.75mm filaments were used as the feed and the surfaces were printed at 190°C and 210 °C respectively. The layer thickness of the prints was 0.1 mm. A total of 21 tiles were printed and used for cultivation, 7 of which were flat (3 ABS and 4 PLA tiles) and 14 had an array of 45 equally distanced concave hemispheres with diameter of 9.2 mm (7 ABS and 7 PLA).

6.2.2 Cultivation experiment

The photobioreactor set up and algae inoculation method described in Chapter 5 was used for this experiment. Substrate surfaces were placed in the photobioreactor lanes (numbered 1 - 3) with assigning a lane to flat surfaces and two lanes to textured surfaces. Within each lane, ABS and PLA tiles were placed in sequences selected by random permutation. A total concentration of 0.01 g/L (dry biomass basis) of *Stigeoclonium tenue* was added to 50 liters of BBM as the inoculum. Average flow rate in the lanes was 107± 7 mL/s. The cultivation period was 12 days. Algae grown attached to substrates were collected from each sample and dry biomass content was determined using the method described in Chapter 3.4.1.

6.2.3 Results

Similar to the results from Section 6.1, the cultivation experiment with flat and textured substrates resulted in more biomass growth per nominal substrate surface area on substrates with concave hemispheres (Figure 6.3). Dividing the biomass per actual area (total surface area of the substrates that includes the area of hemispheres in the case of the textured substrates) did not show increased biomass amount because of addition of the hemispheres. The described trends were found for both ABS and PLA substrates. Linear regression modeling of the system relating the

effect of material type and topography to the amount of biomass obtained confirmed the significant effect of the topography ($p < 0.001$), while the effect of material type was non-significant ($p = 0.57$). Even thought, further analysis on the material effects through ANOVA showed significant difference between the biomass harvested from flat PLA and ABS substrates ($p = 0.023$), the material type did not significantly impact the biomass harvested from the textured substrates and the overall result.

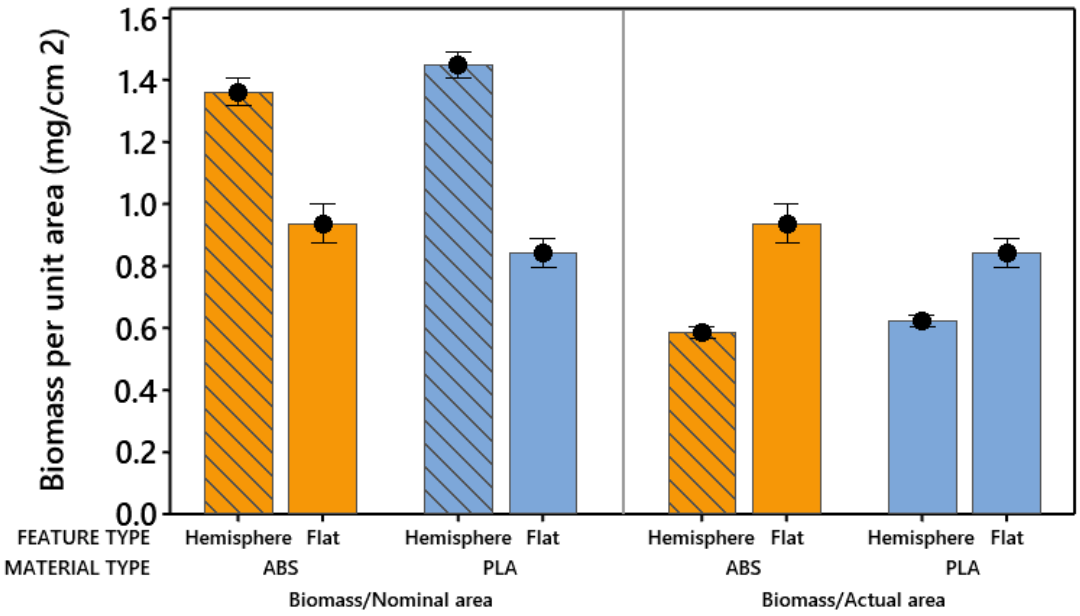


Figure 6.3 Harvested biomass from flat and textured (concave hemisphere) substrate surfaces per nominal unit area (left) and actual unit area (right).

6.3 Combined effect of topography feature size and material on attached growth

In this section a cultivation experiment was conducted on substrate samples manufactured from PET-G, PLA and ABS. Substrates with concave hemispherical features with diameters in the range of 4.4 to 7.6 mm were manufactured to simultaneously investigate the effect of material

properties and macroscale topographical feature sizes on attached cultivation of periphytic filamentous algae *Stigeoclonium tenue.*

6.3.1 Substrate surface preparation and characterization

PLA, ABS and PET-G substrate surfaces of 5 by 9 by 0.7 cm were 3D printed with concave hemisphere topographical features of different diameters using a Prusa i3 MK3S+ 3d printer. Monoprice white PLA Plus, Hatchbox white ABS and Overture white PET-G 1.75mm filaments were used as the feed, and the surfaces were printed at 190 °C, 210 °C, 245 °C, respectively. The layer thickness of the prints was set to 0.1 mm. Three different diameters were selected for the hemispheres within the range of 2.4 to 9.2 mm based on a Sobol sequence generated in Python. The lower end of the diameter range was defined based on the limitations of the 3D printer in generating a precise hemisphere and the upper range was defined based on the previous experimental observations (Sections 6.1 and 6.2) and area limitations of the samples for fitting the hemispheres. The Sobol sequence is a low discrepancy sequence that enables sampling for feature sizes uniformly spread across the targeted diameter range [130]. It also is advantageous in terms of selecting feature sizes within the target range for prospective experiments without losing uniformity due to addition of extra sampling points.

The selected feature diameters through the sampling process were 4.4, 6 and 7.6 mm. The number of concave hemispheres on each substrate sample was 45 and each feature size had 2 replicas per material type, except for the flat tiles which did not have any replicas and were present as control. The following summary describes the naming of the categorical variables and the number of substrate samples:

- Total number of substrates samples/tiles: 21 (7 per reactor lane/channel)
- Number of tiles per material type: 7 (1 flat tile and 2 textured tiles per each feature diameter)
- Number of features sizes: 4 (flat, d = 4.4 mm, d = 6 mm, d = 7.6 mm)

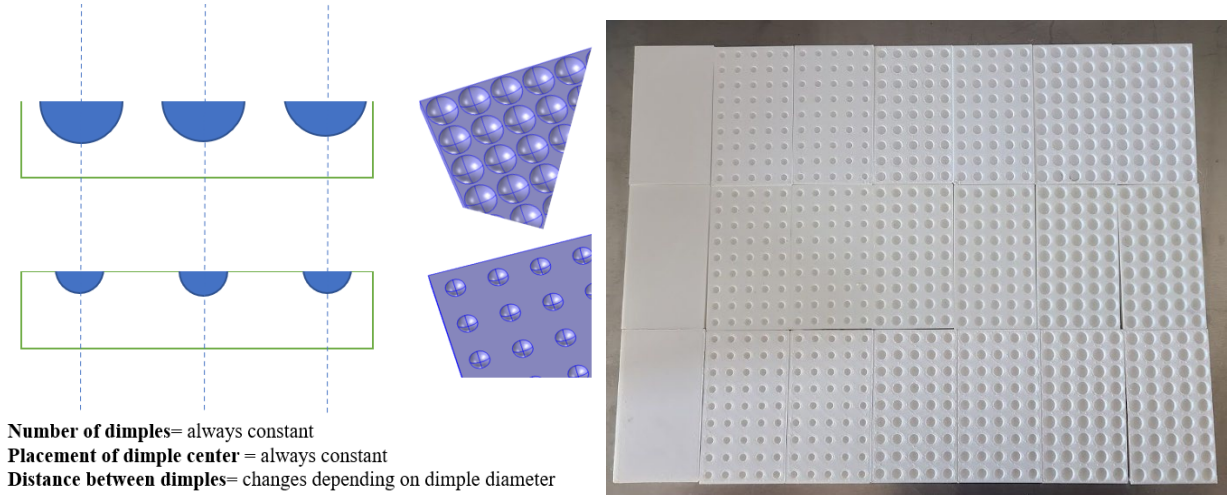
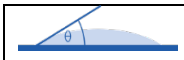


Figure 6.4 Susbstrate design criteria (left) and 3D printed substrates with a range of macroscale topographical feature sizes (right).

The surface energy of the polymeric substrates was measured through sessile contact angle measurement with ethylene glycol, water and diiodomethane on heat pressed filaments of PET-G, PLA and ABS (see section 3.2.2 for surface energy calculation details). Zeta potential values were measured via streaming potential measurement in standard 0.01M KCl electrolyte at the pH of 6.8. The results of the surface characterization of the polymeric substrates are presented in Table 6.1.

Table 6.1 Summary of obtained physicochemical properties for PLA, PET-G and ABS.

	**Contact angle ◦**			ζ	γ^{LW}	γ-	γ+	γ
Material	Water	Ethylene Glycol	Diiodomethane	(mV)	(mJ/m^2)			
PLA	90° ± 4°	70° ± 3	63 ° ± 4°	-45	26.6	5	0	26.6
PET-G	83° ± 3°	66° ± 4	46° ± 3	-53	35.1	6.1	0	35.1
ABS	103° ± 1°	80 ± 1°	62° ± 1°	-56	26.8	0.5	0	26.8

(Zeta potential (ζ**), **total** surface energy (**γ**), surface energy components:* Lifshitz-van der Waal *(γ^{LW}), electron acceptor (γ-) and electron acceptor (γ-))*

6.3.2 Cultivation experiment

The photobioreactor set up and algae inoculation method described in Chapter 5 was used for this experiment. Three lanes of the photobioreactor (lanes 1-3) were used for experimentation with each lane holding nine equally distanced substrate surfaces. First and last substrate placements in each lane were assigned to dummy tiles to omit placement related effects as previously discussed in Chapter 5. Therefore, each lane held seven substrate samples. The placements were chosen in a way that the 5th placement (middle of the reactor) down each lane was occupied by the control flat substrate and each lane contained two of each feature diameters. A total concentration of 0.01 g/L (dry biomass basis) of *Stigeoclonium tenue* was added to 50 liters of BBM as the inoculum. Average flow rate in the lanes was measured to be 100 ± 3 mL/s. The cultivation period was 11 days. Algae grown attached to substrates were collected from each substrate sample and dry biomass content was determined using the method described in Chapter 3.4.1.

6.3.3 Results and discussion

The results obtained by harvesting biomass from the surfaces with different feature sizes show that the attached biomass per nominal area will increase with increasing the feature diameter size (Figure 6.5).

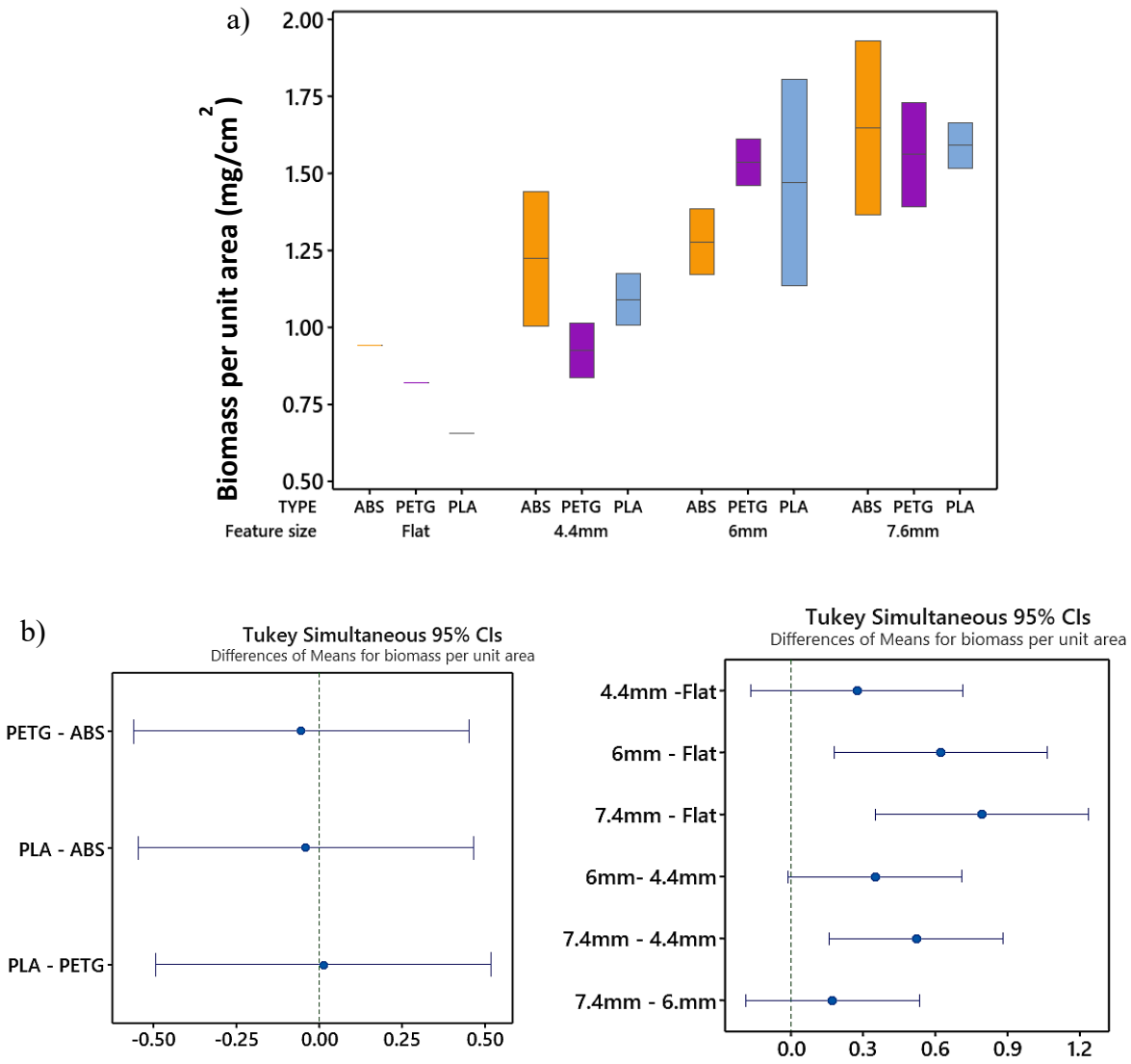


Figure 6.5 a) Harvested biomass per unit area (nominal area in case of textured substrates) from substrates of different material and topographical feature sizes. b) Tukey's test results for pair-pair comparison of biomass per unit area means between material types (left) and feature sizes (right), inclusion of zero in an interval suggests a non-significant difference between the means.

Linear regression model relating the amount of harvested biomass to material type and feature size reveal that material type does not significantly impact the amount of attached biomass ($p = 0.9$), whereas the feature diameter does ($p = 0.002$). Conducting Tukey's HSD post hoc analysis on the obtained biomass data reveals that even though the pairs with the closest feature sizes within the range do not have significantly different amount of attached biomass per unit area, pairs with larger feature size differences do exhibit differences in adhered biomass (Figure 6.5b). The non-significance of material related effects might be tied into similarities between the substrate surface charge and surface energy of the tested polymeric materials (Table 6.1) but are in accordance with findings of Section 6.2.

6.4 Conclusions

The results from this chapter highlight the importance macroscale topographical features of the substrate on attached growth of filamentous algae *Stigeoclonium tenue*. In addition, it can be concluded that the variations in surface energy and zeta potential values of the tested polymeric substrates of PLA, PET-G and ABS were possibly not large enough to significantly impact the amount of attached biomass under the implemented experimental conditions. Overall, the findings suggest that addition of macroscale topographical features can be a solution for increasing the algae biomass cultivation amount without increasing the footprint area of the photobioreactor/cultivation environment. Even though the use of additive manufacturing for creating favorable substrates for attached algae cultivation might not be a viable solution for large scale biomass production, the concave hemispherical topographical features presented in this chapter can be recreated for industrial scale applications with other polymer manufacturing methods such as heat pressing. Additionally, the concave hemispheres sized within the tested diameter range do not create difficulties for mechanically harvesting algae which is another

important factor in determining the practicality of a substrate for being utilized in algae cultivation systems.

Chapter 7: Assessment of the effects of physicochemical surface properties of the substrate and algae on attachment favorability through XDLVO theory

The results of Sections 7.1 and 7.2 have been published in Auburn University Journal of Undergraduate Research in 2019 as part of the article titled "Understanding the Growth and Attachment of Algae on Nanocomposites" [131].

Ever since its introduction decades ago, the XDLVO thermodynamic model [92, 132] has been frequently used in studying bio-adhesion phenomena in aqueous media, and algal adhesion is no exception [80, 132-134]. Treating an algae cell as a colloidal entity and measuring the total interaction energy between an algal cell and the substrate surface (G^{TOT}) for predicting the favorability of adhesion has gained popularity in recent years [80, 89, 135, 136]. This is partially because of the vast application potentials of attached algal systems as a cost- and energy-efficient way of cultivating algae and the importance of understanding algae-substrate surface interactions and adhesion favorability in yield enhancement in these systems [5]. Moreover, understanding algae-substrate adhesion helps in reducing membrane fouling in harvesting algae in non-attached cultivation systems that require separation of algae from the liquid growth media. This can contribute to a more cost-efficient biomass production process [137, 138]. As discussed in Chapter 2, in the XDLVO modeling approach, the total interaction energy G^{TOT} as a function of distance (d) between the interacting bodies (Equation 7.1) is theorized to be a linear sum of Lifshitz-van der Waals (LW) interactions (G^{LW}), which originate from instantaneous asymmetrical distribution of electrons in molecules; electric double layer/electrostatic (EL) interactions (G^{EL}) from the electrostatic interactions between the cell and the substrate; and acid-base (AB) interactions (G^{AB}),

which originate from polar interactions in the aqueous media [92]. Negative G^{TOT} values indicate attraction, while positive values indicate repulsion between the interacting bodies.

$$G^{TOT}(d) = G^{LW}(d) + G^{AB}(d) + G^{EL}(d) \quad (7.1)$$

The XDLVO model measurements rely heavily on the knowledge of surface energy, and surface energy components and surface electric potential for the interacting entities/bodies/surfaces. A common approach for measuring the G^{LW} and G^{AB} interaction energy values is measurement of surface energy and surface energy components through contact angle measurements [139]. On the other hand, the G^{EL} interaction energy cannot be determined without the knowledge of the electric potential of the surfaces and requires an electrokinetic measurement of some type [117, 140]. Since electric potential of the surfaces cannot be measured directly, zeta potential (ζ) which is defined as the electric potential at the slipping plane is measured and used for estimation of the surface electric potential (ψ_0) [141].

Despite the existence of reliable methods of surface energy/contact angle and zeta potential measurement in the literature for many common and conventional surfaces and particles, there is a lack of either methodology or validated measurements for many biological entities such as algae. The existing methods and measurements have only been evaluated with a few algal species and are sometimes not suitable for some algal morphologies. In addition, interpretation of practical implications of the obtained interaction energy values in large-scale/real-life attached cultivation systems is yet challenging. In this chapter, the attachment of microalgae species, *Scenedesmus dimorphous* to PLA-based nanocomposite substrate surfaces containing CNC was modeled using the XDLVO approach. In addition, the obtained results were compared to experimental attachment studies conducted to determine the practical implications of the modeling approach. Additionally, the use of the XDLVO model for calculating the interaction energy of periphytic filamentous algae

with diverse cell morphologies was explored, focusing on *Stigeoclonium* as the target species. This required development and validation of methods for surface energy and zeta potential measurement for *Stigeoclonium.*

7.1 *Scenedesmus dimorphus* static attachment to PLA and PLA nanocomposites

7.1.1 Polylactic acid and polylactic acid nanocomposite preparation

Following a method by Kamal and Khoshkava [142], 8 wt % CNC/PLA nanocomposites were prepared using both sulfated and lignin-coated CNC. An aqueous suspension of sulfated cellulose nanocrystal (SA-CNC) containing (11.8 wt %/7.7 vol. %) CNC and 1.2 wt. % sulfur (specified by the manufacturer) U.S. Forest Products Laboratory (FPL) (Starkville, MS), supplied through University of Maine process development center were used to make a 3wt% CNC suspension. The dispersion was tip sonicated using a Vibra-Cell tip sonicator (Sonics & Materials, Newtown borough, CT) at 30% amplitude and 5:3 seconds on-off cycles. A regular commodity spray bottle was used to spray the dispersion into a constantly stirred liquid nitrogen bath. The resulting frozen droplets were collected, and freeze dried overnight at -45 ºC, under 0.01 mbar vacuum, using a FreeZone 4.5-liter freeze dry system (Labconco, Kansas City, MO). The freeze-dried powder was then mixed with ground PLA pellets (Ingeo grade resin, Nature works) to create an 8 wt% mixture of SA-CNC and PLA. The mixture was fed to a HAAKE MiniLab twin-screw compounder (Thermo Fisher Scientific, Waltham, MA) and continuously mixed at 190 ºC and 60 rpm for 20 mins and ultimately extruded into 1.7 mm filaments. The same procedure was followed to make the 8wt% lignin coated CNC (L-CNC) and PLA nanocomposite except for substituting the SA-CNC with L-CNC (Bioplus, Altamonte Springs, Florida) and eliminating the tip sonication step to avoid separation of the lignin coating from the CNC. PLA pellets, L-CNC/ PLA and SA-CNC/PLA filaments were then heat pressed using an electric tortilla press (Brentwood Appliances,

Los Angeles, CA) to make thin plastic films. The thickness of the pressed films varied between 0.2-0.9 mm.

7.1.2 Attachment test

An attachment test was developed based on modifications to a procedure used for a similar purpose in the literature [36]. A 1000 mL beaker was filled with a 750 mL algae solution with a concentration of 100,000 cells/mL (determined through absorbance). The composites and PLA were cut into 1.5 cm x 1.5 cm samples. These samples were each placed in the bottom of the 1000 mL algae-solution beaker, using one sample per type. The beaker was then placed in a dark environment for 24 hr to allow the cells to settle on the surface. The samples were then removed from the beaker and washed on an orbital mixer to remove any unattached or loosely attached cells. The test was repeated for four times. Each composite was analyzed by taking 10 images across the sample using reflected microscopy and a 20X/0.45 LU Plan Fluor objective using a Nikon Eclipse 80i microscope (Melville, NY) with Nikon Elements software. The number of algae cells in each 85,300 μm^2 image were counted; a total of 40 images per substrate were acquired for analysis (except for PLA/L-CNC for which 29 images were analyzed).

7.1.3 Chemical Surface characteristics of PLA and PLA nanocomposites

Figure 7.1 shows an image of the pressed samples. Table 7.1 summarizes the results obtained from contact angle and zeta potential measurements. Lignin is known to be hydrophobic [143], and addition of lignin-coated CNC to PLA not only has the potential of increasing dispersion and compatibility of CNC with PLA [144], but also has the potential of making the composite more hydrophobic. The increase in the contact angle value of water with PLA/L-CNC substrate in comparison to plain PLA substrates validates this point (Table 7.1). On the other hand, sulfonated

CNC is known to be hydrophilic, and it is expected to increase the hydrophilia of the associated composite.

Based on the results, the surface energy components of PLA were not dramatically impacted by addition of CNC (Table 7.1). However, addition of CNC to PLA did impact the surface zeta potential (Table 7.1). Zeta potential is a measure of electrostatic potential at or very near to the beginning of the diffuse double layer created by surface charges at the solid/liquid interface in a polar medium and can be used for making interpretations about charge and adsorption characteristics of a surface [113, 114]. The obtained zeta potential values were also used for determination of G^{EL} values. The sessile drop contact angle of three probe fluids (water, ethylene glycol, and hexadecane) was measured on the prepared films using a goniometer (ramé-hart Instrument Co., Mountain Lakes, New Jersey) using equations provided in Section 3.2.2. The contact angle values were measured every 10 seconds over the course of first 30 seconds of contact and averaged to result a single value. The measurements were repeated 10 times. The obtained contact angle values were later used to calculate the surface energy and surfaced energy components of the PLA and PLA nanocomposite films.

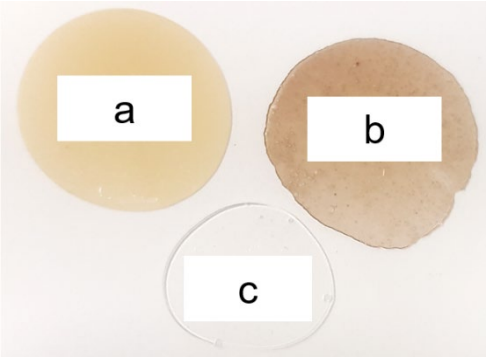
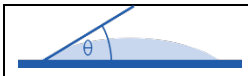


Figure 7.1 Heat pressed substrates: (a) PLA/SA-CNC (b) PLA/L-CNC (c) PLA

Table 7.1 Summary of measured physicochemical properties for PLA and PLA nanocomposite substrates.

	**Contact angle ◦**			ζ	γ^{LW}	γ-	γ+	γ
Material	Water	Ethylene Glycol	Hexadecane	(mV)		(mJ/m^2)		
PLA	78° ± 1°	56° ± 1°	19° ±1°	-45	26.1	9.3	0.7	31.2
PLA/SA-CNC	78° ± 1°	53° ± 1°	17° ± 1°	-51	26.2	8.7	1.1	32.4
PLA/L-CNC	82° ± 1°	59° ± 2°	22° ± 2°	-54	25.5	6.7	0.8	30.1

(Zeta potential (ζ**), **total** surface energy (**γ**), surface energy components:* Lifshitz-van der Waal *(γ^{LW}), electron acceptor (γ-) and electron acceptor (γ-))*

Thermogravimetric analysis of PLA and PLA nanocomposites was performed to investigate the effect of the nanomaterial and processing on PLA properties (Figure 7.2). Since the PLA was not extruded in an inert environment it was expected to undergo some hydrolytic degradation. Typically, however, the incorporation of a nanomaterial stabilizes the polymer chains resulting in an increase in the decomposition temperature T_d. Table 7.2 shows the decomposition temperature (T_d) of samples (at the 5% loss of total sample weight) obtained from heating the samples in an inert medium (N_2 gas). Both nanocomposites had lower T_d than equivalently processed PLA. Exploring the reasons for this has not been a priority, but it is likely due to the surface chemistry of the CNC catalyzing degradation.

Table 7.2 Decomposition temperature of PLA and PLA composites.

Material	T_d(°C) (5% weight loss)
PLA	329
PLA/SA-CNC	279
PLA/L-CNC	315

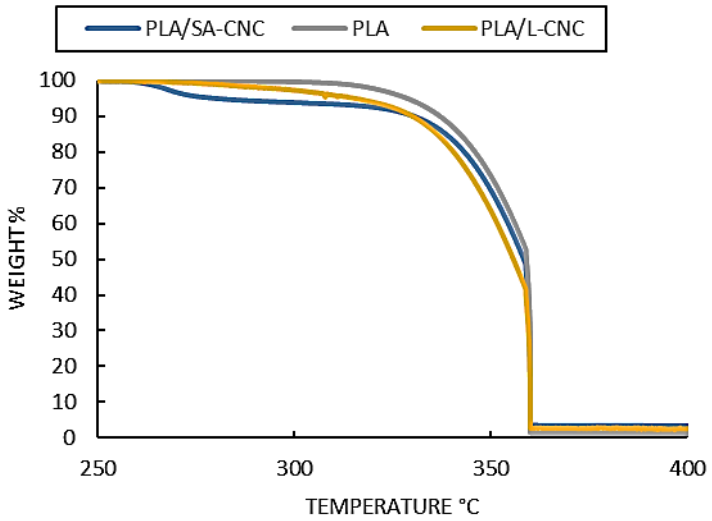


Figure 7.2 Results obtained from thermogravimetric analysis (TGA) on the substrates in N_2.

7.2 XDLVO modeling of *Scenedesmus dimorphus* attachment to PLA and PLA composites

The use of XDLVO approach describes the interaction energy after transport of algae cells near the substrate and within the interaction range of the considered forces. In this approach the interaction energy components of G^{EL}, G^{AB}, G^{LW} should be determined and measured separately and added together to find the G^{TOT} value. One important step in this process is to choose a geometrical configuration that accurately represents the interacting entities. Treating the substrate surface as a semi-infinite plate with a thickness much larger than the separation distance and the microorganism (algae cells in the case of interest subject of this work) as a sphere is a common

practice in the field [80, 89]. Assuming the algae cells to be a sphere, following equations can be used for finding the G^{TOT} value between a cell and the substrate [139]:

$$G^{LW}(d) = -\frac{A}{6}\left[\frac{r}{d} + \frac{r}{d+2r} + \ln\left(\frac{r}{d+2r}\right)\right] \quad (7.2)$$

$$G^{AB}(d) = 2\pi r\lambda\Delta G_{adh}^{AB} \exp[(d_0 - d)/\lambda] \quad (7.3)$$

$$G^{EL}(d) = \pi\varepsilon r(\psi_m^2 + \psi_s^2)[\frac{2\psi_m\psi_s}{\psi_m^2+\psi_s^2}\ln\frac{1+e^{-\kappa d}}{1-e^{-\kappa d}} + \ln(1 - e^{-2\kappa d})] \quad (7.4)$$

In Equation (7.2), d is distance between the interacting bodies, r is the diameter of algae cell, and A is the Hamaker constant. In Equation (7.3), d0 represents the minimum distance between the two entities, λ is the gyration radius of water molecules in a solution. In Equation (7.4), subscripts m and s stand for microbe (algae) and substrate, respectively. ε is the permittivity of the medium, ψm and ψs are the surface potential of algal cells and substrate respectively, and κ −1 is the double layer thickness.

The surface energy components and zeta potential values of algae surface are required for measurement of total interaction energy between the substrate and algae. These values are readily available for *Scenedesmus dimorphus* in the literature [118], and alongside obtained zeta potential and surface energy components for PLA and PLA composites, they can be used for calculating the total interaction energy (refer to Appendix 1 for details). Figure 7.3 shows the total energy values as a function of distance between the algal cell and substrate. Generally, a negative G^{TOT} value indicates attraction, and a positive value indicates repulsion. Although, none of the modeled interactions exhibited a negative primary minimum (attractive forces overcoming the repulsive forces at short distances), all three samples had a negative secondary minimum value after the initial energy barrier, suggesting the dominance of attractive forces in longer distances and favorability of attachment. The difference between the G^{TOT} value of the three tested materials are not drastic. This was not surprising since the formulations had more similar surface energy

components and the zeta potentials than initially expected; the greatest difference was observed between the values obtained for PLA/L-CNC and the two other material (PLA and PLA/SA-CNC). Increasing the content of hydrophobic materials such as lignin or hydrophilic materials such as CNC-SA or the incorporation of other hydrophilic polymers would enable a wider range of surface energies and zeta potentials and greater differences in G^{TOT}.

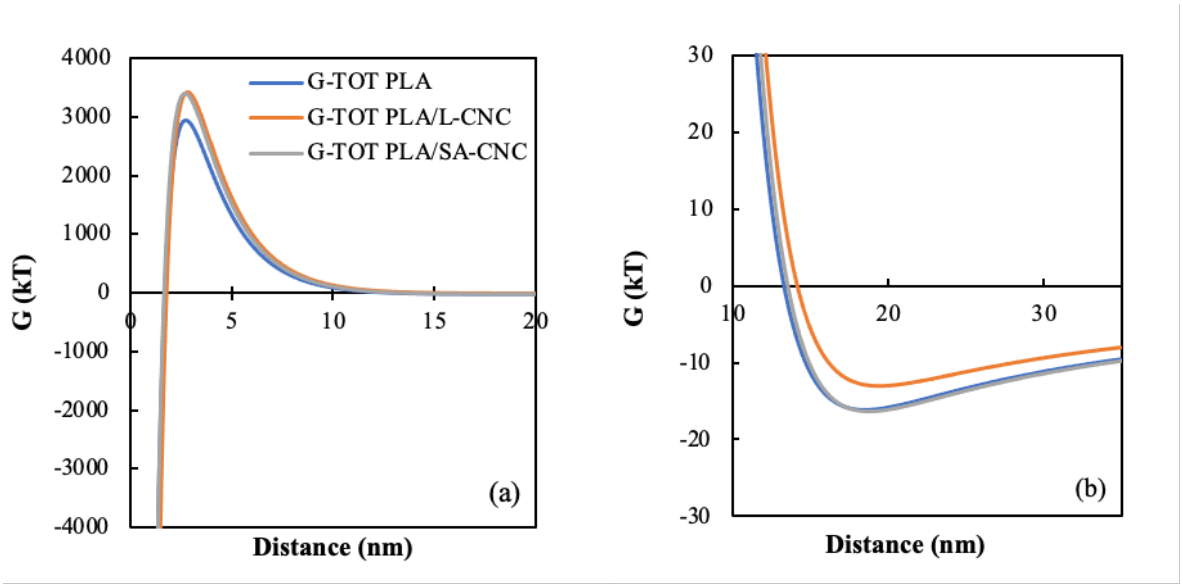


Figure 7.3 (a) Total interaction energy of PLA and PLA based composites as a function of distance from the substrate surface; (b) Secondary energy minimum.

7.2.1 *Scenedesmus dimorphus* attachment to PLA and PLA composites in flask

The following two graphs summarize the results obtained from the static attachment experiments. As depicted in Figure 7.4 (a), the average number of cells attached to the substrates was influenced by the substrate material, with PLA having the highest number of cells attached. Due to the high error values, Fisher's statistical significance test was conducted for sample pairs. The results suggest that the differences observed in the number of cells attached to the PLA substrate is significantly different from the composites with the larger difference being observed between PLA and PLA/L-CNC composite. This is in agreement with the model results, which also

suggested that PLA was more favorable for attachment than PLA/L-CNC. However, the model predicted only negligible difference between the PLA and PLA/SA-CNC. This discrepancy may be due to the significant error in the measurements, suggested by the broad error bars. One source of error was that the color and opacity of the composites made it difficult to visualize the algal cells, therefore inducing errors to the counting process.

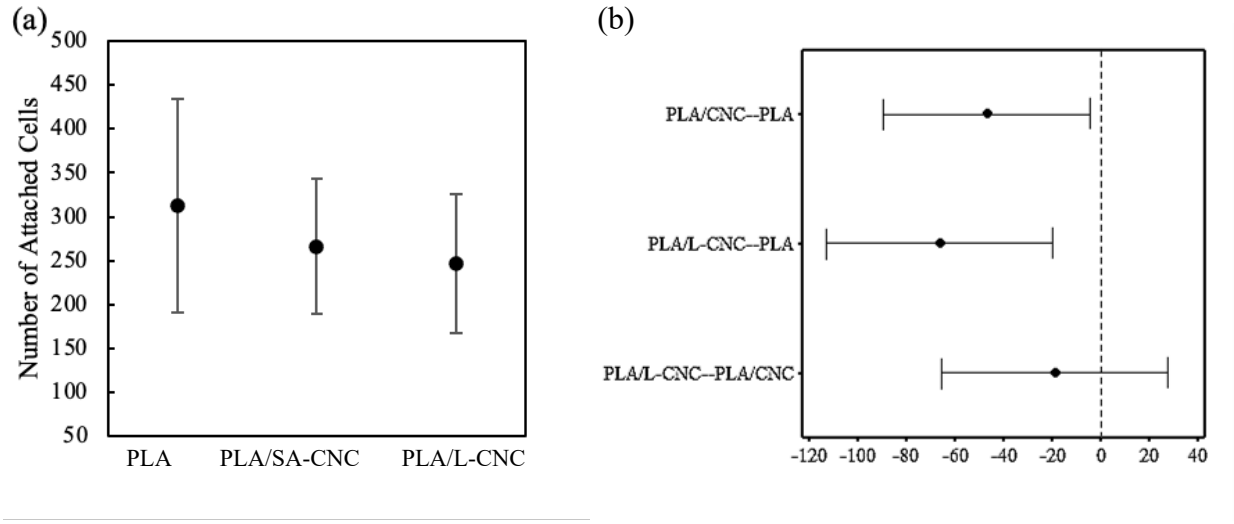


Figure 7.4 (a) Number of algae cells per image for composites; error bars represent standard deviation (b) Fisher's post hoc analysis for pairwise comparison of means (number of attached cells) between material types (intervals not containing zero, indicate significantly different means).

7.3 XDLVO interaction energy modeling for periphytic filamentous algae

Stigeoclonium is a filamentous green alga that is often found growing attached to other surfaces in periphytic algal communities. Algae of *Stigeoclonium* genus has been frequently reported to be present in many periphyton-based attached algae water remediation systems. In addition, *Stigeoclonium* has potential as a source of valuable bio-compounds. Yet not much is known about interaction of this algae with different substrate surfaces asides from its preference in its natural habitats for being epiphytic [145]. Understanding the interaction of this algae with

the substrate surface may enable designing substrate surfaces optimized for prosperous cultivation of this algae. Currently, yield consistency and efficiency of many algae-based process and products suffer from the lack long-term control over the cultivated species. Contamination with unwanted algae is a problem that hurdles downstream processing of algal biomass and efficiency of algal cultivation systems, especially open systems. Hence in this work, the physicochemical surface properties of *Stigeoclonium* required for predicting attachment substrate surface preferences of this algae using the XDLVO theory were measured and utilized in the modeling. Surface energy and its components were measured via sessile contact angle measurement on the algae film. In addition, streaming potential measurement was explored as a suitable method for finding the surface zeta potential of this filamentous algae.

7.3.1 Contact angle measurement on *Stigeoclonium tenue*

A method by Busscher et.al [146] for preparation of bacterial films for contact angle measurement, later adapted to algae films/mats [88, 118] was followed. Washed *Stigeoclonium tenue* cells were collected on a 0.45 micron Whatman cellulose acetate membrane via vacuum filtration. The collected algae mat was allowed to air dry for 1.5 hr to eliminate the excess water. The filter was then fixed on a glass slide using double-sided tape. Glycerol (purity ≥ 99.0%, VWR Life sciences), ethylene glycol (purity ≥ 99.0%,VWR Life sciences) and diiodomethane (purity ≥ 99.0%,VWR Life sciences 99%, stabilized, Thermo Scientific) were used as probe liquids for contact angle measurement on the algae mat. The sessile contact angles were measured using a Data Physics OCA50 optical contact angle goniometer with 2-5 μL droplets.

7.3.2 Streaming potential measurement

Streaming potentials were measured over the range of 200 to 400 mbar using an Anton Paar SurPASS 3 Eco electrokinetic analyzer using Ag/AgCl electrodes. The algae samples were

prepared by being washed three times over a stainless-steel tea strainer with Type I water. Additionally, the algae on the filter were collected and resuspended in the electrolyte (0.01 M KCl or BBM depending on the experiment) and left for 15 minutes to accustom the cells to the electrolyte. The algae were then filtered through 300 micron woven mesh nylon filter (Spectrum Laboratories) to separate longer filaments and larger pieces to be loaded onto the SurPASS sample holder. One final step of filtration was performed by resuspending the algae in the electrolyte and filtering through a Whatman CFP1 cellulose filter. The filtrated algae cells/filaments were then loaded onto the instrument's 10 mm cylindrical granular sample holder. This sample holder sandwiches the sample particles/fibers between two 25 micron nylon woven mesh filters to create a porous environment for electrolyte passage while preventing sample leakage to the electrolyte (Figure 7.5). The loaded algae sample was left soaking in the electrolyte for five minutes and rinsed with the electrolyte after loading onto the instrument for assuring equilibria between the sample and the electrolyte. The pH of the electrolyte was adjusted before each measurement cycle by addition of either 0.05 M NaOH or 0.05 M HCl solution depending on the target pH value. The permeability index associated with each measurement cycle was monitored closely to meet the requirement of the instrument (permeability index of 100 ± 10) for adequate passage of electrolyte through the sample and accurate zeta potential measurement.

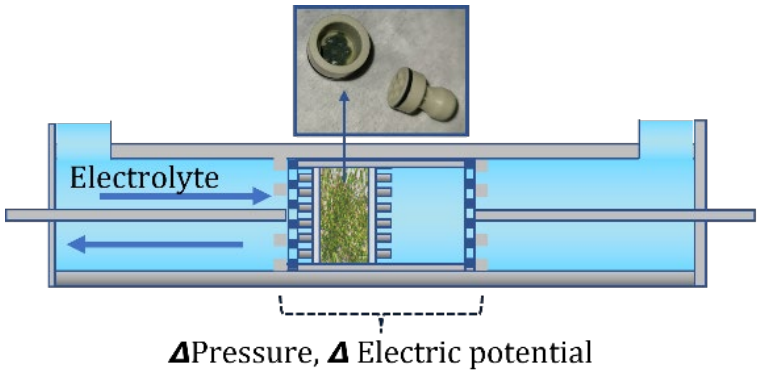


Figure 7.5 Algae sample configuration for streaming zeta potetnial measurment.

7.3.3 XDLVO modification

Accurate modeling of the algae substrate interaction requires the knowledge of the cell morphology at the substrate interface during the early stages. The effectiveness of the model in predicting algae's substrate references is expected to be impacted by the precision of the model. The species of interest in this study, *Stigeoclonium tenue* is a branched filamentous green alga. Despite lacking some structural complexities of land plants, *Stigeoclonium* cells change in shape and size during their life cycle and not all the cells are morphologically the same [69, 147]. In natural environments and attached systems, filamentous algae like *Stigeclonium* are rarely found as a singular cell and they are usually found as entangled chains of filaments. Additionally, different cells of these algae can look vastly different from one another depending on factors such as life cycle stage and proximity to the substrate surface. Therefore, it is necessary to choose a geometrical configuration that represents these algae accurately. Additionally, including the morphological complexities of these algae can potentially improve the accuracy of the model in predicting the adhesion behavior. In case of large, entangled pieces of algae, the plate-to-plate geometrical configuration might be a better representation for *Stigeoclonium tenue* cells in comparison to modeling the adhesion for a single spherical cell with a semi-infinite plate.

For two semi-infinite plates interacting with one another, interaction energy components per interacting area can be measured as provided by the following equations [92, 140, 148]:

$$G^{LW}(d) = -\frac{A}{12\pi(d)^2} \tag{7.5}$$

$$G^{AB}(d) = \Delta G_{adh}^{AB} \exp[(d_0 - d)/\lambda] \tag{7.6}$$

$$G^{EL}(d) = \varepsilon\kappa\left[\left(\frac{\psi_m+\psi_s}{2}\right)^2\left\{1-\tanh\left(\frac{\kappa d}{2}\right)\right\} - \left(\frac{\psi_m-\psi_s}{2}\right)^2\left\{\coth\left(\frac{\kappa d}{2}\right)-1\right\}\right] \tag{7.7}$$

Where d is distance between the interacting bodies, A is the Hamaker constant and λ is the gyration radius of water molecules in a solution. Subscripts *m* and *s* stand for microbe (algae) and substrate respectively. ε is the permittivity of the medium, ψ_m and ψ_s are the surface potential of algal cells and substrate respectively, and κ^{-1} is the double layer thickness (see Appendix 1 for further details).

7.3.4 Results and discussion

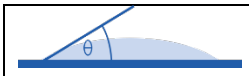
Contact angle measurement on the filtered *Stigeoclonium tenue* films with the three select probe liquids of glycerol, ethylene glycol and diiodomethane resulted are shown in Table 7.3 (at least 6 measurements were performed on three individually prepared algae lawns per probe liquid and the standard deviation of the measurement is provided in the table). The measured contact angles and known literature values of energy components for each liquid [92] were plugged into the modified Young's equation (Equation (7.8)) to determine the surface energy and surface energy components for the algae (refer to Section 3.2.2 1 for further details).

$$(1+\cos\theta)\gamma_l = 2\left(\sqrt{\gamma_s^{LW}\gamma_l^{LW}} + \sqrt{\gamma_s^{+}\gamma_l^{-}} + \sqrt{\gamma_s^{-}\gamma_l^{+}}\right) \tag{7.8}$$

Plugging in the measured contact angle values and using liquid surface energy components available in the literature for the probe liquids used, results in negative square root values for the acid-base energy components of the algae surface energy which have been previously reported in the literature for both abiotic and biotic surfaces including algae surfaces [118]. There are extensive arguments around interpretation of the negative square root values in the literature. Proposed reasons for negative square root values include lack of accuracy of contact angle measurements on the biofilms, lack of accuracy in the reported probe liquid surface energy component values, the mathematical method used for solving Equation (7.8) for multiple liquids or even general flaws of the acid-base approach in defining surface energy components and

estimating them [118, 149, 150]. Nonetheless a common interpretation and treatment of the negative values in the literature is assigning them zero values [118, 150, 151] . In this research, the same approach was followed for obtaining acid-base components of the algae surface energy.

Table 7.3 Contact angle and surface energy components of *Stigeoclonium tenue*. Eight contact angle measurements per liquid were carried on three separately prepared films.

	**Contact angle** ◦			γ^{LW}	γ-	γ+	γ
Material	Glycerol	Ethylene Glycol	Diiodomethane	(mJ/m^2)			
Filtered *Stigeoclonium tenue* film	89±3	60±6	52±4	33	0	0	33

(Zeta potential (ζ**), **total** surface energy (**γ**), surface energy components:* Lifshitz-van der Waal *(γ^{LW}), electron acceptor (γ-) and electron acceptor (γ-))*

Even though most of the zeta potential values reported for the algae in the literature are measured by determining the electrophoretic mobility of algae cells [118], the use of conventional electrophoretic light scattering (ELS) based instruments for measurement of zeta potential is difficult for *Stigeoclonium tenue*. This is mainly because most of these instruments are designed and calibrated for measuring the zeta potential by finding the electrophoretic mobility particles for particles smaller than 100 microns. Theoretically, the electrophoretic mobility of a particle is not affected by its size or shape, but there are some conditions under which the shape and size might influence the mobility. That is when the particle size is significantly larger than the diffuse ionic double layer thickness (determined by the Debye length) [117] . Even though individual cells of *Stigeoclonium tenue* are smaller than 100 microns in all their dimensions, because of the filamentous nature of this algae, cells are connected to one another and form thin chain-like

structures that can grow up to centimeters long. Filamentous algae usually end up getting entangled because of aeration during initial inoculation in the flask, and in washing, handling, filtering, etc. Additionally, the branched morphology of *Stigeoclonium tenue* contributes to the entanglement issues. Therefore, it is very difficult to have a significant concentration of singular cells of *Stigeoclonium tenue* and many other filamentous algae like it without complex and labor-intensive procedures such as multistep cutting and filtration of the algae, which causes stress on the cells or controlled inoculation to produce germinating spores. On the other hand, filamentous algae are the dominating species found in some of the most effective attached algae water remediation systems designed to date. Therefore, it is valuable to explore alternative methods of measuring zeta potential for these algae. Upon some preliminary experimentation for obtaining the zeta potential of *Stigeoclonium tenue* using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., GB) through ELS, the author faced repeated issues with algae sedimentation in the measurement cell and noise despite the effort to cut the *Stigeoclonium tenue* cells into bits and piece within the viable measurement range of the instrument (100 microns).

Flow of an aqueous solution over a charged solid surface generates an electrokinetic response that can be measured as the streaming potential or streaming current. Ultimately, the streaming potential or streaming current can be used for measuring the zeta potential values using the Helmholtz-Smoluchowski (HS) equations [152]. Streaming potential measurements through HS approach are widely used for solid planar samples and rely on the exact knowledge of the geometry and dimensions of the electrolyte streaming pathway. However, the streaming potential measurements can also be used for irregularly shaped samples with unknown cell constants and dimensional values such as fibers and powders using the Fairbrother and Mastin approximation of the HS Equations (7.9) [152].

$$\xi = \frac{dU_{str}}{d\Delta p} \times \frac{\eta}{\varepsilon \times \varepsilon_0} \times \kappa_B \quad (7.9)$$

Where, U_{str} represents streaming potential, p represents pressure, η is the dynamic viscosity of the electrolyte, κ_B is the bulk electrolyte conductivity and ε and ε_0 are relative electric permittivity of the electrolyte solution and the electric permittivity of vacuum, respectively. There are a scarce number of studies which report zeta potential measurement on living cells, through measurement of the streaming potential [153].

As a result of cell growth and entanglement, many filamentous algae appear like thin fibrous material in aqueous media and are visible to the naked eye. In our proposed methodology (Section 7.3.2) the streaming potential of *Stigeoclonium tenue* was measured by loading the filtered algae onto the SurPASS Eco 3 cylindrical sample cell, sandwiched between the two inert 25 microns pore size filters. The pre-filtration of algae for selection of larger filaments ensures that algae will not leak into the electrolyte. In addition, the conductivity of the electrolyte was monitored for changes due to contamination. As a result, there was no apparent evidence of algae leakage into the electrolyte during the zeta potential measurement cycles that would significantly throw off the measuring variables. Additionally, microscopy was performed on the electrolyte at the end of measurement cycles for monitoring algae cell leaks. Figure 7.6 shows the result of measurement of zeta potential of *Stigeoclonium tenue* using both standard KCl (ionic strength = 0.01 M) and BBM growth media (ionic strength = 0.009M) in a range of pHs often observed during *Stigeoclonium tenue* cultivation in BBM (each zeta potential measurement was performed three times and the presented results were obtained by testing at least seven and three individual algae sample loading for the BBM and KCl electrolytes, respectively).

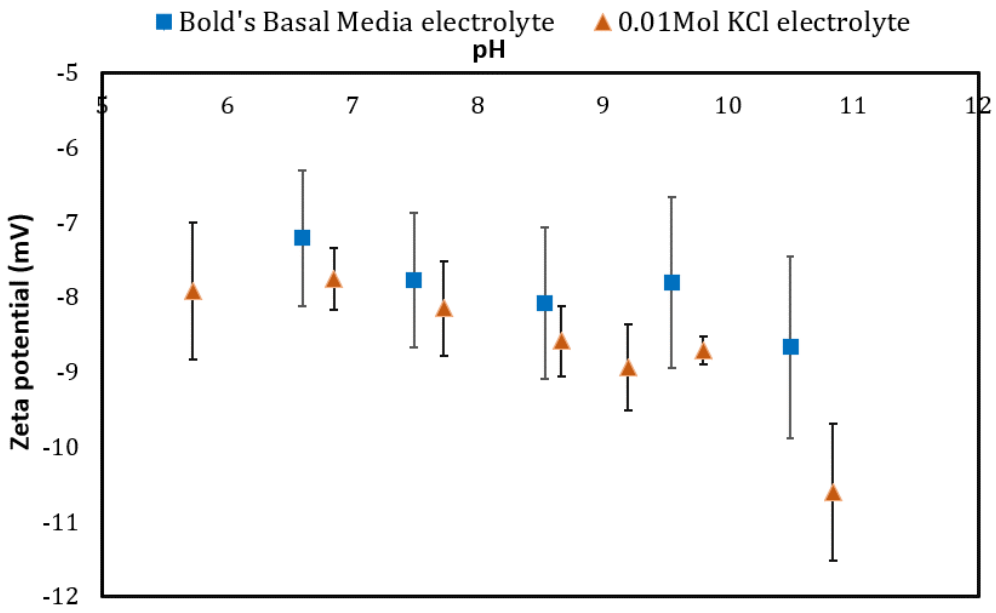


Figure 7.6 Streaming zeta potenial values of algae *Stigeoclonium tenue.*

As shown in Figure 7.6 the measured zeta potential of *Stigeoclonium* using the standard KCl electrolyte does not change dramatically (less than 1 mV decrease with pH increase of 4 units) over the tested pH range and is also similar to the obtained value for the BBM electrolyte. The BBM standard growth medium is a multi-salt buffer. The presence of multivalent ions such as of Ca^{+2}, Mg^{+2} in the BBM media can create a complex ionic structure around the tested surface and potentially interfere with the zeta potential reading. Therefore, the measured zeta potential with BBM electrolyte should be considered as the apparent zeta potential. However, the measured zeta potential values were not vastly different from the standard electrolyte in the tested pH range.

In order to measure the XDLVO interaction energy values for *Stigeoclonium tenue* and some common surfaces used for attached cultivation, there is a need for estimation of the cell dimensions. As discussed, *Stigeoclonium tenue* is a branched filamentous alga with cells connected to each other to form long chains. Moreover, different cells of this algae look morphologically different from one another and have different sizes (Figure 7.7).

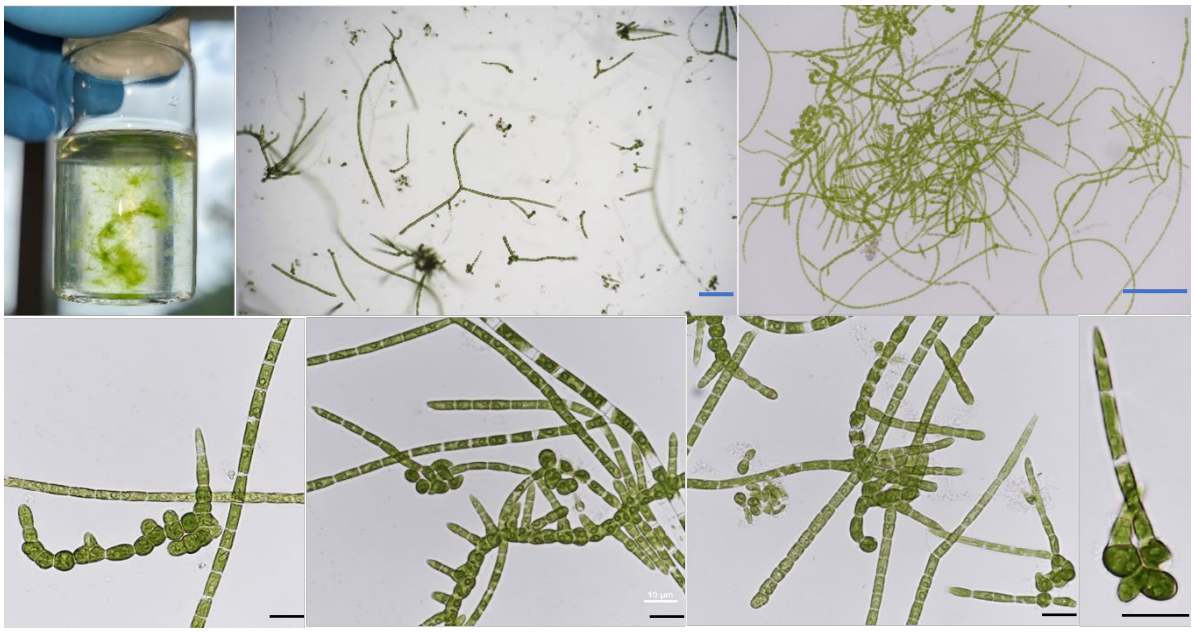


Figure 7.7 *Stigeoclonium tenue* from macro to micro scale. The black and blue bars represent 10 and 100 microns, respectively.

Filament cells are mostly cylindrical, with acute apical cells; prostate cells on the other hand are round and form an axis for outward growth of filaments. Depending on the dominant reproduction mode of the culture there may be some additional cell types present such as zoospores. Therefore, estimation of equivalent diameter as previously done by Ozkan et al. (2013) for some filamentous cyanobacteria, ellipsoid diatoms, and green algae may not be the most accurate geometrical representation for *Stigeoclonium*. According to phycological references, *Stigeoclonium* attaches to substrate surfaces from the prostrate cells and branch out [64, 69, 147, 154]. Therefore, the diameter of the prostrate cells might be a better estimation of the dimension of the algae cells for the purpose of XDLVO model. Measurement of the diameter of 15 prostate cells resulted in an average of 4.3 ± 0.5 microns.

Using the obtained streaming zeta potential values and surface energy components for *Stigeoclonium tenue* (Figure 7.6 and Table 7.3), the interaction energy of PLA and *Stigeoclonium*

were modeled in an aqueous media with ionic strength and pH values similar to the BBM algae growth media 0.009M and 6.8 respectively. The PLA substrate properties used in the modeling were based on the surface characterization results reported in Table 6.1 (see Chapter 6). Both plate-plate and sphere-plate configurations were used in modeling. As shown in the Figure 7.8, adhesion of on single *Stigeoclonium tenue* to the PLA substrate is not thermodynamically favorable at separation distances smaller than 24 nm as indicated by the positive total interaction energy. G^{tot} eventually shows negative values in distances between 24 and 35 nm indicating favorability of loose attachment. At separation distances associated with the secondary minimum the acid-base interactions have already decayed, yet electrostatic (repulsive) and van der Waals forces (attractive) influence the magnitude and placement of the secondary minimum more prominently.

In the case of plate-plate configuration and modeling the interaction between the PLA substate surface and a 400 μ^2 plate of *Stigeoclonium tenue*, an overall trend similar to sphere-plate configuration was observed (Figure 7.9) with a secondary minimum at larger separation distances.

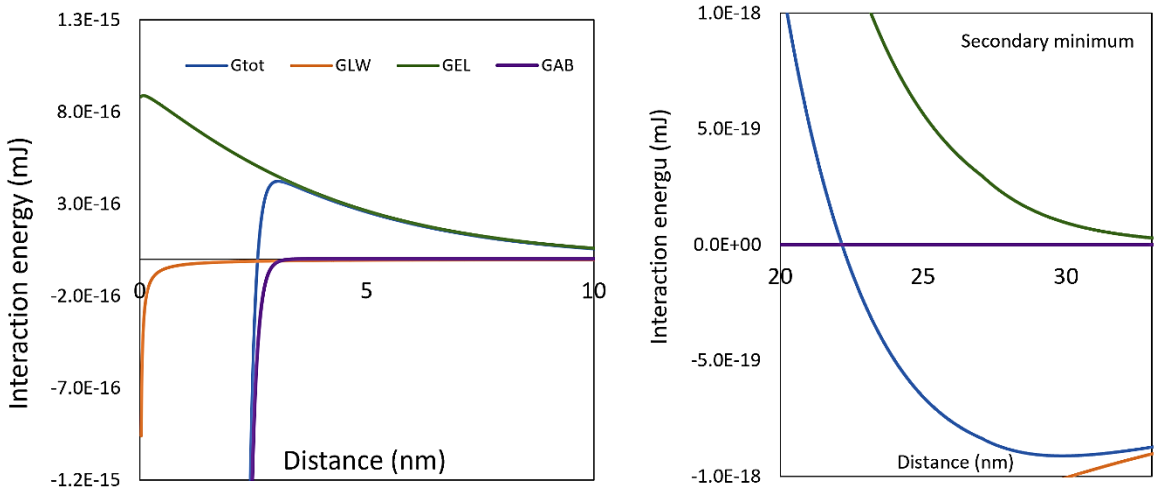


Figure 7.8 Interaction energy between a single *Stigeoclonium tenue* prostate cell and PLA substrate as a function of separation based on XDLVO model (sphere-plate geometrical configuration).

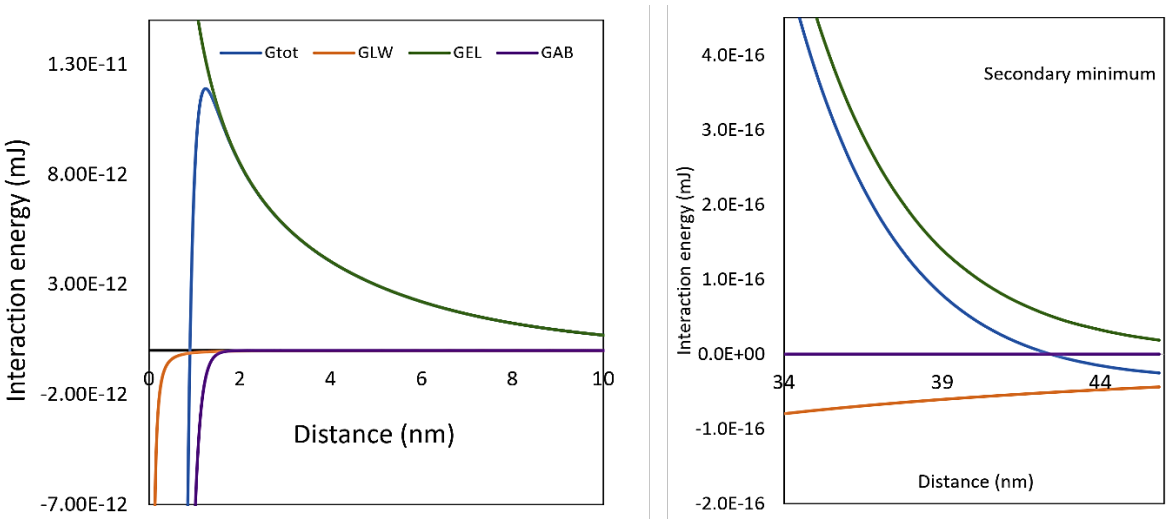


Figure 7.9 Interaction energy between a 400 µm² plate-like group of *Stigeoclonium tenue* cells and PLA substrate as a function of separation based on XDLVO model (plate-plate geometrical configuration)

7.5 Conclusions

A novel method was developed for zeta potential measurement of filamentous algae based on streaming potential measurements. This method was used for zeta potential measurement on *Stigeoclonium tenue*. This was the first time that surface zeta potential of filamentous algae cells was measured through streaming potential values. Additionally surface energy of *Stigeoclonium tenue* was measured by modifying the existing methods. The obtained zeta potential and surface energy values enabled modeling the algae substrate interaction for *Stigeoclonium tenue*. The XDLVO modeling of microalgae *Scenedesmus* and filamentous algae *Stigeoclonium* both highlighted the importance of accurate measurement of physicochemical characteristics of the interacting bodies in accurate modeling. Moreover, the results lay the foundations for substrate energy components and charge optimization for targeted adhesion of algae tested. Due to negative substrate and algae surface charges electrostatic interactions were always repulsive. The acid-base component of the interaction even though attractive decayed at smaller separation distances than

the electrostatic and Lifshitz van der Waals (LW) components which is expected given the testing conditions [117]. There is an opportunity to optimize the acid-base component of the substrate to enhance the attraction at shorter separation distances. Combined with the Lifshitz van der Waals component, this can lead to a primary energy minimum of the total interaction energy which is an indicator of a thermodynamically favorable interaction. Comparing the thermodynamic modeling results to the observations in Chapter 5 and 6 on successful attached cultivation of *Stigeoclonium* to PLA substrate surfaces reveals the complicated nature of the adhesion and necessity of further investigations of the adhesion behavior. In the photobioreactor system a combination of factors such as hydrodynamic characteristics of the flow and changes of algae cell properties overtime can impact the adhesion that is when the thermodynamic modeling captures the adhesion only at initial contact of algae with the substrate. Therefore, it will be valuable to conduct future experiments comparing attachment substrates selected based on the modeled XDLVO interactions to growth on PLA.

Chapter 8: STEM education outreach

This chapter is written based on a proceeding by Karimi et al. titled "Water Quality: Adaptable Modules for Engaging K-16 Students" submitted to the Frontiers in Education (FIE) conference in April 2022.

The author has been a part of several education outreach activities and research during her doctoral education at Auburn. Her activities ranged from training teachers, analysis of grant-related data, and designing and exhibiting STEM activities for K-12 students and college freshmen. Given the main research focus of the author and her interest in the sustainable water remediation process, access to clean water and water quality assessment-related educational activities became the focus of her education outreach work. This chapter describes a group of water quality activities that the author has been involved in developing, adapting, and executing of them for at open houses for K-12 students and their families, summer camps, and freshman engineering students. The required time for the activities ranged from 5 minutes for open houses to 3 hours for summer camps and 5 hours for an Auburn University freshman Introduction to Engineering Course. Similarly, the activities ranged from completely qualitative to requiring the use of spreadsheets and basic statistical methods. All these activities garnered student interest, and the longer activities were associated with increased knowledge of and interest in engineering.

8.1 Introduction

According to goal congruity theory (GCT), students' interest in a career field can be enhanced when the perceive that their values and the values associated with that profession are aligned. GenZ students, underrepresented minorities, women, first-generation college students,

and students from rural areas all tend to value helping others. As a result, they may not identify with fields that they perceive as being focused on achieving high income, social status, and having a low societal impact. Over the last decade, there have been numerous efforts by the National Academy of Engineers (NAE) and other organizations to highlight the role that engineers have in improving quality of life, social justice, and solving local and global challenges. These efforts include identifying 14 Grand Challenges for Engineering and establishing a Grand Challenge Scholars Program to facilitate undergraduates developing the skills needed to address these challenges [155]. In spite of these efforts, many parents, teachers, and students perceive engineering as a career void of collaboration and best suited for people who are driven by material values often at the expense of the well-being of others and the planet. Activities that connect engineering concepts and careers to societal needs such as medicine, clean energy, carbon dioxide sequestration, infrastructure improvements, and clean water can highlight that "engineers make a world of difference," and "engineering is essential to our health, happiness, and safety" [156]. Short experiences at festivals and STEM open houses as well as more in-depth interventions at summer camps and in courses can increase student and public awareness that engineering is an altruistic, pro-social profession, that plays a key role in social justice issues such as unequal access to resources.

Amongst all the societal challenges, the need for clean water is perhaps the one that most people can easily relate to since everyone needs to drink water. Water is abundant on earth, but the distribution of water and access to clean water is vastly different from region to region. Regardless of where one lives, sustainable access to safe and clean water is a perpetual global challenge. Nearly everyone has experienced a time when they have been inconvenienced by not having readily available clean or drinkable water. This could be something as simple as

accidentally swallowing saltwater at the beach. A significant proportion of the population has been affected by times of being told to conserve water due to droughts or boil water because of municipal water contamination incidents. Even those who have not been directly affected may have heard of news stories related to these topics such as the lead contamination crisis in Flint Michigan [157]. There is also significant awareness of how communities have been affected by water contamination with lead, arsenic, per- and polyfluorinated substances (PFAS), and other contaminants. Therefore, people from early childhood through adulthood can relate to activities related to the need for access to clean water.

As of the year 2017, about 2.2 billion people did not have safely managed drinking water service [158]. That is when the human right to clean water and sanitation was recognized by United Nations (UN) in 2010 and the new UN sustainable development goals aim for affordable access to clean and safe water for all by 2030 [159]. At the same time water access problem is not exclusive to regions without well-established water service systems. Due to active growth in the population, introduction of pollution to water sources and aging of water related infrastructure, sustainable access to clean water remains to be an ongoing issue even in regions with well-established water distribution and treatment systems [160]. In United States, the 1972 Clean Water Act set goals and guidelines for fighting pollution and restoring water quality in the nation by imposing effluent discharge limitations and standards for water quality protection [161]. Even though United states is a relatively water abundant country it is anticipated that U.S. public drinking water supplies will face challenges related to public water infrastructure, global climate effects, waterborne disease (including emerging and resurging pathogens), land use pressure, contamination and depletion of groundwater aquifers, surface water contaminations and necessity of regulation updates [162].

8.2 Short activities

The simplest activity focused on the concept of size-based filtration with natural materials using only visual observation to assess changes in water quality. The core concept is that size-based filtration relies on physical removal of suspended contaminants by porous media. Pollutant particles have different sizes, and they can be eliminated from the water by passing through a media with adequately small pores to prevent their passage. In this activity, participants are asked which group of materials would clarify a container of muddy water containing debris such as crushed leaves and why. This tabletop activity has been used at open houses for students, teachers, and families with attendees ranging from 20 participants to over one thousand participants. The activity is best suited for smaller group sizes due to the need for fresh filtration media for each participant. The set-up requires preparing a dirt water mixture using either potting soil or natural dirt. Crushed leaves or other small objects may be added as well. For safety, it is advisable to add some bleach to the water and have participants use hand sanitizer after the activity. The set-up also includes synthetic or natural filtration materials, funnels, or containers for holding the filtration media, as well as containers for collecting the water. Towels for cleaning the possible spills should also be present. The materials can include synthetic materials such as cheesecloth, kitchen towels, coffee filters and filter papers. Depending on time constraints students may hold the materials up to the light for examining the texture or be shown cell phone images of the materials such as the ones shown in Figure 8.1. If time allows, they can take their own images using their cell phones. Students can then see the water poured through one of the materials using a standard ring stand and funnel. Emphasis is placed on how water that looks clear may not be safe to drink by showing them pictures of bacterial test strips or agar plates from water that was filtered but did not contain bleach or other sterilizing agents. They are then told how clean water requires additional treatment

and they should always check with an adult before drinking from a water source. In another variation of the activity, natural filtration is described using materials such as sand, pebbles, and larger rocks. They can then either pack or watch a facilitator pack, a column with the materials and then pass the water through and see how it clarifies with multiple passes. For this activity, a clear 1 L PET bottle that has had the top cut off and holes in the base can work well (Figure 8.2). To ensure the containment of small sand and rock particles, placing a coffee filter at the bottom can be helpful. Ideally, the filtration column should be packed in a way that water goes through the media with larger objects first for the filtration column to work effectively without getting clogged. The use of a ring stands for holding the filtration column/setup can reduce the likelihood of spills. An example of a set-up using natural and synthetic materials is shown in Figure 8.2.

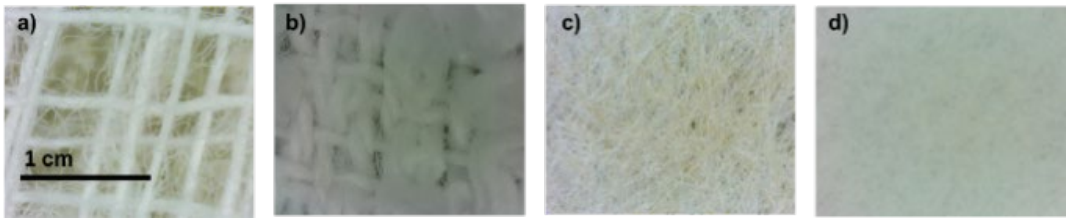


Figure 8.1 Cell phone microscope images of a) cheesecloth, b) dish towel, c) coffee filter, d) Number 2 Whatman qualitative cellulose filter paper.

Figure 8.2 Materials for activity on designing a filter column with natural and synthetic materials.

Assessment of activities at open houses is challenging because collecting the participants feedback should be quick and not disrupt/interfere with participation in other activities of the event. Informal assessment can include student engagement with the facilitator or coming back to the activity with a family member or friend to explain it to them. Quick pick assessments based on pictograms such as emojis on tablets/interactive screens or even slips of paper can be an effective way of getting Likert scale feedback. Both types of assessments have shown that this activity is enjoyed by a wide range of age-groups. In 2018, the water quality enhancement using the water filtration column described in this section and water quality assessment using the commercially available test strips was used as a demonstration activity at the Auburn GRAND Expo with students in grades 2 - 7. Over 100 students attended the event. Twenty students completed an optional survey on their enjoyment of this station. The water filtration activity compared favorably to other activity stations. When asked whether the activity made them more or less interested in engineering, 88% responded favorably.

8.3 Middle and high school classes and summer camps

Expansions of the short activities were used in middle school classrooms as well as middle and high school summer camps. The in-school activity was aligned with teaching standards of the state of Alabama (USA) for 7^{th} grade life science [163], but is easily adoptable for other courses. It includes an engagement activity highlighting how odors can go through latex balloons, but not mylar balloons. Students then perform an augmented version of the short activity with cheese cloth, kitchen towels, and filter paper using bleach-free dirt-water suspension. The augmented version included the additional use of filter papers that had been impregnated with silver nanoparticles via a simple microwave synthesis method [164]. In this version, analysis extends beyond visual observation of water clarity; qualitative measures of bacterial contamination are also

included. To reduce costs, potato slices which had been quickly boiled in water and placed in petri dishes or bags replace the use of agar plates [11]. Students place drops of the initial and filtered water on potato slices and close the container. After approximately two days of incubation at room temperature (preferably in a dark place), all samples except the ones filtered through the silver nanoparticle impregnated papers show significant bacterial growth. The bactericidal action of the silver nanoparticles is discussed as well as the low-cost technology used to make the nanoparticle impregnated filter papers and how communities with limited access to resources might use the technology. As part of the discussion, a video on "The Drinkable Book" for water decontamination using silver nanoparticle impregnated paper is shown. The Drinkable Book contains information about water quality, the pages can be used as biocidal filers, and the book is shipped in a box that serves as the filter holder. [165, 166] After the video, students are engaged in a discussion about the current and future availability of clean water locally and globally. Students are also asked to discuss times they experienced challenges due to excess water, not enough water, or not enough clean water.

At teacher training events, teachers appreciated the hands-on nature of the activity for teaching about size scale while incorporating environmental science and biological science concepts. Assessment of the classroom activity was primarily in the form of post activity tests on students' knowledge and in a format available in the online resources [163]. In summer camps, the water filtration activity was conducted after an assessment of students' attitudes towards engineering followed by an engagement activity called "What's the Challenge?"[167]. In this activity, students brainstorm on important social and engineering challenges and problems they think somebody should prioritize and solve. The results are often a list that include problems and challenges ranging from minor inconveniences and local issues to global issues. They are then

asked to group and prioritize the list as a large group. This invariably leads to many of the items listed as Grand Challenges for Engineering or UN Sustainable Development Goals [159] including access to clean water to be prioritized on students' list. The first few iterations of the activity in summer camps used the same filter materials as the 7th grade life science activities. In one iteration, creating the filter column was a design challenge among groups of students. The students were given the freedom to pick their column packing materials (filtering media) and sequences and they could compete based on criteria such as fastest flow rate, or cleanest water in the fewest passes. Regardless of the execution details described, knowledge pre-test and post-tests were taken by students. Testing of biological contamination using test strip, potatoes, or agar plates depended on whether it would be possible for students to see the results after the two-day incubation period. For this reason, when less time available, a greater emphasis was placed on chemical analysis. Water known to contain chlorine, carbonates, or nitrates is used and the chemical analysis is conducted using chemical test strips and Exact iDip meters. Regarding water analysis for chemical properties, the authors suggest addition of agents such as vinegar (for increased acidity), salt for increased chloride content, or bleach (for increased hypochlorite salt content and reduced bacterial load) to create a variety of water samples that will yield significantly different results. Either the chemical test strips or the Exact iDip meters can be used to provide similar information, but each method has different advantages and disadvantages. The test strips are inexpensive, readily available, and easy to read. They rapidly provide information on several water quality parameters (e.g., pH, alkalinity, hardness, chlorine, and nitrate content) in the form of color changes on an adsorbent pad. Semi-quantitative information can be obtained by comparing the color to that on a key provided on the test strip bottle. In contrast to test strips, the Exact iDip meters are quantitative handheld spectrophotometers. The cost is considerably less than

professional instruments, but still on the order of $100 - $300 and they also require disposable test strips to enable analysis. Therefore, meters are only cost effective for recurring events where they will be used many times. For the meters, a few milliliters of water is placed in the sample chamber and a test strip for specific parameters like free chloride and nitrate is used to stir the water. Turbid water samples or water containing too much sediment are not suitable for being read in the meter due to issues with light passage in the meter chamber. Depending on the parameter being measured the meter displays the result in a few seconds to a few minutes and also uses Bluetooth to send it to a cell phone app that logs the result, date, time, and geolocation. The app also allows for storing historical data to look for trends. Therefore, the meters enable a discussion of citizen science and tracking historical data in natural or municipal systems. Students also typically enjoy the cell phone interface.

The first formal evaluation of the water activities in camps was for a half-day activity session in a week-long residential engineering camps sponsored by Auburn University. This included a camp consisting of 27 high school females and a co-ed camp with an additional 29 participants. At the end of the lesson, students completed an attitudes survey of their enjoyment of the activities and a short post-test of their knowledge. Findings indicate many of the student participants of the camp found the water quality assessment and enhancement introductory lesson and lab activities interesting and educational (Table 8.1). In the surveys collected from the students after their participation, 86% of the participants of the co-ed camp and 96% of the participants of the camp for female high school students found the lab activities focusing on water filtering and quality assessment either very interesting or somewhat interesting. In addition, 97% of the co-ed camp participants and 100% of participants of the camp for female high school students indicated that they have either learned something or learned a lot from taking part in the activity.

Table 8.1 Assessment of clean water activities for summer camps.

Assessment questions		Co-ed camp		Female high school student camp	
Was it interesting?	1=needs improvement; 2=somewhat interesting; 3=very interesting	Mean	% selecting 2/3	Mean	% selecting 2/3
	What is nano	2.3	97%	2.4	89%
	lecture on water purification	2.1	97%	2.2	89%
	lab activities	2.2	86%	2.3	96%
Did you learn something?	1=didn't learn much; 2=learned some; 3=learned a lot				
	What is nano	2.4	100%	2.5	100%
	lecture on water purification	2.3	93%	2.4	96%
	lab activities	2.3	97%	2.4	100%

8.4 Freshman introduction to engineering classes

The activities used in the summer camps were further extended for use in a university freshman "Introduction to Engineering" course classes. Each week the course consisted of a 50-minute lecture for approximately 120 students and two 75-minute laboratory or problem-solving sessions. As part of a larger research assessment [168, 169], students completed surveys on engineering knowledge and attitudes during the first week and last week of the course as well as surveys and knowledge tests before and after activities related to the engineering grand challenges. The water module consisted of an industry speaker from Nalco Water and 75 min labs similar to what conducted in the summer camps using the Bluetooth Exact iDip meters. In addition, students borrowed the meters to obtain data for water sources on and around the campus. The resulting data was used to teach or reinforce key chemical engineering principles such as unit conversion, computing moles from ppm, and calculating mixture compositions. This took the place of

homework exercises on these same topics. All the data from the class, was collected into a master excel spreadsheet and used to reinforce concepts related to analyzing and plotting data. Freshman student experience with spreadsheets varies widely at this university, so initial calculations and plots were made as a class exercise with instructor facilitation and peer-to-peer teaching. Specifically, students used the group water quality data to calculate averages and standard deviations from specific collection locations. They also made bar graphs of these parameters that included error bars. Finally, all the information including additional research on water quality issues was compiled in a lab report. This activity was conducted multiple semesters over a three-year period. Representative assessment data is shown in Table 8.2. When asked to rate the activity from 1 to 3 based on whether it was interesting and whether it helped in learning about engineering students gave the activity 2.40 and 2.17 respectively.

Table 8.2 Introduction to Engineering Evaluations.

	Was it interesting?	**How did it help you learn about engineering?**
	M (scale 1 to 3)	M (scale 1 to 3)
Lecture by E. Parola of Nalco	2.33	2.04
Assigned Reading	1.86	1.91
Water Lab	2.40	2.17
Report Writing	1.75	1.90

8. 5 Conclusions

Access to clean water is an important educational topic that is a perpetual challenge, affecting people around the globe. Due to the social, economic, environmental, and technical significance of water quality and access to clean water, there is an opportunity to use this topic as a means of engaging K-13 students' interest into real life engineering challenges. Teaching

students about water quality enhancement and assessment methods through interactive activities ranging from a few minutes to hours can be used for raising awareness toward these challenges. These activities can also help K-13 students gain basic understanding of water quality parameters and teach them methods for water quality enhancement and assessment, data analysis and basic statistics. Simple water quality enhancement methods such as size-based filtration coupled with silver nano particle-based filtration can be easily adapted into classroom lessons and be presented as demonstration STEM activities. Readily available tools, such as pool test strips, water quality meters and bacterial culturing can be used independently or paired with the described water quality enhancement methods to give students some hands-on-experience with assessment of various water quality parameters. The results from surveys filled by K-16 students who have participated in the water quality enhancement and assessment activities described in this work, show the positive impact of the activities in engaging the students' interests into the topic of water quality and teaching them about water quality enhancement and assessment methods. Additionally, the activities have positively impacted students' attitudes towards engineering career paths.

Chapter 9: Conclusions and summary

This research enabled significant advances in the tools and methods needed for improved understanding of periphyton-substrate interactions and the implications for more efficient engineering of periphyton-based algae systems. Attached algal cultivation systems remain an interesting avenue for further exploration of commercial algae-based products and processes. Understanding key parameters in controlling attached growth and using them to design cultivation systems with improved efficiency and inclusive of diverse algae species and community structures remains a challenge. One of the crucial parameters in attached algae growth that can be leveraged for controlling and improving the cultivation is the condition of the substrate surface. Gaps in the body of knowledge about substrate effects on attached algal cultivation, especially for periphyton-based systems, call for the design of experiments and systems suitable for studying these effects. This work has addressed these challenges and contributed to the field by introducing a robust framework for studying the attached cultivation of periphytic filamentous algae with commercial application potentials.

A primary goal of this research was to present and experiment with tools and methods to investigate substrate-related effects and algae-substrate interactions for periphytic filamentous algae. Periphyton-based algae systems are effective in water remediation and rely on algae species that have natural adaptations for attached growth. The three-dimensional networks of the algae often present in these systems (dominated by filamentous algae) are structurally different from the more comprehensively explored and studied prostrate microalgae biofilms. Therefore, there are limitations in applying the body of knowledge available on the influences of substrate characteristics on algal bio-adhesion and biofouling to periphyton-based systems. This work

addressed some of these limitations by highlighting the unique characteristics of periphyton algae such as morphological diversity within cells of the same species and attachment adaptations, it also made advances in modifying cultivation and algae surface characterization methods to capture the influence of these characteristics.

The main focus of this work was understanding the effect of substrate physicochemical properties such as surface energy and charge on the attached growth of periphytic algae. The results presented in Chapter 4 from the cultivation of green filamentous periphytic algae on polymeric and non-polymeric substrates such as glass, PLA, PTFE, and PMMA indicate the importance of physicochemical characteristics of the substrate in promoting algae adhesion at early stages with effects becoming less significant as the attached community grows and matures. This could be important from the application standpoint in selecting substrates for attached cultivation of periphytic algae in systems such as algal turf scrubbers, depending on the targeted harvesting frequency and the importance of cultivation time in overall efficiency.

Another major focus of this work was to create tools and methods required for studying substrate influences on attached growth of filamentous periphytic algae that were appropriate for the morphological characteristics and attachment preferences of these algae. In this regard, *Stigeoclonium tenue* was chosen as a model periphytic filamentous algae for designing a cultivation system and modeling the adhesion behavior based on its application potentials. Chapter 5 presented an intermediate scale photobioreactor setup that has been designed and tuned for investigating the substrate effects in periphytic algae. The performance of this photobioreactor in terms of providing a suitable environment for testing substrate effects was assessed via attached cultivation of an algae community dominated by *Stigeoclonium tenue* on PLA substrate surfaces. Results from Chapter 5 indicate the effectiveness of the designed setup/photobioreactor and tested

cultivation conditions in successfully growing attached communities dominated by the periphytic filamentous algae *Stigeoclonium tenue*. This is important because such a framework enables studying substrate interaction and preferences at a species level which has not been widely explored for periphytic filamentous algae before. This framework can be adapted for cultivation and studying other periphytic filamentous algae. The photobioreactor design presented in Chapter 5 was used as the cultivation environment for further understanding the substrate effects on the attached growth of periphytic filamentous algae in Chapter 6.

The influences of topographical characteristics of the substrate on the attached growth of periphytic algae were also a topic of interest in this work. The effectiveness of simple macroscale topographical substrate features on attached biomass was explored through algae cultivation on 3D printed polymeric substrates made from PLA, ABS, and PET-G. The results of this investigation, presented in Chapter 6, indicate that concave hemispherical substrate topographical features with millimeter-scale diameters are effective in increasing attached biomass amount without changing the areal footprint of the cultivation surface. From the application standpoint, this result presents an opportunity to enhance the production of periphytic algal biomass through the introduction of easily manufacturable substrate feature characteristics that will not interfere with the mechanical harvesting process.

In Chapter 7, the use of the XDLVO thermodynamic approach for describing the algae cell-substrate surface interactions was explored. The correlation of the modeled adhesion behavior with the experimental observations on the attachment of microalgae *Scenedesmus* to PLA and PLA-based nanocomposites was investigated. The results of this part of the research demonstrated the effectiveness of XDLVO-based approach in predicting the algae adhesion preferences while highlighting the need for method development and modeling modifications to adapt the method

for periphytic filamentous algae. Subsequently, *Stigeoclonium* was used as a model species for developing measurement methods of surface physicochemical properties for periphytic filamentous algae. More specifically, a novel method was developed for zeta potential measurement on algae filaments via streaming potential measurements. Additionally, the complex and diverse cell morphologies among *Stigeoclonium tenue* cells were used to assess the appropriateness of the use of sphere-plate geometry for modeling thermodynamic interactions between algae cells and the substrate surface for filamentous species. Ultimately, the use of plate-to-plate geometry for modeling the interaction was also explored. Results from this chapter indicate the importance of adaptation and development of methods for determining physicochemical characteristics of filamentous periphytic algae cells and modeling their interaction with the substrate in understating the early-stage adhesion. This information can be used for the optimization of substrate surface characteristics to design substrates that are thermodynamically favorable for the adhesion of specific algae species.

In addition to the main focus of this work on attached algae cultivation, a chapter of this dissertation (Chapter 8) was devoted to describing the author's development and implementation of a series of engineering education activities focused on water quality and access to clean water. These activities were designed to engage K-16 students in different learning environments such as summer camps, open houses, and first-year engineering classrooms. The results from this work showed that water quality assessment and enhancement concepts are readily adaptable to the educational needs of a wide range of age groups and time constraints. Assessment of the activities showed that they effectively gained students' interest in engineering topics and highlighted that engineering is an altruistic career path.

Recommendations for future research

Despite the collective efforts to understand and engineer the process of algae adhesion and cultivation, including this work, there are still gaps in the body of knowledge about attached algae systems that need to be addressed to make algae-based products and processes commercially feasible and widely accessible. These are as follows:

a) As mentioned in Chapter 2, the adhesion of algae to surfaces is theorized to be preceded by bacterial adhesion and pre-conditioning of the substrate. Bacteria are known to exude EPS material on the substrate, so the algae might not be interacting with the pristine substrate surface. There is a possibility that physicochemical characteristics of the substrate, such as surface energy and zeta potential, are changed by the preconditioning process. This is important because most of the studies relating the physicochemical characteristics of the substrate to the adhesion of algae, including this work and research on using the XDLVO modeling approach for microalgal biofilms, have neglected the potential influences of bacterial conditioning on surface characteristics. Additionally, one can argue that the influence of topographical characteristics especially at a scale influencing the bacterial adhesion (microscale) can also impact the bacterial preconditioning of the substrate. Therefore, the investigations of the effects of bacterial preconditioning including investigation of the potential differences in the bacterial community composition between different substrate materials is required for effectively describing the algal adhesion behavior.

b) In addition to bacterial conditioning, it is important to remember that algae-substrate interaction is a dynamic process involving living cells which can change throughout the colonization process. These changes could be in the cell shape and even surface properties. Therefore, knowledge of the influences of the sequence of chronological changes of the

attached algae community on the algae-substrate interactions might be another avenue for better understanding the attached cultivation.

c) One important aspect of the algal adhesion which is often neglected in investigating the effect of substrate characteristics on attached systems is existence of specialized attachment adaptations of the algae. Even though studies in natural systems and in the field of phycology have reported and studied these adaptations, investigation into the practical implications of these adaptations on algae-substrate interactions in cultivation systems has not been done. This is especially true for periphyton-based species that prefer attached growth and have specialized attachment strategies, such as mucilage pads or root-like structures, that can impact the adhesion behavior significantly. Therefore, incorporating the morphological characteristics that aid adhesion into models of attached growth systems might advance the understanding of algae- substrate interactions.

d) Most attached algae systems rely on hydrodynamic flow for nutrient advection, cell transport and more. Substrate interactions with the flow can create hydrodynamic zones that are favorable or unfavorable by affecting local flow velocity and shear stress, ultimately impacting algae settlement and growth. Moreover, in the case of the three-dimensional turf-like structure of the periphytic communities dominated by filamentous algae, these interactions are even more complex. This is mainly due to the ability of the filamentous algae network to grow outward from the substrate, extend far beyond the substrate surface, and move with the flow. Hence, understanding the combined effect of substrate characteristics such as topography, algal community structure, and the flow dynamics is required for predicting favorable cultivation conditions and optimizing the substrate for enhanced attached growth. This goal can be potentially achieved through CFD modeling of the flow.

e) The effectiveness of concave hemispherical topographical features (millimeter scale diameter) in increasing the amount of *Stigeoclonium* biomass per nominal area of the cultivation substrate was demonstrated in Chapter 6. In many periphyton-based systems such as ATS, repeated and targeted harvesting is a common practice performed for regulating the algal community, recovering biomass, and boosting productivity [27, 170]. Depending upon the mechanical harvesting method, there is a possibility that presence of concave substrate surface features in the scales tested in this work could serve as a somewhat protected refuge for algae cells, speeding up reemergence of the community after a harvest. This could be tested through subjecting the textured substrates to repeated growth and harvest cycles with methods similar to systems like ATS.

f) Attachment strength is also a parameter that could be affected by the substrate properties. Even though XDLVO modeling can be an effective tool in predicting existence of attractive forces between algae cells and the substrates, it only explains the early stages of adhesion in simplified systems. As mentioned previously, attached algae communities are dynamic in their response to environmental factors and experience lifecycle -elated changes. Therefore, they could respond to the substrate properties and adapt to the attachment environment in different ways. These responses could be in the form of changes to the basal cell in the proximity of the substrate [99] or secretion of mucilaginous material, all of which can potentially change the strength of their adhesion to the substrate. The presence of other microorganisms such as bacteria in the community and their metabolic activities may also impact the strength of algae cell adhesion. As an example, in some of the preliminary observations made by the author on long-term attached cultivation of *Stigeoclonium tenue* on PLA, glass, and PTFE, the algae

community on the PTFE were observed to peel off from the substrate after a period of cultivation, while the communities on PLA and glass remained attached.

g) One of the key achievements of this study was finding the optimum cultivation conditions and environment for growing attached communities heavily dominated by one periphytic filamentous algae species at an intermediate scale. This achievement enables understanding substrate effects at a species level that can help in designing substrates suitable for cultivation of a target periphytic filamentous species. However, cultivation experiments in this work, even though open to the surrounding environment, were performed in indoor conditions where the possibility of contamination by other organisms, especially other algae, was less than outdoor systems. Additionally, standard nutrient media devoid of other algae species was used for the cultivation. It would be valuable to know whether the findings about the substrate effects will also apply to outdoor systems or wastewater-based attached cultivation. In the latter, the presence of other organisms, especially algae, and the nutrient composition and concentration would promote or suppress the growth of the target periphytic filamentous species. Some preliminary cultivation experiments and observations by the author on the use of wastewater from a tilapia pond aquaculture for attached photobioreactor cultivation of *Stigeoclonium tenue* on PLA substrates have shown some promise in the ability and effectiveness of the developed methods for achieving attached communities dominated by the target algae. Moreover, enhancement of attached growth induced by concave hemispherical features was also observed to be present. Nonetheless, further studies are needed for testing and understanding the practical implications of the knowledge obtained in the photobioreactor systems of this work for more complex mixed community periphyton systems.

In summary, this research advanced the development of the characterization tools and laboratory methods for the cultivation of periphytic algae. The results have advanced the understanding of substrate interactions particularly for *Stigeoclonium tenue* and established a framework for future research. Algae are a valuable source of biomaterial and have a profound ecological role as indicators and remediators of water quality, therefore it is hoped that this work and these tools will inspire successive refinement in the understanding of attached algae cultivation and enabling feasible commercial use of algae.

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Appendix 1 : Surface physicochemical properties and XDLVO modeling

This appendix will go through the steps involved in using the experimental surface physicochemical properties measured in finding the interaction energy components of the XDLVO model. Note that the reference numbers provided are in accordance with dissertation reference list (See page 124 to access corresponding references).

The XDLVO thermodynamic modeling approach measures the favorability of bio-adhesion by treating the algae and the substrate as colloidal entities and ultimately measuring the total interaction energy between an algal cell and the substrate as a function of distance. The total interaction energy G^{TOT} (Equation A-1) is the linear sum of three interaction energies that are functions of distance comprising the following: Lifshitz-van der Waals (LW) interactions (GLW), which originate from instantaneous asymmetrical distribution of electrons in molecules; electric double layer/electrostatic (EL) interactions (G^{EL}) from the electrostatic interactions between the cell and the substrate; and acid-base (AB) interactions (G^{AB}), which originate from polar interactions in the aqueous media [92]. Negative G^{TOT} values indicate attraction, while positive values indicate repulsion between the cell and the substrate.

$$G^{TOT}(d) = G^{LW}(d) + G^{AB}(d) + G^{EL}(d) \quad \text{(A-1)}$$

Assuming a spherical particle such as algae interacting with a semi-infinite plate, following equations can be used for finding each of the energy components [112, 117]:

$$G^{LW}(d) = -\frac{A}{6}\left[\frac{r}{d} + \frac{r}{d+2r} + \ln\left(\frac{r}{d+2r}\right)\right] \quad \text{(A-2)}$$

$$G^{AB}(d) = 2\pi r\lambda\Delta G_{adh}^{AB} \exp[(d_0 - d)/\lambda] \quad \text{(A-3)}$$

$$G^{EL}(d) = \pi\varepsilon r(\psi_m^2 + \psi_s^2)\left[\frac{2\psi_m\psi_s}{\psi_m^2+\psi_s^2}\ln\frac{1+e^{-\kappa d}}{1-e^{-\kappa d}} + \ln\left(1 - e^{-2\kappa d}\right)\right] \quad \text{(A-4)}$$

Where d is distance between the interacting bodies, r is the diameter of the particle (algae cell in the case of this work), A is the Hamaker constant, d0 represents the minimum distance between the two entities (0.157nm is a commonly used literature value for similar systems), λ is the decay length for water, corresponding to gyration radius of clustered water molecules (0.6 nm in case of hydrophilic interactions and up to 1.3 nm for hydrophobic interactions based on measurements int the literature) [80, 92] . Subscripts m and s stand for microbe (algae) and substrate, respectively. ε is the permittivity of the medium, ψm and ψs are the surface potential of algal cells and substrate respectively, and κ^{-1} is the double layer thickness.

The G^{LW} and G^{AB} components of the interaction can be found by plugging in the surface energy component values into the following equations where the subscript adh refers to the interaction energies at the surface (minimum distance) [92]:

$$\Delta G_{adh}^{LW} = -2\left(\sqrt{\gamma_m^{LW}} - \sqrt{\gamma_l^{LW}}\right)\left(\sqrt{\gamma_s^{LW}} - \sqrt{\gamma_l^{LW}}\right) \quad \text{(A-5.a)}$$

$$A = -12\pi d_0^2 \Delta G_{adh}^{LW} \quad \text{(A-5.b)}$$

$$\Delta G_{adh}^{AB} = 2\left(\sqrt{\gamma_m^+} - \sqrt{\gamma_s^+}\right)\left(\sqrt{\gamma_m^-} - \sqrt{\gamma_s^-}\right) - 2\left(\sqrt{\gamma_m^+} - \sqrt{\gamma_l^+}\right)\left(\sqrt{\gamma_m^-} - \sqrt{\gamma_l^-}\right) - 2\left(\sqrt{\gamma_s^+} - \sqrt{\gamma_l^+}\right)\left(\sqrt{\gamma_s^-} - \sqrt{\gamma_l^-}\right) \quad \text{(A-6)}$$

The surface energy components required can be found through contact angle measurements with several probe liquids using the acid-base approach [92, 112, 139]:

$$(1 + \cos\theta)\gamma_l = 2\left(\sqrt{\gamma_s^{LW}\gamma_l^{LW}} + \sqrt{\gamma_s^+\gamma_l^-} + \sqrt{\gamma_s^-\gamma_l^+}\right) \quad \text{(A-7. a)}$$

$$\gamma = \gamma LW + 2\sqrt{\gamma - \gamma +} \quad \text{(A-7.b)}$$

The G^{EL} component requires the knowledge of surface charge values and the double layer thickness (Debye length) of the media. The double layer thickness can be found using equation

(A-8) if information about ionic composition and ionic strength of the interaction media are available.

$$1/\kappa = \sqrt{\frac{\varepsilon kT}{e^2 \sum v_i^2 n_i}} \qquad \text{(A-8)}$$

where k is the Boltzmann's constant and e is the elementary charge. T is the absolute temperature in kelvin, v_i and n_i are the valency and the number density (per volume of bulk liquid) of each ionic species present.

Since the surface charges cannot be measured directly for the interacting entities, zeta potential is measured and converted to the surface charge through estimations available in the literature. For example, for a spherical particle in a media with known double layer thickness Equation (A-9) can be used for obtaining the surface charge through the zeta potential value [117]:

$$\psi = \zeta\left(1 + \frac{\upsilon}{r}\right) e^{\kappa\upsilon} \qquad \text{(A-9)}$$

Where ζ is the zeta potential, r is the radius of the particle and υ is the hydration layer thickness which could be estimated based on the ionic strength of the media [80].

In case of changing the configuration from sphere-plate to plate-plate, equations in table A-1 can be used for finding the interaction energy components.

Table A-1 XDLVO mode for plate-plate and sphere-plate configuration

Plate- Plate Interactions [116,139]

- $G^{LW}(d) = -\frac{A}{12\pi(d)^2}$
- $G^{AB}(d) = \Delta G^{AB}_{adh}\, exp[(d_0 - d)/\lambda]$
- $G^{EL}(d) = \varepsilon\kappa \left[\left(\frac{\psi_m+\psi_s}{2}\right)^2 \left\{1 - \tanh\left(\frac{\kappa d}{2}\right)\right\} - \left(\frac{\psi_m-\psi_s}{2}\right)^2 \left\{\coth\left(\frac{\kappa d}{2}\right) - 1\right\}\right]$

Sphere –Plate Interactions [116]

- $G^{LW}(d) = -\frac{A}{6}\left[\frac{r}{d} + \frac{r}{d+2r} + ln\left(\frac{r}{d+2r}\right)\right]$ (A5)
- $G^{AB}(d) = 2\pi r\lambda\Delta G^{AB}_{adh}\, exp[(d_0 - d)/\lambda]$ (A6)
- $G^{EL}(d) = \pi\varepsilon r(\psi_m^2 + \psi_s^2)\left[\frac{2\psi_m\psi_s}{\psi_m^2+\psi_s^2} ln\frac{1+exp(-\kappa d)}{1-exp(-\kappa d)} + ln\{1 - exp(-2\kappa d)\}\right]$