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Development and optimization of biofilm based algal cultivation

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Development and optimization of biofilm based algal cultivation

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ABSTRACT

This dissertation describes research done on biofilm based algal cultivation systems. The system that was developed in this work is the revolving algal biofilm cultivation system (RAB). A raceway-retrofit, and a trough-based pilot-scale RAB system were developed and investigated. Each of the systems significantly outperformed a control raceway pond in side-by-side tests. Furthermore the RAB system was found to require significantly less water than the raceway pond based cultivation system.   Lastly a TEA/LCA analysis was conducted to evaluate the economic and life cycle of the RAB cultivation system in comparison to raceway pond. It was found that the RAB system was able to grow algae at a lower cost and was shown to be profitable at a smaller scale than the raceway pond style of algal cultivation. Additionally the RAB system was projected to have lower GHG emissions, and better energy and water use efficiencies in comparison to a raceway pond system.

Furthermore, fundamental research was conducted to identify the optimal material for algae to attach on. A total of 28 materials with a smooth surface were tested for initial cell colonization and it was found that the tetradecane contact angle of the materials had a good correlation with cell attachment. The effects of surface texture were evaluated using mesh materials (nylon, polypropylene, high density polyethylene, polyester, aluminum, and stainless steel) with openings ranging from 0.05–6.40 mm. It was found that both surface texture and material composition influence algal attachment.

CHAPTER 1. INTRODUCTION: DISSERTATION FORMATTING

This dissertation presents a comprehensive review of biofilm based algal cultivation. The major topics include; (1) evaluation of algal productivity between a revolving algal biofilm (RAB) based cultivation system and a raceway pond, (2) evaluation of water use efficiency between a RAB based cultivation system and a raceway pond, (3) identification of an optimal material that promotes algal attachment and biofilm growth, (4) TEA/LCA comparison between a raceway pond algal cultivation and RAB cultivation.

Thesis Organization

Chapter 2 is a literature review outlining the current stat of the art of algal biofilm based cultivation systems. Chapter 3 reports a yearlong comparison of the RAB cultivation system in comparison to a raceway pond based cultivation. Chapter 4 reports on the water use efficiency of the RAB system in comparison to raceway pond based culture. Chapter 5 investigates the identification of an optimal material that promotes algal attachment and biofilm growth. Chapter 6 describes a TEA/LCA comparison between a raceway pond and RAB system.

CHAPTER 2. BIOFILM-BASED ALGAL CULTIVATION SYSTEMS: A REVIEW

A paper published by *Applied Microbiology and Biotechnology*

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Abstract

Biofilm-based algal cultivation has received increased attention as a potential platform for algal production and other applications such as wastewater treatment. Algal biofilm cultivation systems represent an alternative to the suspension-based systems that have yet to become economically viable. One major advantage of algal biofilm systems is that algae can be simply harvested through scraping and thus avoid the expensive harvesting procedures used in suspension-based harvesting such as flocculation and centrifugation. In recent years, an assortment of algal biofilm systems have been developed with various design configurations and biomass production capacities. This review summarizes the state of the art of different algal biofilm systems in terms of their design and operation. Perspectives for future research needs are also discussed to provide guidance for further development of these unique cultivation systems.

Introduction

Microalgae cultivation has been extensively researched for production of fuels, chemicals, feeds, and nutraceuticals. It has also been used as an effective process for wastewater treatment (Mata et al. 2010; Kesaano and Sims 2014; Pittman et al. 2011) as well as for mitigation of CO_2 emissions (Brune et al. 2009) and ammonia emissions (Kang et al. 2014).

Currently, the majority of the microalgae cultivation processes are based on open ponds or enclosed photobioreactors in which the algal cells are suspended in liquid medium. The algal cell densities in these systems are typically low. For example, cell concentrations can be as little as 0.5 g/L (0.05% dry basis) in open ponds and 2–6 g/L (0.2–0.6% dry basis) in photobioreactors (Davis et al. 2011). To harvest algal biomass from this diluted broth, the culture is commonly concentrated through sedimentation, flocculation, or flotation to around 1% (dry basis) and then further concentrated to 20% through centrifugation (David et al. 2011; Dassey and Theegala 2013; Barros et al. 2015). These multiple operations are time consuming and cost prohibitive. In a recent techno-economic study of microalgae production for fuel, it was estimated that biomass harvesting costs alone accounts for 21% of the total capital cost of an open pond cultivation system (Davis et al. 2011).

As an alternative to suspension-based culture systems, algal biofilm culture systems have drawn interest in recent years. This style of cultivation system has particular benefit in reducing the costs related to algae harvesting (Blanken et al. 2014; Gross and Wen 2014; Gross et al. 2015; Lin et al. 2003). In the algal biofilm system, algae are attached on a material surface and harvested by scraping (Christianson and Sims 2012; Johnson and Wen 2010). The harvested algae has a solid content of 10–20% (dry basis), which is similar to that of the post-centrifuged biomass (Gross et al. 2013; Johnson and Wen 2010). In addition to the benefit of easy

harvesting, algal biofilm systems also have a variety of unique features such as minimization of light limitation and enhancement of CO_2 mass transfer. Separation of the algal cells and liquid medium also increases the solids retention time of the cells without being washed out. Algal biofilm reactors were first reported in the 1980s (Przytocka-Jusiak 1984) with a rotating polystyrene disk design used for removing nitrogen and phosphorus from municipal wastewater. To date, various algal biofilm reactors have been reported with applications in wastewater treatment (Kesaano and Sims 2014; Godos et al. 2008; Mulbry and Wilkie 2001; Posadas et al. 2013; Gao et al. 2015) and biomass production (Gross et al. 2013; Blanken et al. 2014; Liu et al. 2013). The aim of this review is to summarize the state of the art of the design and operation of various algal biofilm systems. This includes: the reactor design configurations, the pros and cons of the systems, and the factors affecting biofilm growth performance. The perspectives of the algal biofilm system are also discussed to provide guidance for further research and development needs. It should be noted that while there are other reviews of algal biofilm systems available (Kesaano and Sims 2014; Hassard et al. 2015), they mainly focused on the application aspects of the biofilm systems such as wastewater treatment.

Different configurations of algal biofilm systems

Various algal biofilm systems have been developed over the past few years. Depending on the application of the system, the design rationale, geometry configurations, and attachment materials employed have varied widely. For example, some biofilm systems use attachment materials with many crevasses that create additional attachment area, but this additional area does not have access to light. This system is commonly used in the wastewater treatment (Fitch and England 2002), with a consortium of algae and bacteria involved (Gullicks et al. 2011). The

biomass in this system is not harvested but rather sloughed off into the liquid (Hassard et al. 2015). Other biofilm systems use flat attachment materials that are designed to allow maximal light exposure to the biofilm. The biomass in these systems can be easily harvested. The algal cells in biofilm are predominant due to the favorable photosynthesis conditions, although it has been reported that algal biofilms will have some level of bacterial contamination if organic carbon is present in the wastewater (Pohlon et al. 2009; Irving and Allen 2011). The geometries of the algal biofilm systems are also highly variable, from a flat plate to a rotating drum. The materials used for cell attachment include glass (Schnurr et al. 2013), cotton (Gross et al. 2013; Kesaano et al. 2015), stainless steel (Blanken et al. 2014), and polyvinyl chloride (Posadas et al. 2013). A comprehensive summary of the different algal biofilm systems is provided in Table 1. In this review we categorized the different types of algal biofilm systems based on the relative movement of the algal biofilm to the liquid medium, i.e., stationary biofilm or rotating biofilm (see Table 1).

Stationary biofilm design

In the stationary biofilm system, a pump or other device is used to move liquid over the surface of a biofilm, which can be positioned either horizontally or vertically. A typical representation of the stationary biofilm system is an algal turf scrubber (ATS), in which a flat and slightly slanted attachment sheet such as polyethylene is placed horizontally. Biofilm forms on this flat surface and a thin layer of liquid is flowed over the biofilm (Mulbry et al. 2001; Kebede-Westhead et al. 2003; Mulbry et al. 2008). ATS systems have been studied for their capacity to treat wastewater and surface water. Since ATS uses few or no moving parts, it minimizes the capital requirement as well as the energy use and operating costs. However, scale up of the ATS

requires a significant footprint, which may not be a viable option in areas where land is limited such as many municipal wastewater treatment plants.

In contrast to the horizontally-oriented ATS system, the stationary attachment material can be vertically oriented to create a "flat plate biofilm reactor" (Genin et al. 2013; Schnurr et al. 2013; Liu et al. 2013; Zamalloa et al. 2013). The attachment material is usually submerged in a reservoir in which liquid is circulated with a pump or mixed with bubbled air. This biofilm reactor appears to be similar to the suspension-based flat plate photobioreactor except that a supporting attachment material is placed inside to promote biofilm growth. The vertical orientation of the algal biofilm drastically reduces the land footprint. The flat plate biofilm reactor can also reduce contamination because the liquid reservoir can be sealed off from the outside environment. However, the transparent liquid reservoir will increase the capital costs for this system. Additional energy for liquid circulation or air bubbling also increases the operating costs.

An alternative to flowing liquid over a stationary biofilm is to deliver liquid to the attached algal cells through spraying devices. The most notable version of a spray-based biofilm system is the one developed by Bioprocess Algae LLC. (Bioprocess H$_2$O LLC 2014). In this system, the biofilm is positioned vertically; the spraying nozzles provide the appropriate amount of the liquid to the biofilm to keep it moist. In the misting system, the costly transparent reservoir capable of holding liquid, which is required in a flat plate biofilm reactor, is not needed. In addition, compared to the flat plate biofilm reactor, in which the entire liquid volume in the reservoir is circulated, the spraying system delivering the liquid is less energy intensive. A major disadvantage of this system is that the spray nozzles could be susceptible to clogging. The

shading caused by the "mist" and the mutual shading of parallel stacked vertical biofilm sheets may also negatively affect the photosynthetic activity of the algal cells.

Rotating biofilm design

The rotating algal biofilm (RAB) is another typical biofilm-based system. The attachment material in the RAB system alternatively rotates between the air phase and liquid medium; algal biomass in the RAB system can be harvested through scraping the biofilm when it is in the air phase (Gross et al. 2013). The configuration of the attachment materials in the RAB system can be flat rotating discs (Blanken et al. 2014), a cylindrical drum that is rotated from its center axis (Christianson and Sims 2012), or a conveyor belt rotated by a plurality of drive shafts (Gross et al. 2013).

One typical example of a rotating biofilm design is the rotating biological contactor (RBC), which has been widely used in wastewater treatment (Gullicks et al. 2011; Hassard et al. 2015). In RBC systems, a drum is continuously rotated between liquid and air using a drive shaft and the algae cells attach to the drum. The earlier version of the RBC was used for treating wastewater and harvesting biomass was not crucial. Therefore, the algae biomass was not collected, but rather allowed to slough off into the wastewater (Spengel and Dzombak 1992). A modified RBC bioreactor has also been used for algal biomass production where harvesting of biomass was conducted. Blanken et al. (2014) recently reported a RBC-based Algadisk system using rotating discs as the attachment material and they achieved very high biomass productivity (~20 grams of biomass per m^2 of disc surface per day).

Other types of RAB systems have also been reported. For example, researchers at Utah State University have developed an algal biofilm system by wrapping ropes around a cylinder rotating

between the gas and liquid phases (Christenson and Sims 2012). The algal biomass on the rope can be harvested by passing the rope through an adjustable diameter scraper that can remove the biomass from the rope.

Researchers at Iowa State University have reported another configuration of a RAB system based on a vertical conveyor belt design (Gross et al. 2013; Gross and Wen 2014; Michael et al. 2015; Gross et al. 2015). In this system, a sheet of attachment material is rotated through liquid with a plurality of drive shafts. The height of the conveyor can be increased without increasing the footprint area, unlike the cylinder configuration system where an increase in height results in an increase in footprint area.

Evaluation of algal growth in algal biofilm systems

Algal growth in open pond systems is usually evaluated by biomass productivity based on pond surface area, i.e., mass of biomass per unit of pond area per unit of time. However, for algal biofilm systems the measurement for productivity is not always that straightforward, especially when the attachment materials are vertically oriented. In general, two criteria for biomass productivity in vertically-based algal biofilm systems have been used: (1) the surface biomass productivity ($P_{surface}$), which is based on the surface area of the attachment material, and (2) footprint biomass productivity ($P_{footprint}$), which is based on the footprint area of the biofilm reactor. Evaluating the biomass productivity of algal biofilm reactors, therefore, needs to be consistent and explicit. For example, Liu et al. (2013) designed a flat plate attached reactor resulting in 80 g-m^2 day^{-1} of footprint biomass productivity; while Schnurr et al. (2013) reported 2.8 g-m^2 day^{-1} of surface biomass productivity using a similar flat plate parallel horizontal photobioreactor. The large discrepancy between these two sets of data was due to the different

baselines used in calculating the biomass productivity. The surface biomass productivity reported by a majority of references was in the range of 2 to 6 $g\text{-}m^{-2}$ day^{-1}, with an exceptionally high value (20.1 $g\text{-}m^{-2}$ day^{-1}) being reported by Blanken et al. (2014). In terms of footprint biomass productivity, however, the values varied widely because the footprints accommodating the different algal biofilms are different. For example, Christenson and Sims (2012) reported a footprint biomass productivity of 31 $g\text{-}m^{-2}$ day^{-1} with the drum-rope based rotating algal biofilm system, while other algal biofilm systems reported 5.0 $g\text{-}m^{-2}$ day^{-1} (Mulbry and Wilkie 2001), 9.9 $g\text{-}m^{-2}$ day^{-1} (Boelee et al. 2013), and 80 $g\text{-}m^{-2}$ day^{-1} (Lui et al. 2013). The biofilm system developed by Iowa State University researchers achieved a footprint productivity of 46.8 $g\text{-}m^{-2}$ day^{-1} which outperformed a standard raceway pond by 824% in a side-by-side growth comparison (Gross et al. 2015).

A summary of the two types of biomass productivities obtained by different algal biofilm system was provided in Table 1. The wide range of the biomass productivity reported here was due to the variation of strains, the operation conditions (light intensity, CO_2 concentration, and medium source), and experimental set up (reactor scale, attachment materials). It should be noted that the majority of the biomass productivity data in Table 1 were not specified as ash-containing- or ash-free-dry-weight (AFDW). For a consistent comparison of the different systems, it is encouraged to use AFDW in the further reports to eliminate the inflated values of algal yield and productivity resulting from the soil particles, metals, and other inorganic constituents in the medium. Also, the biomass productivities reported in Table 1 were strongly related with the scale of the system. Extrapolation of the lab-scale data to pilot scale need to be treated with great caution.

Operational factors in algal biofilm systems

In general, the performance of an algal cultivation system depends on the specific design configuration and the purpose for growing algae. For example, in a raceway pond, keeping the algae suspended is crucial in order to reduce the light limitation and enhance CO_2 mass transfer; in an algal biofilm system, the liquid mixing in the reservoir is still needed, but it is not as critical as other parameters because the algae is attached to material. The algal biofilm system has its own unique factors that affect overall performance including algal species, the properties of attachment material, the algal biofilm thickness, and the shear stress applied to the biofilm.

Algal species

In algal biofilm systems, the propensity of algae strains to attach to the surface of the attachment material is a major factor to consider. The attachment of algae to a substrate depends on the surface properties of both the algae and the substrate. Algal cells generally have a negative surface charge that helps repel other negatively charged cells, and thus, minimize undesirable natural flocculation (Gerde et al. 2014). The relative strength of the negative charge also determines the hydrophobicity of the cell membranes (Ozkan and Berberoglu 2013). In turn, cells that are more hydrophobic tend to be superior at forming biofilms (Ozkan and Berberoglu 2013).

In addition to the physiochemical properties of the cell membrane, extracellular polymeric substances (EPS) produced by the algal biofilm also play an important role in cell attachment. EPS are carbohydrate and protein based polymers that can promote adhesion of cells to the attachment material and also "glue" other cells together (Tian et al. 2006). Algal strains capable of producing a large amount of EPS are expected to form biofilm easily.

Attachment materials

Various mechanisms such as hydrophobic interactions (Palmer et al. 2007) or acid-base interactions (Ozkan and Berberoglu 2013) have been proposed to explain the cell attachment to a substrate (attachment material). Among various surface properties, the water-material contact angle (Irving and Allen 2011) and surface energy (Christianson and Sims 2012; Genin et al. 2013) have been extensively studied for their role in cell attachment. However, understanding of the exact surface properties that promote algal attachment has not been fully determined. In addition to the physiochemical properties of the material, texture (surface roughness) also plays an important role in algal attachment (Sekar et al. 2004; Kohler et al. 1999; and Cao et al. 2009; Cui et al. 2014). In general, increasing the surface texture creates zones where liquid velocity is decreased and algal cells can have enough time to settle on the surface and subsequently attach (Cao et al. 2009). Such a texture also minimizes the sheer forces imposed on the biofilm when fluid is flowed over it, which reduces cell sloughing.

In general, the initial attachment of algal cells to the fresh surface of the materials is crucial and can be time intensive. Once the initial colonization occurs, attachment of additional algal cells to the existing algal biofilm layer is relatively easier (Ozkan and Berberoglu 2013). The durability of the attachment materials is another important factor, particularly for the long-term operation of an algal culture. The attachment material will have to resist moist conditions, sometimes with high salinity. The material needs to withstand the physical force applied during mechanical harvest. For example, cotton-based duct canvas and ropes have been used for algal biofilm culture systems (Gross et al. 2013; Christenson and Sims 2012). While these materials show superior algal attachment characteristics, the cotton material deteriorates within 2–3 months, and

thus needs to be replaced frequently (Gross et al. 2013; Gross and Wen 2014), which increases operation down time, reducing productivity and causing additional costs.

Biofilm thickness and sloughing

Once the initial biofilm is established on the surface of the fresh attachment material, it is important to maintain the existing biofilm at an appropriate thickness (Katarzyna et al. 2015). Algal biofilms are photosynthetic and require sufficient light to grow and reproduce. When the biofilm grows, new layers of cells are stacked on top of existing layers, which will eventually increase the shading of the bottom cells as the thickness increases. The bottom cells could also become nutrient deprived because of limited nutrient/mass transfer rates through the biofilm layers. After harvest the bottom cells will serve as inoculum for the next growth cycle and it is critical to maintain a healthy bottom layer of cells. If these cells are unhealthy, an extended lag in the growth phase will be observed during the next growth cycle.

As the biofilm thickens, the structural integrity of the biofilm decreases, which increases the risk of sloughing. Sloughing happens for both stationary and rotating biofilm systems due to the shear stress caused by movement between the liquid flow and the biofilm. This sheer force can be minimized by controlling the velocity of the liquid flow (for the stationary biofilm) or the rotation speed (for the rotating biofilm) (Gross et al. 2013).

Advantages of the algal biofilm systems

Algal biofilm systems have many advantages in comparison to suspension-based systems. The most evident is the simple biomass harvest. In a typical algal biofilm system, algal cells are naturally concentrated in the biofilm, with solids contents ranging from 10–20% (Johnson and

Wen 2010; Gross et al. 2013). The algae can be harvested by scraping, which avoids the expensive harvesting and dewatering processes used for suspension cultures. However, the majority of harvesting operations for current algal biofilm systems are still based on manual operations. Scraping large surface area of the algal biofilm, particularly stationary biofilm systems, is labor intensive. Therefore, a mechanized harvest device needs to be developed in order to mitigate this labor cost.

In addition to the advantage of simple harvest, algal biofilm systems can use light efficiently (Guzzon et al. 2008; Hill et al. 2009; Liu et al. 2013; Blanken 2014). In suspension cultures and in biofilms, light is attenuated from maximal levels at the light-exposed surface to near darkness in the interior of the suspension or biofilm; the exact light gradient depends on light path and biomass density in a suspension (biofilm thickness in case of a biofilm). In theory, there is no fundamental difference between suspension cultures and biofilm cultures with respect to the light gradient. In practice, however, the unique feature of the biofilm system enables the possibility of attenuating this light gradient. This is because in the algal biofilm system, the biofilm thickness can be controlled by frequent harvesting so the biofilm will not get too "thick" and the interior layer will not be completely dark. The biomass from this frequent harvest will still have a high solids content. On the contrary, in the open pond systems, the frequent harvesting of biomass from the dense broth (to avoid light limitation) will only result in a dilute algal density with a large amount of water, and will enviably increase the harvest cost.

The CO_2 mass transfer in the algal biofilm, is a completed issue. On one hand, the mass transfer from the bulk gaseous phase to the cells on the biofilm surface is greatly improved compared to the open ponds due to (1) the very thin liquid boundary layer covering on the surface of the biofilm; and the small eddies (crashing waves) formed when this liquid layer flows over the

bioflm; and (2) a large gas-liquid interface area compared to open ponds. On the other hand, unlike the suspension systems where the dissolved CO_2 molecules can be directly adsorbed by the algal cells, the dissolved CO_2 on the biofilm surface will have to diffuse into the interior layers of the biofilm, which is usually a bottleneck step. However, similar to the light limitation problem discussed previously, the CO_2 diffusion within the biofilm can be alleviated by reducing the thickness of the biofilm (through frequent harvesting). It should also be noted that the above mass transfer feature of the algal biofilm is not confined to the CO_2, other gas species (such as oxygen) and nutrients will also experience the similar process. For example, Bernstein et al, (2015) have experimentally determined an oxygen gradient in the algal biofilm, shortening the thickness of the biofilm will facilitate the photosynthesis of the interior biofilm cells.

Another advantage of algal biofilm systems is the differentiation of hydraulic retention time (HRT) and solids retention time (SRT). In suspension-based systems where algae cells are homogeneously suspended, a specialized cell-liquid separation device is needed to separate the cells from the liquid and return them back to the reactors. In the biofilm systems, cells are retained on the attachment materials without being washed out with the liquid. This is a particular advantage in wastewater treatment applications where a high SRT to HRT ratio is desired.

Vertically-oriented algal biofilm systems can also significantly reduce the footprint area as compared to a conventional raceway pond. This feature has an obvious advantage when land is limited, such as for wastewater treatment facilities in urban areas. The reduced footprint concurrently leads to enhanced aerial biomass productivity. To address this hypothesis, we assume we work with sunlit systems and the cell growth is limited by the light intensity. Because one unit of ground surface (footprint) receives a fixed amount of sunlight energy, the only way

for increasing the biomass productivity per unit of the ground surface is to enhance the efficiency of sunlight conversion to microalgae, i.e., the photosynthetic efficiency (PE). For most plants including microalgae, the theoretical maximum PE value is around 10–12% (Dau and Zaharieva, 2009; Tredici, 2010; Schultze et al, 2015). However for most photobioreactors or raceway ponds, the actual PE value is often lower than the theoretical value due to multiple reasons such as light reflection from culture surface, light penetration limitation due to mutual shading, inefficiencies in photorespiration and limitations in photosynthetic machinery within the cell (such as photo-saturation and photo-inhibition).

As stated earlier, algal biofilms can potentially reduce the light limitation problem by frequent harvesting biomass to keep the biofilm "thin". Also, vertical biofilm systems increase the growing surface area within a given aerial (incidence) area; therefore, the fixed amount of light energy that a given incidence area receives will be diluted when it strikes the larger growing surface area. Commonly direct sunlight results in photo-saturation of photosynthetic machinery within the algae cells and thus reduces PE. However in the vertical biofilm system the direct sunlight is diluted throughout the large biofilm surface area, thus decreasing photo-saturation and increasing PE.

Finally, implementation of a vertical algal biofilm on an open pond can also reduce the impact of light reflection. This is because when the light hits the traditional open pond surface, part of the light will be reflected back to the ambient environment and wasted. While when a vertical biofilm is implemented on the open pond, there is significant chance that reflected light from the pond surface will strike the biofilm surface and be utilized.

All the above factors will increase the PE value within the same light incidence area. This increased PE, together with a fixed amount of sunlight energy per unit of ground surface

(footprint) receives, will lead to an increased biomass yield within the same footprint area. The results from the most recent reports (Blanken et al., 2014; Gross et al., 2015) also support this hypothesis, i.e., rotating algal biofilm results in a higher biomass yield on light compared to suspended systems.

In our most recent study, it was shown that the rotating algal biofilm can increase water use efficiency (Gross et al. 2015). This study found that the water evaporative loss of the rotating algal biofilm system was higher compared to a raceway pond control with the same footprint. However, the biofilm system had a much higher biomass productivity than the raceway pond. As a result, the water consumption per unit of biomass produced in the biofilm system was only 26% of the water required to produce the same unit of biomass in a raceway pond.

Perspective and conclusions

Algal biofilm-based culture systems have drawn renewed interest as an alternative to conventional suspended raceway ponds for algae production. Various algal biofilm systems have shown great promise in minimizing harvesting costs. In addition, these biofilm systems have shown other benefits such as reduced light limitation, enhancement of CO_2 mass transfer, extended biomass retention time, smaller footprint, and better water utilization efficiency. However, the development of algal biofilm systems is still immature compared to suspension-based systems which have received significant funding and resources over the past several years. Future research on algal biofilm systems needs to focus on (1) identifying the appropriate strains and materials for optimal biofilm formation and growth, (2) exploring the value of specialty compounds such as EPS in the biofilm derived biomass, (3) CO_2 transfer and light penetration within the biofilm, (4) long duration pilot- and demonstration-scale studies, and (5) the

economics and sustainability of algal biofilm systems. A better understanding of the above key areas will evolve algal biofilm systems into economically viable alternatives for algal cultivation.

Conflict of interest

Authors Z. Wen, M. Gross, and D. Jarboe have equity interests and management roles in Gross-Wen Technologies, LLC. The terms of this arrangement have been reviewed and approved by Iowa State University in accordance with its conflict of interest policies.

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Table 1. Summary of different algal biofilm systems.

Biofilm System	Growth Medium	Algal Species	Scale	Attachment Material	Footprint Biomass Productivity (g/m²-day)	Surface Biomass Productivity (g/m²-day)	Organization	Reference
Stationary Biofilm Design								
Polystyrene rocker system	Dairy wastewater	*Chlorella sp.*	Lab	Polystyrene	NA	2.59	Virginia Tech University	Johnson and Wen, 2010
Flat plate parallel horizontal photobioreactor	Synthetic medium	*Nitzschia palea, Scenedesmus obliquus*	Lab	Glass	NA	2.80	University of Toronto	Schnurr et al., 2013
Parallel plate air lift reactor	Synthetic medium	Polyculture	Lab	Cellulose acetate	NA	2.08	University of Toronto	Genin et al., 2013
Twin-Layer biofilm photobioreactor	Synthetic medium	*Halochlorella rubescens*	Lab	Printing paper	51	31	University of Cologne	Schultze et al., 2015
Twin-Layer biofilm photobioreactor	Synthetic medium	*Isochrysis, Tetraselmis, Phaeodactylum, Nannochloropsis*	Lab	Printing paper	NA	1.8	University of Cologne	Naumann et al., 2013
Twin-Layer biofilm photobioreactor	Municipal wastewater	*Halochlorella rubescens*	Lab	Nylon filter sheets	1.2	6.3	University of Cologne	Shi et al., 2014
Algal disk/vertical plate attached photobioreactor	Synthetic medium	*Scenedesmus obliquus*	Pilot	Glass plate, filter paper	80	NA	Chinese Academy of Sciences	Liu et al., 2013
Concrete slab algae biofilm photobioreactor system	Synthetic medium	*Botryococcus braunii*	Lab	Concrete	0.71	NA	University of Texas	Ozkan et al., 2012
Flow lane biofilm reactor	Synthetic medium	Polyculture	Lab	Polyfelt sheet	9.9	NA	Wageningen University	Boelee et al., 2013

Roof installed parallel plate microalgae biofilm reactor	Domestic wastewater	*Scenedesmus obliquus*	Lab	Polycarbonate sheet	2.5	NA	Ghent University	Zamalloa, 2013
Enclosed Biofilm Tubular Reactor	Swine slurry	*Chlorella sorokiniana*	Lab	Polyvinyl chloride	NA	NA	University of Valladolid	Godos et al., 2008
Algal Turf Scrubber	Dairy wastewater	Polyculture	Pilot	Polyethylene mesh	5.0	NA	United State Dept. of Agriculture	Mulbry and Wilkie, 2001
Algal based immobilization reactor	Municipal wastewater	*Scenedesmus sp.*	Lab	Polypropylene	NA	NA	Shanghai Jiaotong University	He and Xue, 2010
Flow lane biofilm reactor	Municipal wastewater	Polyculture	Lab	Polyvinyl chloride	NA	NA	University of Valladolid	Posadas et al., 2013
Algal biofilm membrane photobioreactor	Municipal wastewater	*C. vulgaris*	Lab	Flexible fiber bundles	NA	NA	Zhejiang Ocean University	Gao et al., 2015
Suspended-solid phase photobioreactor	Synthetic medium	*Scenedesmus sp.*	Lab	Cotton, Linen, Mohair	NA	NA	Tsinghua University	Zhuang et al., 2014
Single layer attached photobioreactor	Synthetic medium	*Botryococcus braunii*	Lab	Cellulose acetate	NA	6.45	Chinese Academy of Sciences	Cheng et al., 2014
Rotating Biofilm Design								
Revolving algal biofilm cultivation system	Synthetic medium	*Chlorella vulgarus*	Lab	Cotton duct	10.5	3.51	Iowa State University	Gross et al., 2013
Revolving algal biofilm cultivation system	Synthetic medium	*Chlorella vulgarus*	Pilot	Cotton duct	46.8	5.80	Iowa State University	Gross and Wen, 2014; Gross et al., 2015
Rotating algal biofilm reactor with spool harvester	Municipal wastewater	Polyculture	Lab	Cotton rope	20	NA	Utah State University	Christenson and Sims, 2012

Rotating algal biofilm reactor with spool harvester	Municipal wastewater	Polyculture	Pilot	Cotton rope	31	NA	Utah State University	Christenson and Sims, 2012
Rotating algal biofilm reactor	Municipal wastewater	Polyculture	Lab & Pilot	Cotton rope	NA	NA	Utah State University, Montana State University	Bernstein et al., 2014
Photo rotating biological contactor	Copper mine wastewater	*Klebsormidium sp.*	Lab	Polyvinyl chloride	NA	NA	University of Adelaide	Orandi et al., 2012
Algadisk	Synthetic medium	*Chlorella sorokiniana*	Lab	Stainless steel, polycarbonate	NA	20.1	Wageningen University	Blanken et al., 2014

CHAPTER 3. YEARLONG EVALUATION OF PERFORMANCE AND DURABILITY OF A PILOT-SCALE REVOLVING ALGAL BIOFILM (RAB) CULTIVATION SYSTEM

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Abstract

Current algal cultivation has been mainly performed in open ponds or photobioreactors in which algal cells are suspended and harvested through flocculation and centrifugation. A unique attachment based Revolving Algal Biofilm (RAB) cultivation system was recently developed for easy biomass harvest with enhanced biomass productivity. The objective of this research was to evaluate the performance (durability, algal growth, and the geometry) of the RAB system at pilot-scale. A yearlong test of the RAB system was successfully conducted at a greenhouse facility at Boone, Iowa, USA. The RAB resulted in an average of 302% increase in biomass productivity compared to a standard raceway pond, with a maximum biomass productivity (ash free) of 18.9 g/m^2-day being achieved. The RAB with a vertical configuration generated higher productivity than the triangular RAB. Collectively, the research shows that the RAB as an efficient algal culture system has great potential for being deployed at commercial scale.

1. Introduction

Microalgae have been identified as a promising biomass feedstock that can produce a variety of fuels, feeds, and chemicals. While, biodiesel is the most common fuel produced from microalgae, other types of biofuels can also be produced from microalgae such as alcohol via fermentation of carbohydrates (Wang et al., 2011), biogas through anaerobic digestion (Gunaseelan, 1997) and bio-oil from thermochemical processing (Yang et al., 2004). Other products for microalgae biomass include fertilizers (Mulbry et al., 2008), nutraceuticals (Hudek, 2014), and aquacultural feed (Duerr et al, 1998, Hemaiswarya et al, 2010).

Commercial algal production, however, is still facing the challenge of high capital and operational costs associated with growth and processing of the algal biomass. This is primarily due to the high costs of harvesting algal cells from conventional suspension culture systems where the cell densities are typically very low. For example, algae cell density can be 0.5 g/L (0.05% dry weight or 99.95% moisture content) in open ponds and 2-6 g/L (0.2-0.6% dry weight or 99.4-99.8% moisture content) in photobioreactors (National Algal Biofuels Technology Roadmap, 2010). To harvest algae from these systems, the algal culture broth is first concentrated to 1% solids via flocculation, floatation, or sedimentation, the algal slurry is further concentrated to 10-20 wt% using a centrifugation or filtration (Davis et al., 2011). These multiple operations are time consuming and expensive. Molina Grima et al. (2003) reported that algae harvest and dewatering contribute up to 30% of total production costs. In a recent report by Davis et al. (2011), biomass harvesting costs contribute to approximately 21% of the total capital costs in an open pond system.

As an alternative to suspended algal growth systems, biofilm based growth systems show promise in decreasing the high costs associated with harvest. In biofilm cultivation systems,

algae cells are attached on the surface of a supporting material and harvested by scraping. The biomass harvested from these systems usually has a water content between 80-90%, similar to centrifuged algal biomass (Gross et al, 2013 & Johnson and Wen, 2010); therefore, the expensive concentrating and dewatering steps utilized in suspended algal culture can be avoided.

In algal biofilm systems, the attachment of algal cells to the supporting material depends on the surface properties of both the algal cells and the material, which can either inhibit or promote attachment (Ozkan and Berberoglu, 2013). Various attachment mechanisms have been proposed such as hydrophobic interactions (Palmer et al, 2007) or acid-base interactions (Ozkan and Berberoglu, 2013). Material surface properties such as water-material contact angle (Irving and Allen, 2011) and surface energy (Christianson and Sims, 2012) have also been reported to alter algal attachment rate.

Algal biofilm reactors were first reported in 1980s with rotating disks of polystyrene being used to grow algal biofilm to reduce nitrogen and phosphorus in municipal wastewater (Przytocka-Jusiak et al., 1984). Various types of algal biofilm systems have been developed since then. For example, the Algal Turf Scrubber (ATS) system was designed around the concept of surge flow in coral reef communities and has been explored for treating municipal and animal wastewater (Mulbry and Wilkie, 2001). Researchers at Utah State University reported a rope-based rotating drum as an attached algae growth system (Christenson and Sims, 2012). The biomass attached on the rope is harvested by squeezing the algae off the rope. Bioprocess Algae LLC also reported a proprietary biofilm system by placing parallel sheets in a vertical orientation inside a greenhouse-like enclosure that is enriched with CO_2. Algae grow on the surface of the vertical material, while a liquid medium is applied to the algal biofilm through a delivery system (http://www.bioprocessalgae.com).

Recently, a novel Revolving Algal Biofilm (RAB) system was developed in our group (Gross et al., 2013). The RAB cultivation system has two major parts; a flexible belt that is continuously rotating, and a liquid reservoir. The RAB system has proved several advantages over conventional open ponds or photobioreactors including high biomass productivity, easy harvest, high CO_2 mass transfer rate, and enhanced light utilization (Gross et al. 2013). The previous research on the RAB operation mainly focused on laboratory-scale tests. However, in order to explore the feasibility and implement this system at commercial-scale, a pilot-scale RAB system needs to be developed, and the operation under real environmental conditions needs to be evaluated. Therefore, the objective of this work is to fill this gap by performing a pilot-scale RAB test over a one year period, so that the scalability, durability, and productivity of the RAB system can be evaluated.

2. Materials and Methods

2.1 Algal strain and subculture

The microalgae *Chlorella vulgaris* (UTEX #265) was used in this work due to its fast and robust growth. The same strain was also used in our previous laboratory-scale study (Gross et al., 2013) so it can give us an objective evaluation of the algal growth system by eliminating the variation caused by algal strain. The cells were maintained on agar slant at 4°C and transferred to 250-mL Erlenmeyer flasks containing 50-mL modified Bold's Basal Medium (Bischoff and Bold, 1963). The media was sterilized with an autoclave at 121°C for 15 minutes prior to use. The flasks were placed in an orbital shaker set at 200 rpm and 25°C with continuous illumination at 110-120 µmol s^{-1} m^{-2}. The cultures were incubated for 5 days and then stepwise scaled up to a 20-L and a 200-L flat panel photobioreactors, the cells were incubated for 5 days in each reactor.

The culture from the 200-L flat panel photobioreactor was inoculated into 2000-L raceway ponds for pilot-scale testing.

2.2 Design of the algal cultivation systems

The concept of Revolving Algal Biofilm (RAB) design and its operation at laboratory-scale have been described in our previous report (Gross et al., 2013). In brief, a flexible cell attachment material made of cotton duct fabric was stretched around drive shafts to form a triangle configuration. The lowest elevated region of the material was submerged in a nutrient-rich medium reservoir for nutrient supply, while the rest of the attachment material was exposed to the atmospheric conditions. A motor drove the drive shafts which rotated the material between the liquid phase and gas phase, the algal biofilm on the substratum was, therefore, alternatively accessing nutrients and CO_2.

In previous work the RAB operation was only conducted using a triangular geometry rotating belt. In this work the RAB system was also modified into a vertical configuration which resembles a conveyor belt in a vertical orientation. As shown in Figure 1, a raceway pond ($8.5m^2$) was retrofit with two triangle and six vertical RAB systems. Each rotating belt in the triangle and vertical RAB systems were one meter tall with a surface area of 2.94 m^2 and 1.85 m^2 respectively. As a control, a standard raceway pond identical to the RAB retrofit raceway pond was used as comparison baseline.

2.3 Algal production facility

All the algal cultivation systems were operated over a 1 year period (January-December, 2013) in the Iowa State University algal production greenhouse facility located at the Bio-

Century Research Farm in Boone, Iowa, USA. This greenhouse is made of transparent twin wall polycarbonate. The inside temperature was maintained between 11-35 $^{\circ}$C with geothermal heating and cooling. Natural light was used exclusively and fluctuated throughout the year. Throughout experimentation temperature, light intensity, photosynthetically active radiation (PAR) were recorded every hour (Onset HOBO data logger, Massachusetts USA). This data was logged then averaged for each day and represented as daily change throughout the entire year.

2.4 Operation of RAB systems

Each raceway pond was first inoculated with seed from the flat panel photobioreactors, and circulated with a paddle wheel rotating at 50 rpm, which resulted in a liquid liner velocity of 20 cm/sec. The raceway ponds were operated at a 14 day hydraulic retention time (HRT) by replacing half of the pond liquid every 7 days. The RAB systems were then started by rotating the attachment materials (belts) at 1.2 rpm with a linear velocity of 4 cm/sec. The RAB systems were run for the first 14 days to establish the initial growth of algae on the surface of the attaching material. At the end of 14 days, a thick algal biofilm was formed; the biomass was harvested by scraping with a rubber squeegee. After scraping, the residual cells that remained on the material served as inoculum for the next cycle of cell growth (regrowth).

The regrowth operation was repeated from January to June 2013, and biomass was harvested every 7 days. In late June 2013, the cotton duct attaching material was replaced due to material deterioration. From June to December 2013, the RAB system was used for multiple tests such the cell attached growth kinetics and evaluating biomass productivity as a function of harvest time, therefore, the operation of every 7 day harvest/regrowth was run less frequently.

During the operation of the RAB system, the control raceway pond was run in parallel. The suspended culture broths in the raceway ponds (both the RAB-retrofitted-pond and the control pond) were sampled to evaluate cell density.

The sampling of suspended algae in the open ponds was conducted in three replicates, while the sampling of attached algae in the RAB system was conducted in twelve replicates.

2.5 Characterization of algal growth performance in RAB systems

In suspended culture, algal growth performance was evaluated by biomass productivity based on the pond surface area (P_{pond}, unit: g/m^2-day). The value of P_{pond} was determined as follows,

$$P_{pond} = \frac{C \times V}{A \times HRT} \qquad (1)$$

Where C is the cell dry weight concentration, V is the total volume, A is the surface area of the pond (8.5 m^2), HRT is hydraulic retention time (14 day). Cell dry weight concentration was determined by measuring optical density at 680 nm (OD_{680}) first, and converted using a correlation curve between cell dry weight and the OD_{680}.

In the RAB system the algal attached growth performance was based on two criteria, surface biomass productivity ($P_{surface}$), which is based on the surface area of the attachment materials; and aerial biomass productivity (P_{aerial}) which is based on the aerial (footprint) area of the retrofit raceway pond. The calculations of these two parameters are as follows,

$$P_{surface(RAB)} = \frac{Y}{F} \qquad (2)$$

$$P_{aerial(RAB)} = \frac{Y \times S}{A \times F} = (P_{surface(RAB)}) \times \frac{S}{A} \qquad (3)$$

Where Y is the biomass yield per unit of attachment surface area, F is the harvest frequency (7 days), S is the total surface area of the attachment material, A is the surface area of

the pond (8.5 m^2). The value of Y was determined by harvest the biomass from a given area (1 ft^2) of the biofilm, and dividing the biomass by this given area. It should be noted that all the biomass productivity data reported in this work as ash-free basis unless otherwise noted.

2.6 Characterization of chemical composition of the algal biomass

The ash content of the biomass was first determined by heating the biomass in a furnace to 550°C for 6 hours and weighing the remaining matter. The lipid content was quantified according to the Bligh and Dyer method (Bligh and Dyer, 1959). The protein content was estimated by measuring the total Kjeldahl nitrogen (TKN) of the biomass and multiplying by the previously determined conversion factor of 5.95 (Lopez, 2010). The carbohydrate content was determined by subtracting ash, lipid and protein from the total biomass. To determine the fatty acid composition, fatty acid methyl esters (FAME) were prepared following the procedures reported previously Pyle et al., (2008), and quantified by GC-FID using the method developed by Liang et al., (2011). Then, all the chemical composition of the algal biomass was represented as ash-free basis.

3. Results and Discussion

3.1 Yearlong algal growth performance in different algal culture systems

In this work, various algal culture systems were run throughout the year 2013, the biomass productivity as defined in Eqs. (1- 3) is evaluated. Figure 2A shows that the control raceway pond has a higher biomass productivity than the suspended algae in the raceway pond retrofit with the RAB. To make a better comparison, the aerial biomass productivity of the RAB system ($P_{aerial(RAB)}$) was plotted as an indication of the biomass produced in the same size of pond area. It was found that the triangular RAB system generated a similar aerial biomass

productivity, while the vertical RAB system produced a much higher aerial biomass productivity compared to the control pond (Figure 2A). When comparing performance of the suspension and the biofilm algae, the pond-RAB hybrid system significantly outperforms the control raceway with a much higher aerial biomass productivity. This is the case for both the triangular and vertical RAB systems. The yearlong average productivity for the control raceway pond was 2.82 g/m^2-day, triangular RAB retrofit raceway was 5.27 g/m^2-day, vertical RAB retrofit raceway was 11.36 g/m^2-day and maximum values for these three systems were 5.22, 8.51, 18.92 g/m^2-day, respectively.

The biomass productivity of the triangular and vertical RAB systems was further compared to evaluate the effects of varying geometries of the RAB system on the algal growth performance. The aerial biomass productivity of the two systems is shown in Figure 2A. The vertical RAB system outperformed the triangular RAB system throughout the whole year culture. On average the vertical RAB system result in 187% higher biomass productivity then the triangular RAB system. However when surface (attachment area) biomass productivity is evaluated, there is no significant difference between the two systems ($P > 0.05$) (Figure 2B). Since the geometry of the RAB system does not significantly affect biomass productivity per unit of attachment area, the increase in aerial biomass productivity is mainly caused by the increase of total surface area of attachment material.

The above results show that altering the geometries of the RAB reactor is an effective way to improve the aerial biomass productivity. In certain circumstances algal cultivation may be limited to a relatively small foot print area, the RAB system could be a good candidate because it can utilize space in three dimensions by growing algal vertically, and thus increase the aerial biomass productivity without increasing the footprint area. In particular, the RAB

geometries can be manipulated to increase the total amount of attachment area in a given aerial

area (for example, vertical vs. triangular configuration in this work); as a result, the aerial

biomass productivity can be greatly enhanced. However, it should be noted that some

geometries and orientations of the RAB belts will affect surface productivity due to shading of

the biofilm, so a careful design and orientation of the RAB system is needed to maintain the high

biomass productivity. Future study on the quantitative effects of shading on the RAB system

needs to consider all these factors.

In addition to evaluating productivity, the yearlong research also enabled us to evaluate

the durability of the RAB cultivation systems at pilot-scale. Through the entire year long

operation, the RAB system operated well other than the deterioration of the cotton duct

attachment material. Cotton will naturally deteriorate over time due to the physical properties of

the material. In this work, the same cotton belt can sustain for a six month period before the belt

needed to be replaced. In commercial operations this six month belt lifetime may not be

sufficient. Future research to find an appropriate non-degradable attachment material is needed.

3.2 Yearlong environmental conditions and their effects on biomass productivity

Throughout this yearlong study the environmental conditions including temperature,

hours of sunlight, solar radiation, and PAR (photosynthetically active radiation) were recorded

(Figure 3). The purpose of this evaluation was to determine the most influential parameters that

contribute to variation in biomass productivity. The biomass productivities of both triangular

and vertical RAB systems show a similar trend with environmental conditions (Figures 4 & 5).

In general, the biomass productivity showed a positive trend with daylight time (Figures 4A &

5A) and temperature (Figures 4B & 5B), these positive relationships between biomass

productivity and hours of daylight is due to the increased photosynthetic activity at longer

durations of daylight (Seyfabadi et al., 2010). Similarly, increasing temperature leads to an

elevated metabolic activity, and thus, higher biomass productivity until a threshold temperature is

reached. In terms of solar radiation and PAR, the biomass productivity appears to reach to the

highest levels at the mediocre range of these two parameters (Figures 4 & 5), indicating the cells

were under photo-inhibition when solar radiation and PAR were at high levels. Furthermore, the

authors attempted to correlate biomass productivity with the daylight time and temperature using

the linear regression, and with the solar radiation and PAR using the second order polynomial

relationship. However, very low correlation coefficients ($R^2 < 0.5$) were obtained in those

correlations (Figures 4 & 5), indicating quantitative relationships between biomass productivity

and the environments factors cannot be reached.

3.3 Algal growth kinetics on biofilm

During June 10th -20th, 2013, the cell growth in the RAB system reached to the highest

level due to the optimal environmental conditions achieved in this season (Figure 2). During this

period of time, a detailed algal attached growth profile in terms of a time course of biomass yield

and productivity was studied. On June 9th a clean harvest was conducted; the residual algal cells

on the surface of the material were allowed to regrow and the samples were taken every day

from June 13-20. The growth profile was analyzed to evaluate attached growth kinetics and the

optimal harvest frequency. As shown in Figure 6A, the algal cells are in lag phase until day 3

when log phase begins and after day 5 mutual cell shading and sloughing slows growth but yield

is still increasing at day 10. Based on the results in Figure 6A, the specific growth rate for the

triangular and vertical RAB systems were calculated as 0.319 day^{-1}, and 0.357 day^{-1}, respectively.

Figure 6B shows the biomass productivity as a function of culture time. It shows that the productivity is the highest at a 5-7 days of culture time, indicating the harvest frequency at this time frame will result in the highest productivity for both the vertical and triangle systems (Figure 6B). The likely reason for variation in productivity is due to the algae first being in a lag growth phase and once log phase is achieved mutual cell shading and sloughing causes productivity to decrease.

It should be noted that the cell growth performance presented in Figure 6 is the best results among the yearlong test, and is only valid at the environmental conditions during this 10 day period. If this growth profile analysis was done in the winter when the RAB system received much less light, the growth profile may be different (lower yield and productivity). Therefore, future research is needed to evaluate the growth profile during other seasons of the year.

3.4 Chemical composition of algal biomass

In addition to growth performance, the algal composition obtained from the various cultivation systems was also evaluated. For each culture system, eight samples were randomly selected throughout the yearlong operation; the chemical composition of these eight algal biomass samples was relatively constant, indicating the seasonality did not change the chemical composition significantly. An ash analysis (Table 1) shows that ash content in the biomass fluctuates significantly between various growing systems and growth conditions. The ash content in regrowth algae contains less ash than initial growth and the triangular RAB grown algae has a higher ash content compared to vertical grown. The reasoning for this high ash content is not

very well known but a potential explanation could be that the triangular biofilm system grown algae spend more time outside of liquid and will have a higher evaporation rate which could concentrate minerals in the biomass. More research needs to be done to fully understand this variation.

The proximate (carbohydrate, protein, lipid) analysis of the ash-free algal biomass was further determined. As shown in Table 1, the proximate analysis results show the same trend as our previous lab-scale research in that carbohydrate content is higher and lipid content is lower in the RAB system compared to algae grown in suspension (Gross et al., 2013). The high carbohydrate content in the RAB system may be caused by two reasons. First, algae in the RAB system experienced greater light intensities because the cells spend time in the gaseous environment giving direct contact with sunlight. In previous work Bra´nyikova, (2010) showed at high light intensities algae can promote heightened carbohydrate content in the cell biomass. As more carbon was directed to the carbohydrate synthesis pathway, the lipid synthesis of the algae was thus reduced. The second possible reason for high carbohydrate content in attached growth system is that the biofilm produced extra extracellular polymeric substances (EPS), which eventually led to a high level of total carbohydrates. Indeed, EPS contain polysaccarides and is commonly found in biofilms (Genin et al., 2013).

The chemical composition comparison of the initial growth biomass and the re-growth biomass was also compared. As shown in Table 1, the biomass in the initial growth phase had a higher ash content than the re-growth biomass. Additionally, the carbohydrate, lipid and protein content of the Initial Growth Biofilm were located between the levels of Suspended Algae and the Re-growth Biofilm. This is because the suspended algae were used as the seed to inoculate the initial growth biofilm, which eventually reached the re-growth biofilm after multiple

harvests. Therefore, the algae in the initial growth biofilm were transitioning from the suspended algae to the re-growth biofilm, and exhibited the cell composition between these two stages.

Further studies could be conducted to identify whether the carbohydrates are storage carbohydrates such as starch or exopolysaccharide carbohydrates (Suresh and Mody, 2009). The fatty acid compositions of the algal biomass at initial growth vs. regrowth stage in the RAB systems were analyzed (Table 2). It was found that C16:0, C18:1 and C18:3 were the major fatty acids. The algae in the initial growth stage tended to have more saturated and shorter fatty acids than the algae in the regrowth stage, which contained longer and more polyunsaturated fatty acids. In terms of total fatty acid content, initial growth algae contained a much lower TFA content than the regrowth algae, while vertical RAB regrowth algae had a slightly higher fatty acid content than the triangular RAB regrowth algae.

3.5 Comparison of different algal attached cultivation systems

Various attached algal biofilm cultivation systems have been reported in recent years. Table 3 summarizes algal biofilm research published in the past five years. As shown in the table, the majority of these systems are based on lab-scale and indoor environments. The attachment materials varied from cotton based materials to non-degradable polymer sheets.

Table 3 also compared the biomass productivity among different algal biofilm systems. When aerial biomass productivity is considered, the 21.5 g/m^2-day (or 18.9 g/m^2-day based on ash-free biomass) productivity achieved in our pilot-scale RAB system is on the high end of published data, only the algal disk/vertical plate attached photobioreactor (Liu et al., 2013) and the rotating algal biofilm reactor with spool harvester (Christenson and Sims, 2012) achieved a higher aerial productivity. However, both of these two systems only considered the footprint of

the biofilm system when aerial productivity was calculated. In contrast, our aerial productivity was based on the footprint area of the entire raceway pond, which was not fully covered by the RAB system. If only the foot print of the RAB system is considered, our study would result in an aerial productivity of 104.4 g/m^2-day. In addition to the aerial biomass productivity, the surface biomass productivity (the productivity based on per surface area of the attachment material) was also presented. As shown in Table 3, data in this category was presented not as widely as the aerial biomass productivity. Among those limited surface biomass productivity reported, our RAB system achieved 5.8 g/m^2-day, which is higher than other published data.

The authors suggest further research on algal biofilm systems reports both aerial and surface productivity in order to provide a comprehensive and fair comparison. For example, Liu et al. (2013) utilizes a vertical plate attached photobioreactor that grows vertically but does not have a large footprint, and because of the small footprint a productivity of 80 g/m^2-day is achieved. In comparison, Genin et al. (2013) utilized a similar parallel plate photobioreactor but reported a surface productivity of 2.08 g/m^2-day. If these researchers provided both an aerial and a surface productivity a proper comparison could be made. It should be noted that algal biofilm systems have also been researched and developed by various companies (Table 4). However, the detailed information of the system is often not available in the public domain.

4. Conclusion

This work reported yearlong operation of a pilot-scale revolving algal biofilm (RAB) cultivation system. The RAB system was able to operate without major malfunction for the duration of experimentation while achieving a maximum biomass (ash-free) productivity of 18.9 g/m^2-day and an average 12-month ash free biomass productivity of 11.36 g/m^2-day or a 301%

higher productivity compared to the control raceway pond. Due to its inexpensive harvest and high biomass productivity, the RAB system shows promise as an alternative algal cultivation system compared to suspension based algal culture systems.

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Table 1. Ash content (% dry weight) and the chemical position (% ash-free dry weight) of algal biomass at different cultivation systems.

Composition	Initial Growth Biofilm		Re-growth Biofilm		Suspended Algae	
	Triangle RAB	Vertical RAB	Triangle RAB	Vertical RAB	Control pond	RAB Retrofit Pond
Ash (%DW)	39.2 ± 3.2	29.1 ± 3.1	31.2 ± 2.4	12.4 ± 2.5	28.3 ± 3.0	18.5 ± 2.7
Chemical composition (% ash-free DW)						
Carbohydrate	49.2 ± 3.2	52.3 ± 3.3	65.4 ± 3.4	65.6 ± 3.5	27.3 ± 3.3	23.3 ± 3.7
Lipid	10.2 ± 0.9	8.0 ± 1.1	7.2 ± 0.9	7.6 ± 0.5	12.8 ± 0.6	12.8 ± 0.3
Protein	40.6 ± 0.2	39.7± 0.2	27.4 ± 0.4	26.8 ± 0.5	59.9 ± 0.2	63.9 ± 0.7

Table 2. Fatty acid composition of the attached algae (ash-free) of different RAB systems at initial growth and re-growth stage

| Fatty acid (% TFA)	Initial Growth		Re-growth	
	Triangle	Vertical	Triangle	Vertical
C16:0	38.68±0.37	36.62±0.37	35.04±0.06	35.93±0.04
C16:1	6.30±0.20	10.41±0.20	2.65±0.03	3.00±0.04
C18:0	6.32±0.02	9.43±0.02	3.76±0.07	3.92±0.18
C18:1	29.94±0.16	27.01±0.16	37.61±0.62	33.33±0.13
C18:2	8.18±0.14	4.74±0.14	6.79±0.09	8.17±0.10
C18:3	10.57±0.20	12.78±0.20	14.16±0.09	15.65±0.16
TFA (% ash-free DW)	0.69±0.12	0.68±0.11	2.37±0.41	2.67±0.32

Table 3. Comparison of various algal attached growth systems. [a]

| Biofilm System	Scale	Condition	Attachment Material	Aerial biomass productivity (g/m^2-day)	Surface biomass Productivity (g/m^2-day)	Organization	Reference
Revolving algal biofilm cultivation system	Pilot	Greenhouse	Cotton duct	21.5 18.9 (ash-free)	5.8 5.1 (ash-free)	Iowa State University	This study
Revolving algal biofilm cultivation system	Lab	Indoor	Cotton duct	10.5	3.51	Iowa State University	Gross et al.., 2013
Rotating algal biofilm reactor with spool harvester	Lab	Indoor	Cotton rope	20	NA	Utah State University	Christenson and Sims, 2012
Rotating algal biofilm reactor with spool harvester	Pilot	Outdoor	Cotton rope	31	NA	Utah State University	Christenson and Sims, 2012
Polystyrene rocker system	Lab	Indoor	Polystyrene	NA	2.59	Virginia Tech University	Johnsen and Wen, 2010
Flat plate parallel horizontal photobioreactor	Lab	Indoor	Glass	NA	2.8	University of Toronto	Schnurr et al., 2013
Parallel plate air lift reactor	Lab	Indoor	Cellulose acetate	NA	2.08	University of Toronto	Genin et al., 2013
Algal disk/vertical plate attached photobioreactor	Lab	Indoor	Glass plate and filter paper	80	NA	Chinese Academy of Sciences	Liu et al., 2013
Concrete slab algae biofilm photobioreactor system	Lab	Indoor	Concrete	0.71	NA	University of Texas	Ozkan et al., 2012
Flow lane biofilm reactor	Lab	Indoor	Polyfelt sheet	9.9	NA	Wageningen University	Boelee et al., 2013
Roof installed parallel plate microalgae biofilm reactor	Lab	Indoor	Polycarbonate sheet	2.5	NA	Ghent University	Zamalloa, 2013

[a] The productivity values reported here are the maximal productivity of biomass. The productivities reported in this study are based on both ash-containing biomass and ash-free biomass. The data reported in other references are not specifically mentioned as ash-free and ash-containing.

Table 4. Companies reporting in algal biofilm cultivation systems

| Organization	Biofilm System	Reference
European Commission	Algadisk	www.algadisk.eu
Hydromentia	Algal Turf Scrubber	http://www.hydromentia.com
BioProcess Algae	Grower Harvester	http://www.bioprocessalgae.com
GreenShift Corp.	GS CleanTech CO_2 bioreactor	http://www.greenshift.com
OneWater Inc	Algaewheel	http://www.onewaterworks.com
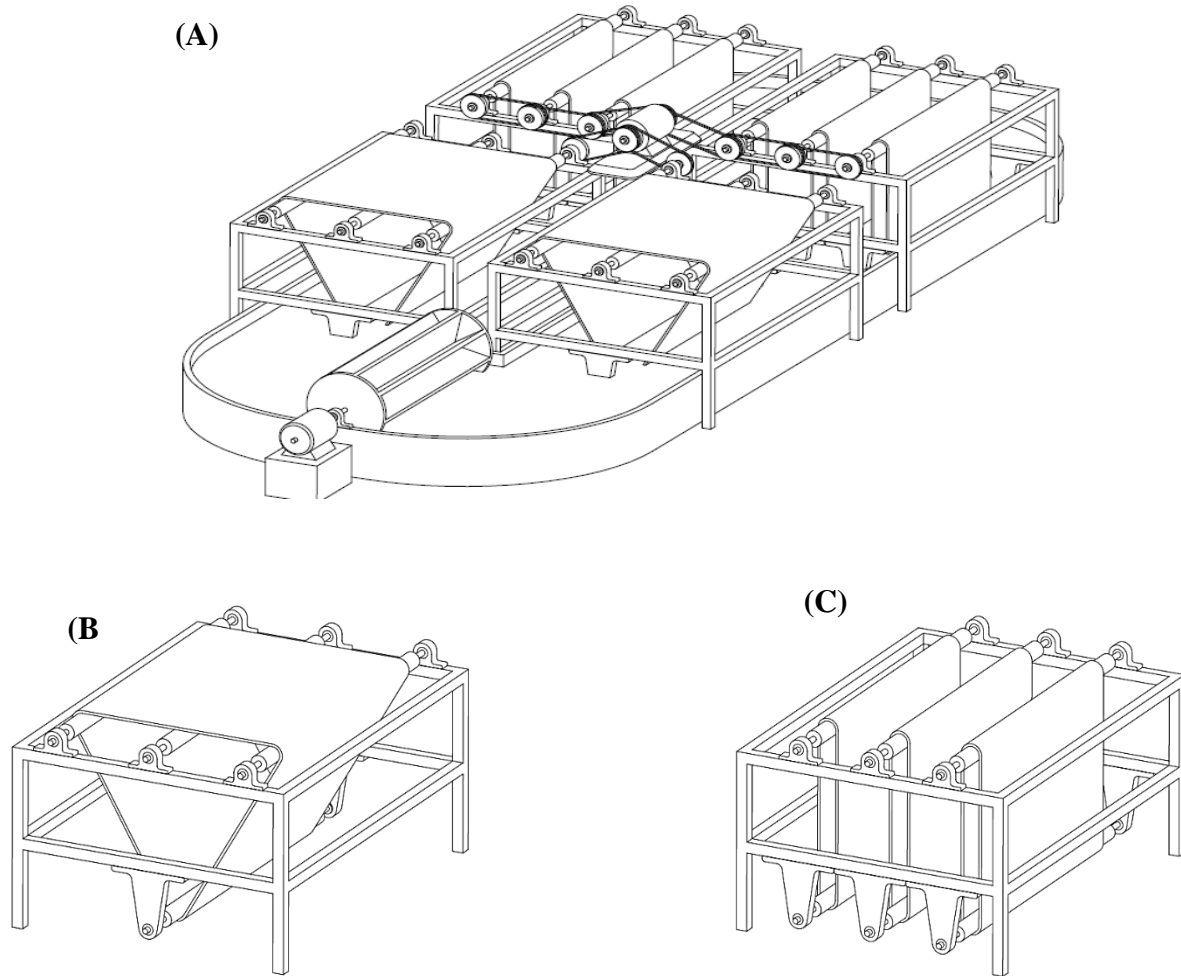
Figure 1. **(A)** Open pond raceway retrofit with triangular RAB system and vertical RAB system **(B)** Schematic of a triangular RAB system **(C)** Schematic of a vertical RAB system
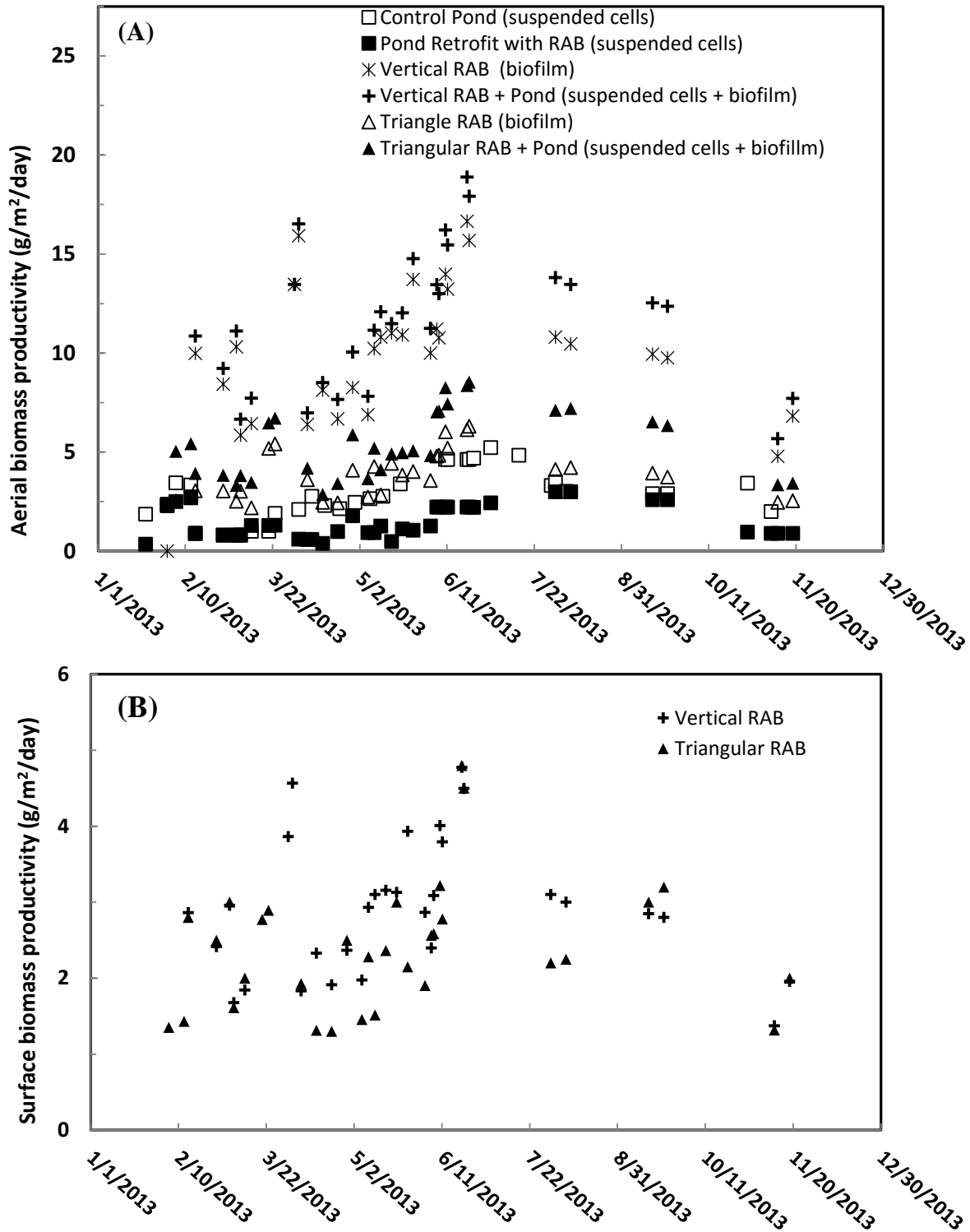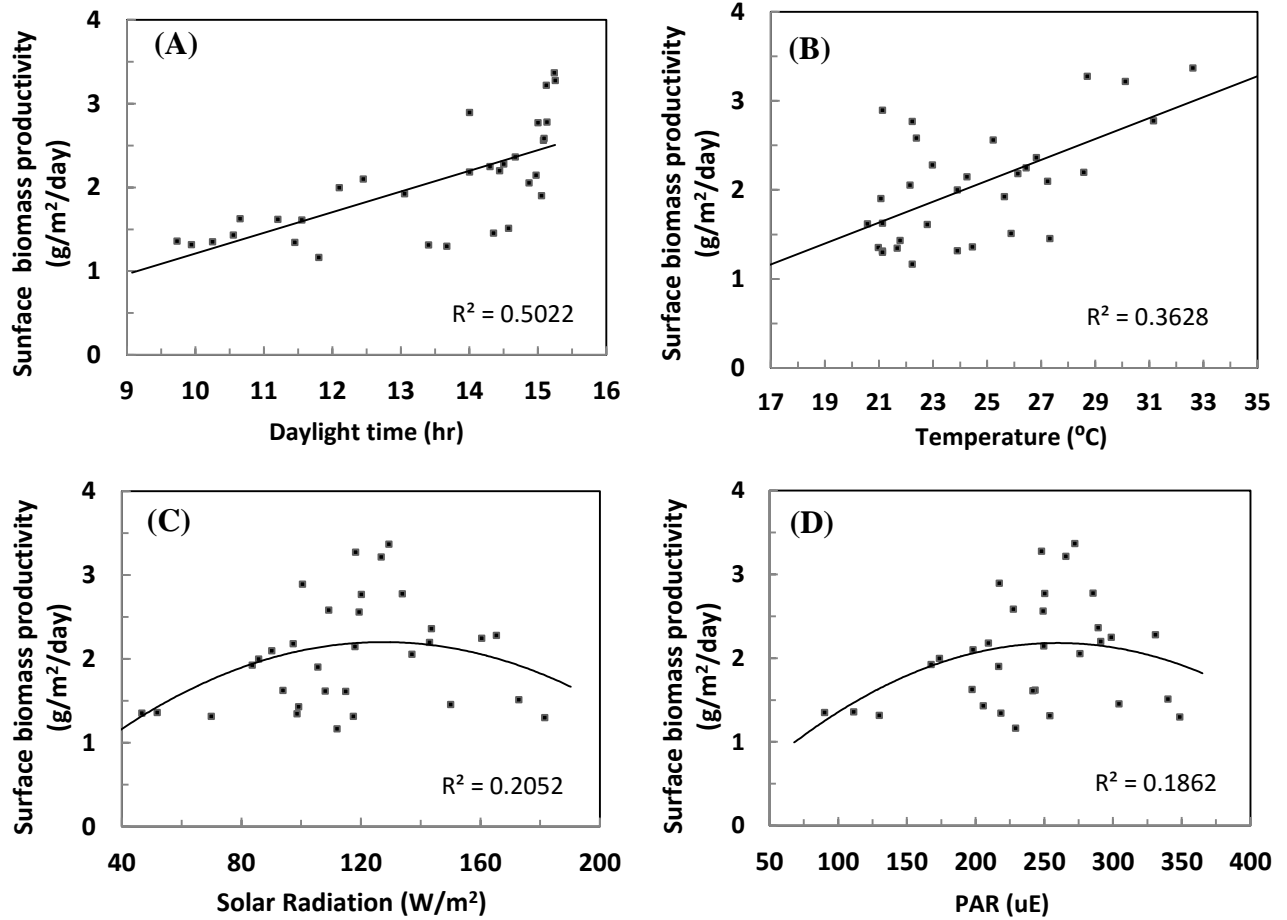
Figure 2. (A) Yearlong aerial biomass (ash-free) productivity obtained in different algal culture systems. **(B)** Yearlong surface biomass (ash-free) productivity comparison between vertical and triangular RAB system
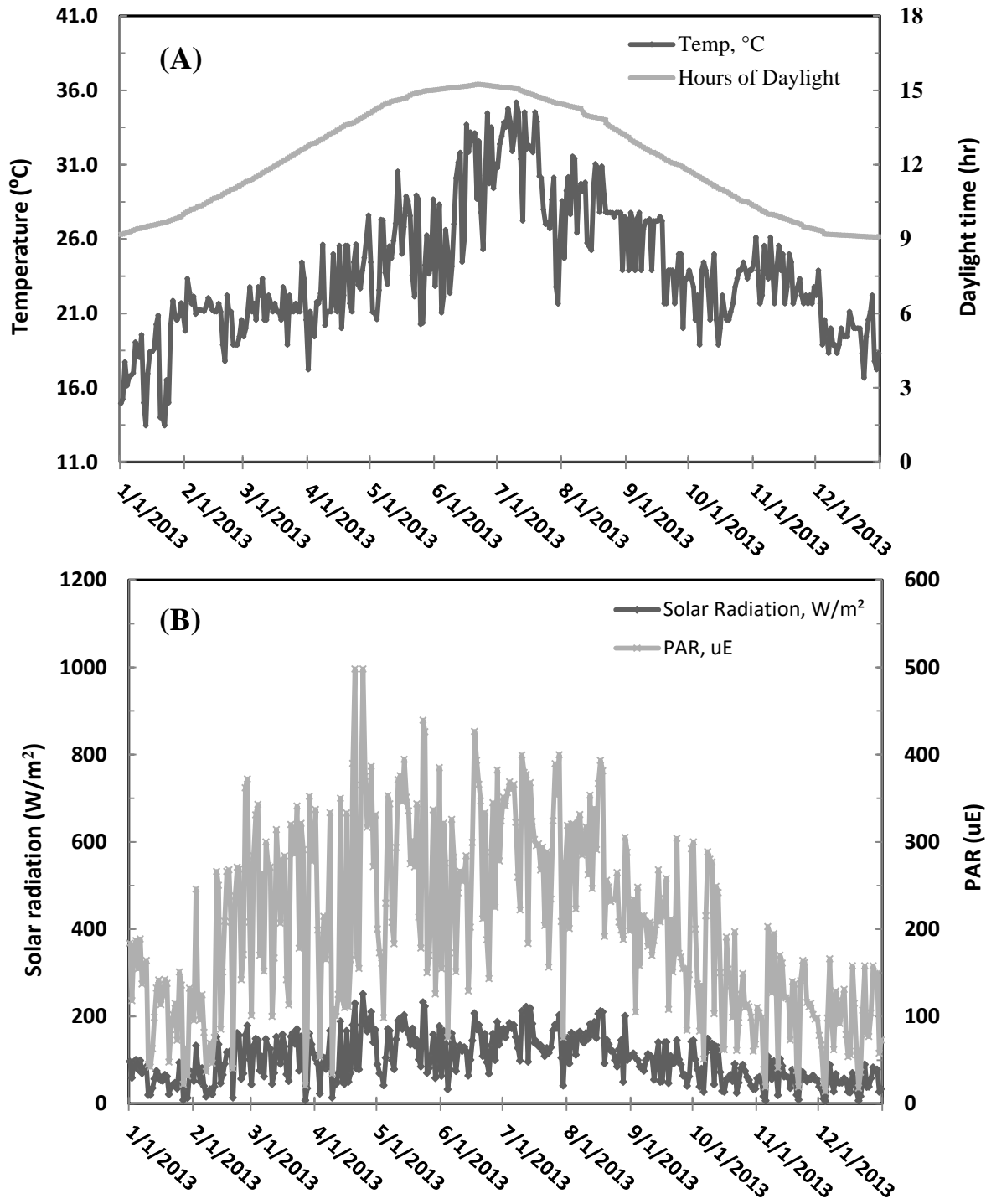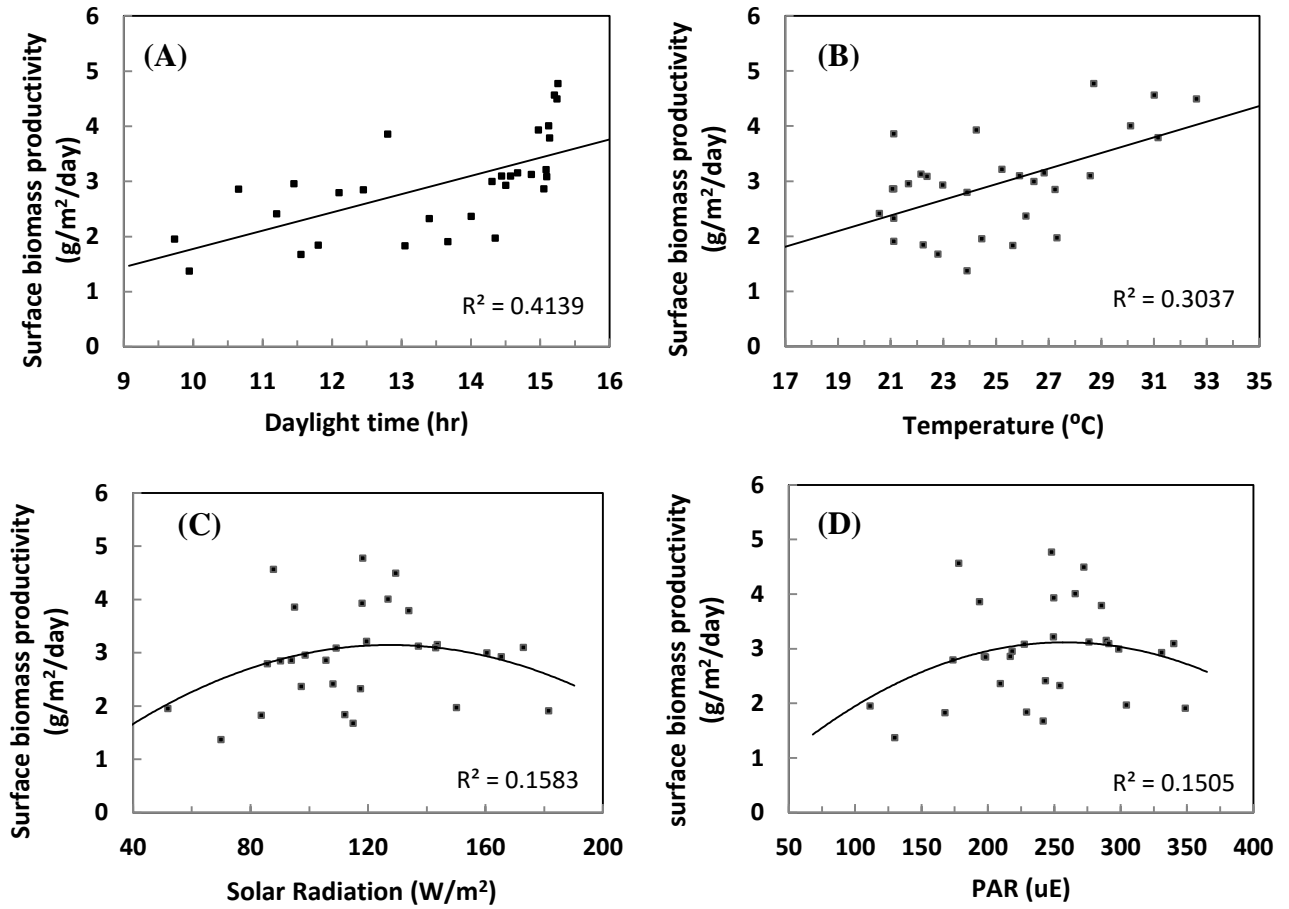
Figure 3. Yearlong environmental conditions. **(A)** temperature and daylight time; **(B)** solar radiation and PAR

Figure 4. Correlation between weather inputs on biomass productivity in triangular RAB retrofit raceway **(A)** daylight time **(B)** temperature **(C)** solar radiation **(D)** PAR

Figure 5. Correlation between weather inputs on biomass productivity in vertical RAB retrofit raceway **(A)** daylight time **(B)** temperature **(C)** solar radiation **(D)** PAR
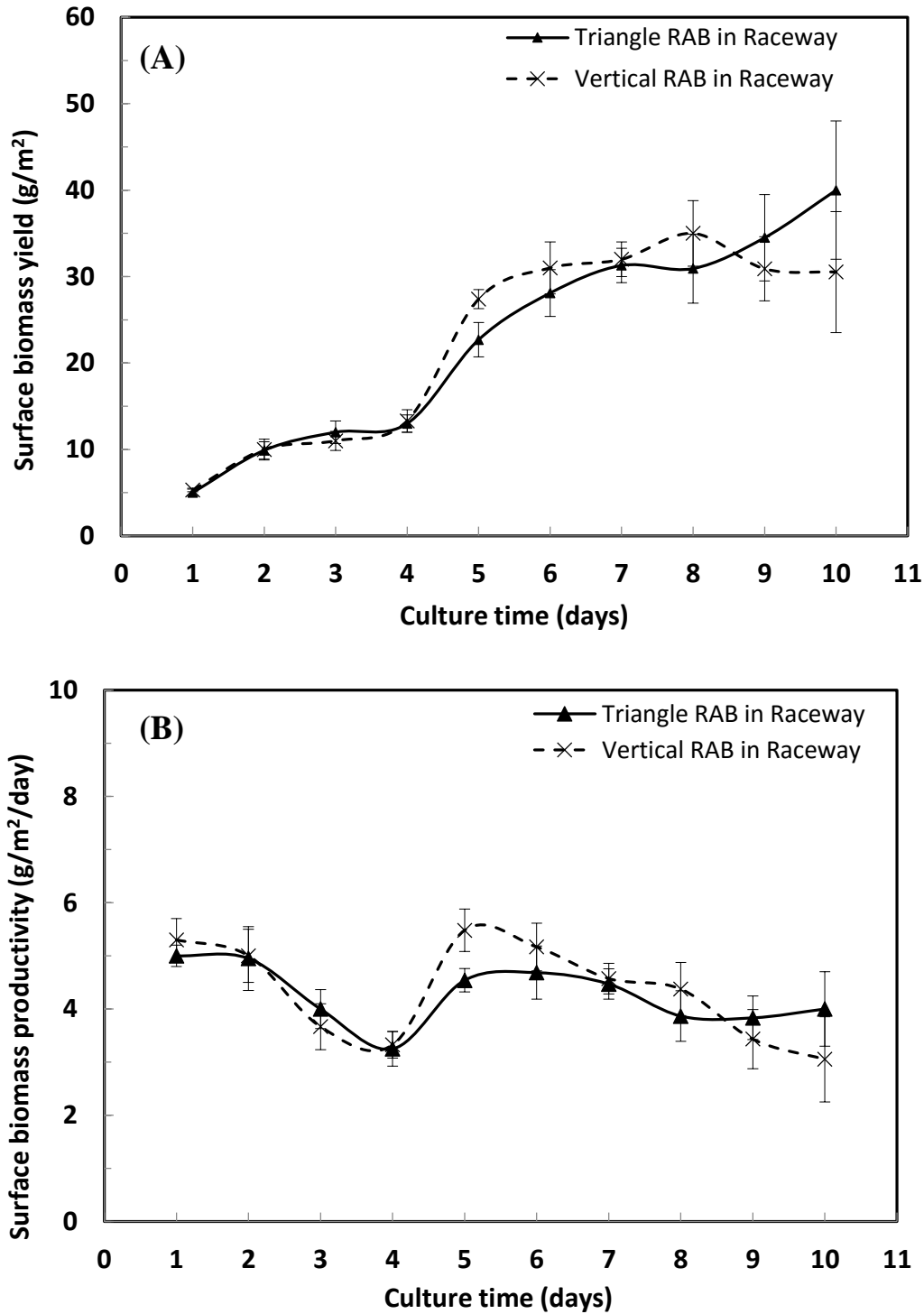
Figure 6. Time course of attached growth on the surface of attachment material (June 10-20, 2013). **(A)** biomass yield **(B)** biomass productivity.

CHAPTER 4. EVALUATING ALGAL GROWTH PERFORMANCE AND WATER USE EFFICIENCY OF PILOT-SCALE ALGAL BIOFILM (RAB) CULTURE SYSTEMS

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Abstract

A Revolving Algal Biofilm (RAB) growth system in which algal cells are attached to a flexible material rotating between liquid and gas phases has been developed. In this work, different configurations of RAB systems were developed at pilot-scale by retrofitting the attachment materials to a raceway pond (2000-L with 8.5 m^2 footprint area) and a trough reservoir (150-L with 3.5 m^2 footprint area). The algal growth performance and chemical composition, as well as the water evaporative loss and specific water consumption were evaluated over a period of nine months in a greenhouse environment near Boone, Iowa USA. Additionally a raceway pond was run in parallel, which served as a control. On average the raceway-based RAB and the trough-based RAB outperformed the control pond by 309% and 697%, respectively. A maximum productivity of 46.8 g m^{-2} day^{-1} was achieved on the trough-based RAB system. The evaporative water loss of the RAB system was modeled based on an energy balance analysis and was experimentally validated. While the RAB system, particularly the trough-based RAB, had higher

water evaporative loss, the specific water consumption per unit of biomass produced was only 26% (raceway-based RAB) and 7% (trough-based RAB) of that of the control pond. Collectively, this research shows that the RAB system is an efficient algal culture system and has great potential to commercially produce microalgae with high productivity and efficient water use.

Introduction

Microalgae have been extensively researched for production of fuels, feeds, and nutraceuticals. The interest in microalgae stems from its unique biological properties such as rapid growth and high lipid content. Microalgae can also be cultivated on marginal land with poor soil quality as long as it receives sufficient sunlight. In addition, microalgae have often been used for mitigating various forms of pollution such as municipal wastewater (Kesanno and Sims, 2014; Mata et al., 2010) and agricultural wastewater (Johnson and Wen, 2010). It has also been used to treat air pollution including off-gas from power plants (Brune, 2009) and remove ammonia gas in concentrated agriculture facilities (Kang et al., 2014).

Microalgae have been commonly grown in open ponds or photobioreactors in which the algal cells are suspended in liquid. In these systems the cell densities are generally low (between 0.5-6 g/L or 0.05-0.6% solids) (National Algal Biofuels Technology Roadmap, 2010). Following growth, specialized harvesting and dewatering operations such as sedimentation, flocculation, flotation, centrifugation, and/or filtration are needed to concentrate the biomass (Davis et al., 2011). However, those operations are costly and can be very time consuming. For example, Molina Grima et al. (2003) reported that water removal contributed up to 30% of total capital and operating costs and Davis et al. (2011) reported that harvesting alone contributes 21% of capital costs in an open pond system.

Biofilm-based culture systems have proved to be effective in reducing the expensive algae harvesting operations. In these biofilm systems, algae are attached on the surface of a material and are easily harvested via scraping. When harvested, the algal paste already has a water content similar to post-centrifuged algal biomass (80-90%), thus the expensive harvesting and dewatering steps can be avoided (Blanken et al, 2014; Christianson and Sims, 2012; Gross et al., 2013). Algal biofilm reactors that used rotating disks of polystyrene to remove nitrogen and phosphorus in municipal wastewater were first reported in the 1980s by Przytocka-Jusiak (1984). Since then a lot of research has been conducted and is summarized by Gross and Wen (2014).

In recent years, our research group has developed a unique revolving algal biofilm (RAB) culture system for attached algal growth which has been shown to be effective at growing concentrated algal biomass with easy harvesting (Gross et al., 2013; Gross and Wen, 2014). The RAB system appears similar to a conveyor belt running vertically. It consists of a revolving belt that algae cells attach to and a liquid reservoir that supplies nutrients and keeps the algae moist. This unique design has shown several advantages that are presented in detail in our previous research. These include simple harvest, high gas mass transfer rates, enhanced light utilization, and enhanced biomass productivity. In a yearlong study, we demonstrated average increased biomass productivity of 302% using the RAB system in comparison to a control raceway pond (Gross and Wen, 2014).

Although increased biomass productivities were obtained using the RAB system, there are still issues yet to be solved. For example, one impediment for widely implementing the RAB system is its perceived high level of water evaporation and water loss. Indeed, high evaporative water loss has been an issue in large-scale algal production, especially in arid climates. In addition, the RAB system reported previously was retrofitted to a raceway and could only be installed in the

straightaways. The raceway requires a lot of water and does not allow maximum utilization of the footprint area. To address these issues, we developed a new version of the RAB system using trough reservoirs to provide algal culture medium with a reduced footprint. The aim of this work was to evaluate the algal growth performance of several RAB systems, and more importantly, evaluate the water loss and water use efficiency of those RAB systems.

Materials and Methods

Algal Strain and Subculture

Chlorella vulgaris (UTEX #265) was used. The cells were maintained on agar slant at 4°C and transferred to Erlenmeyer flasks containing 50-mL of Bold's Basal medium. The medium was autoclaved at 121°C for 15 minutes. The flasks were placed on an orbital shaker set at 200 rpm and 25°C with continuous illumination at 110 µmol s^{-1} m^{-2}. To prepare the seed culture for the pilot-scale RAB system, the flask culture was stepwise scaled up to a 20-L and a 200-L flat panel photobioreactor and transferred into the liquid reservoir of the RAB systems.

Pilot-scale RAB Algal Culture Systems

The RAB system concept is illustrated in Figure 1A. In brief, a flexible material (cotton duct canvas) similar to a conveyor belt was stretched around drive shafts to form a vertical configuration. The lowest region of the belt was submerged in a medium reservoir to supply nutrients, while the rest of the belt was exposed to the gas phase for direct access to light. The shafts were driven by a motor, rotating the belt between the liquid and gas phases.

Depending on the reservoir configuration, the RAB system can be retrofitted in a raceway pond or a trough. Figure 1B shows schematics of a raceway-based RAB system. A raceway pond with 8.5 m^2 of surface area and 2,000-L of working volume was used as a liquid reservoir. The

dimension of each belt was 1 m wide and 0.91 m tall. Figure 1C shows the design of the trough-based RAB system. A serpentine trough was used as the liquid reservoir to supply nutrients for eight vertical belts. The trough was made from PVC pipe (20 cm internal diameter) cut in half that had a total length of 10.5 meters. The footprint area of the trough-based RAB system was 3.5 m^2 with 150-L of liquid. Two configurations of attachment materials were used. From October to December 2013, all the belts were set at 1 m wide and 0.91 meter tall with a surface area of 1.85 m^2 each as shown in Figure 1C. Then, from late December 2013, the height of the attachment materials was varied with each pair of the belts being set as 0.91, 1.22, 1.52, and 1.83 meters tall with a corresponding surface area of 1.85, 2.42, 3.04, and 3.64 m^2, respectively.

To start the algal culture, the seed prepared in the flat panel reactors was inoculated into the reservoir of the RAB system. The liquid in the reservoir was circulated with a paddlewheel. Following inoculation, the conveyor belt was rotated at a linear velocity of 4 cm sec^{-1}. The RAB system was run for the first 14 days to establish a thick biofilm on the belt surface and then the biomass was harvested by scraping with a rubber squeegee. The residual cells remaining on the belt served as inoculum for the next cycle of growth and harvest, which occurred every 7 days. From March 8 to April 11, 2014, the trough-based RAB system was subjected to an attached algal growth kinetic study. During this period the daily growth of the biofilm were monitored. Throughout the experimental period, half the working volume of the reservoir liquid was replaced every 7 days, corresponding to a 14-day hydraulic retention time (HRT). Also, an identical raceway pond (without a RAB system) was run in parallel as a control.

The algal cultures were operated in the Iowa State University Algal Production Facility in Boone, Iowa USA. The facility greenhouse is composed of transparent twin wall polycarbonate. The inside temperature was maintained between 15-30°C with geothermal heating and cooling.

Natural sunlight was used exclusively for the algal growth. Temperature, solar irradiance, and photosynthetically active radiation (PAR) were monitored with an Onset HOBO data logger (Massachusetts USA) every hour throughout all the culture period.

Characterization of Algal Growth in the RAB Systems

The algal growth in the RAB system was determined by combining the attached growth on the attachment materials as well as the suspended growth in the reservoir. The suspended growth was assessed by measuring biomass productivity based on the reservoir surface area (P_{pond}) expressed as:

$$P_{pond} = \frac{C \times V}{A_{pond} \times HRT} \qquad (1)$$

where C is the biomass concentration, V is the liquid volume, A_{pond} is the surface area of the raceway or trough, and HRT is hydraulic retention time. The biomass concentration was determined by measuring the optical density at 680 nm and converting it into dry weight using a correlation curve. The correlation curve of OD 680 vs biomass dry weight was based on using the actual culture from the pilot-scale system, which was at equilibrium state and contained a minimal amount of bacterial contamination due to organic carbon-free medium being used.

The attached algal growth was evaluated by two criteria: (1) surface biomass productivity ($P_{surface}$) which is based on the surface area of the attachment material and (2) footprint biomass productivity ($P_{footprint}$) which is based on the footprint area of the RAB system. These are represented by:

$$P_{surface} = \frac{Y}{t} \qquad (2)$$

$$P_{footprint} = \frac{Y \times A_S}{A_{pond} \times t} \qquad (3)$$

where *Y* is biomass yield per unit of attachment surface area, *t* is the cells culture time before being harvested from the attachment material, A_S is the surface area of the attachment material. The value of *Y* was determined by harvesting the biomass from a given area (1 ft^2) of the biofilm and dividing the biomass by this area. All the biomass productivity results reported in this work are on an ash-free basis unless otherwise noted.

Characterization of the Chemical Composition of the Algal Biomass

The biomass characterization was evaluated by the same methods described in our previous publication (Gross and Wen, 2014). In short, the ash content of the biomass was determined by heating the biomass in a furnace at 550°C for 6 hours and weighing the remaining matter. The lipid content was determined according to the Bligh and Dyer method (Bligh and Dyer, 1959). The protein content was estimated by measuring the total Kjeldahl nitrogen (TKN) of the biomass and multiplying by the conversion factor of 5.95 (Lopez, 2010). The carbohydrate content was determined by subtracting the ash, lipid and protein contents from the total biomass. The fatty acid methyl esters (FAME) were prepared following the procedures reported previously by Pyle et al. (2008) and quantified by GC-FID using the method developed by Liang et al. (2011).

Modeling Water Evaporative Loss in Different Algal Culture Systems

An energy balance model has been developed by Bechet et al. (2011) for predicting the temperature of a shallow algal pond. Here, we derived the water evaporative loss of the RAB systems using this energy balance approach, modifying it for the specific configurations of the RAB system design. The simulation was performed by MATLAB R2014B (MathWorks).

First, the temperature change of the algal culture system was expressed as:

$$\rho_w V C_{pw} \frac{dT_w}{dt} = Q_{rad(air)} + Q_{rad(solar)} - Q_{rad(water)} + Q_{cond} + Q_{conv} - Q_{evap} \qquad (4)$$

where ρ_w, C_{pw}, and T_w respectively represent the density, heat capacity, and temperature of water in either the liquid reservoirs or the liquid thin film of the RAB belt; $Q_{rad(air)}$, $Q_{rad(solar)}$, and $Q_{rad(water)}$ are the radiative heat flux caused by air, solar, and water, respectively; and Q_{cond}, Q_{conv}, and Q_{evap} are the heat flux due to conduction, convection, and evaporation, respectively.

To calculate radiative heat fluxes (Q_{rad}), the following equations can be used:

$$Q_{rad(air)} = \sigma \varepsilon_a \varepsilon_w T_a^4 A \qquad (5)$$

$$Q_{rad(solar)} = (1 - f) H_s A \qquad (6)$$

$$Q_{rad(water)} = \sigma \varepsilon_w T_w^4 A \qquad (7)$$

where σ is the Stefan-Boltzmann constant; ε_a and ε_w are the emissivity of air and water, respectively; T_a is the temperature of ambient air; f is the mutual shading factor caused by the adjacent belts (~0.7), and H_s is the solar irradiance. A is the total surface area contributed to radiative heat transfer including both the liquid reservoir area and the attachment material surface area. For the raceway-based RAB system, A is the sum of the raceway pond area (A_{pond}) and the attachment materials surface area (A_s), that is $A = A_{pond} + A_s$. For the trough-based RAB system, the trough surface area was negligible compared to the attachment material, such that $A \approx A_S$. For the control pond, $A = A_{pond}$ since there is no attachment material involved.

In this work, the conductive heat flux was insignificant compared to convective and evaporative heat flux; therefore, Q_{cond} was negligible. The convective heat flux (Q_{conv}) can be determined as follows:

$$Q_{conv} = h \times A \times (T_a - T_w) \qquad (8)$$

where h is the convective heat transfer coefficient; A, T_a, and T_w have the same concepts as Eqs.

5-7. The value of h can be estimated from the Nusselt number (Nu) (Bergman et al., 2011) using:

$$h = \frac{Nu \times k_a}{L} \qquad (9)$$

where k_a is air thermal conductivity, L is the length of the attachment materials and/or the raceway.

To determine the Nusselt number, the Reynolds number (Re) and Prandtl number (Pr) of the algal

culture systems needed to be determined first using:

$$Re = \frac{\rho_a \, u \, L}{\mu_a} \qquad (10)$$

$$Pr = \frac{\mu \, C_{p(a)}}{k_a} \qquad (11)$$

where ρ_a, μ_a, and $C_{p(a)}$ are density, viscosity, and specific heat of air, respectively, and u is air

velocity. Re values for the raceway pond, pond-based RAB system, and trough-based 0.91-m tall

RAB system, trough-based 1.81-m tall RAB system were determined as 11,744, 11,892, 18,486,

and 24,987, respectively. Since the air flow over the pond surface and liquid film of the RAB

belt is within laminar regime (Re<50,000), Nu can be calculated using the following equation

(Bergman et al., 2011):

$$Nu = 0.664 \times Re^{\frac{1}{2}} \times Pr^{\frac{1}{3}} \qquad (12)$$

The Nu value determined from Eq. 12 was then substituted into Eq. 9 to obtain the value

of h, which was further substituted into Eq. 8 to determine the Q_{conv} value.

Finally, the heat flux due to water evaporation (Q_{evap}) was determined as:

$$Q_{evap} = m_{loss} \times \lambda \qquad (13)$$

where m_{loss} is the mass-based water evaporative loss and λ is the latent heat of water

vaporization. Substituting Eq. 13 into Eq. 4, and assuming the reactor is running at steady state,

i.e., $\frac{dT_w}{dt} = 0$, we get:

$$m_{loss} = \frac{Q_{rad,air} + Q_{rad,solar} - Q_{rad,water} + Q_{cond} + Q_{conv}}{\lambda} \qquad (14)$$

To present the water loss in a more practical way, m_{loss} was further converted into volumetric water evaporative loss based on (1) per unit of algal culture footprint area ($V_{loss\text{-}area}$), and (2) per unit of algal culture volume ($V_{loss\text{-}vol}$), expressed as:

$$V_{loss-area} = \frac{m_{loss}}{\rho_w \times A_{pond}} \qquad (15)$$

$$V_{loss-vol} = \frac{m_{loss}}{\rho_w \times V} \qquad (16)$$

Eqs. 15 and 16 are used to predict the water evaporative loss.

Results

Algal Growth in Different Pilot-scale RAB Systems

Different pilot-scale RAB systems were operated in parallel and biomass productivities were evaluated using Eqs. 1-3. Figure 2A shows the surface biomass productivity of the different RAB systems. From mid-October to mid-December 2013, the raceway-based RAB was run in parallel with the trough-based RAB at 0.91 meter tall. The surface biomass productivities of the two systems were almost identical. From early February through July 2014, the trough-based RAB systems with varying heights were tested. During this period, the raceway-based RAB system and the 0.91-m tall trough-based RAB system still had similar surface biomass productivity. However, a slight decrease in surface biomass productivity was observed with the increasing belt height of the trough-based RAB, although these decreases were identified as statistically insignificant ($P>0.05$). The reason for this slight decrease was most likely due to mutual shading from adjacent belts which resulted in less light reaching the attachment material.

Figure 2B shows the footprint biomass productivity of different culture systems. Throughout the culture period, the control pond resulted in the lowest biomass productivity when compared to the RAB systems; the trough-based 0.91-m tall RAB tended to have a similar level of footprint biomass productivity as the raceway-based RAB ($P>0.05$). From February to June 2014, the trough-based RABs with different belt heights were run for side-by-side comparisons. As the height of the belt increased, so did the footprint biomass productivity (Figure 2B). The average biomass productivity of the trough-based 0.91-, 1.22-, 1.52-, and 1.83-meter tall RAB systems were 17.89, 22.62, 24.09, and 29.58 g m^{-2} day^{-1}, respectively, while the raceway-based RAB achieved an average biomass productivity of 15.17 g m^{-2} day^{-1}. On the contrary, the control pond only produced an average of 3-4 g m^{-2} day^{-1} of biomass. The maximum footprint productivity was 46.8 g m^{-2} day^{-1} in the trough-based 1.88-m tall RAB system.

Biomass Attached Growth Kinetics of RAB systems

We have determined that harvesting the biomass every 5-7 days results in the highest biomass productivity in the raceway-based RAB system (Gross and Wen, 2014). To determine the optimal time for harvesting the biomass in the trough-based RAB system, the kinetics of the attached algal growth were studied by periodically harvesting the attached biomass at different intervals during April 4-11, 2014. The biomass yield from the attachment materials increased as the incubation time increased from day 3 to day 6 and leveled off from day 6 to day 7 (Figure 3A), while the surface biomass productivity reached the highest level around day 5 to day 6 and decreased afterward (Figure 3B). This could be due to cell sloughing, but it is also likely due to mutual cell shading of the innermost cells. The trough-based 0.91-m tall RAB system resulted in the highest biomass yield and productivity. It should be noted that the specific growth rate of algal kinetic growth on the attachment materials was not reported as the exponential growth phase seems be

happening at an earlier time (e.g., day 1 or day 2) when our biomass sampling was not conducted due to very little biomass available to be collected.

Chemical Composition of Algal Biomass in the RAB Systems

The chemical compositions of the algae biomass from various culture systems were evaluated. As shown in Table I, the biomass compositions were significantly different among different systems. For the two RAB systems, the ash contents were similar, but the trough-based RAB had a lower carbohydrate and higher lipid and protein content in comparison to the raceway-based RAB. The ash content of the control pond was much higher than for the RAB systems. A drastic difference in carbohydrate and protein percentage of the ash-free biomass between the suspended cells in the raceway pond and the attached cells in the RAB systems was also observed (Table I).

The chemical compositions of the algal biomass harvested from the trough-based RAB systems with different heights were also compared. As shown in Table II, the chemical composition of the biomass varied with the height. The ash content of the biomass increased with the height of the RAB system. Based on the ash-free biomass, carbohydrate content increased with the height while protein content showed the opposite trend (Table II). The lipid content and fatty acid profile of the RAB systems of different heights did not change significantly.

It should be noted that we did not include nucleic acids in the proximate analysis in Tables I and II as content of nucleic acids in algal biomass is generally very low (as low as 0.4% of DW) (Petra et al. 2011). However, we acknowledge that the nucleic acids (particularly RNA) of algae in a rapid growing system could be higher. Future research is needed to precisely quantify the compounds in order to provide a more precise characterization of the algal biomass.

Water Loss in Algal Culture Systems - Model Validation

The model developed earlier (Eqs. 4-16) were used to predict water evaporative loss. To validate the model, two separate experiments were performed to determine the cumulative water evaporative loss i(.e., $V_{loss\text{-}area}$ multiplying time) over a period of 4-5 days. Since there was no water addition and discharge during the test period, the water volume change was solely the consequence of evaporation. The first validation test was performed between July 7-10, 2014. Figure 4A shows the ambient air temperature and solar irradiance during this period; the daytime temperature and solar irradiance on July 7-8 were very low due to cloudy weather. Figure 4B shows that the water loss of the various culture systems during this 96-hour period. The control raceway pond had the lowest water loss and the raceway-based RAB systems increased water loss due to the contribution from the vertical belt. The trough-based RAB systems had much higher water loss than the raceway-based RAB system due to the large biofilm area relative to the footprint of the trough. As expected, the tallest (1.83 m) had more water loss than the shortest trough-RAB system (0.91 m). Figure 4B also shows that the actual water loss aligned well with our model predictions, indicating the validity of the water loss models developed in this study.

The second validation test was performed on September 16-21, 2014, during which the day/night temperature and solar radiation fluctuation was more consistent (Figure 5A). The water loss of the control pond and the trough-based RAB system (1.83-m tall) was evaluated (Figure 5B). Similar to the previous results, the trough-based RAB resulted in more water loss than the control pond. The model predicted water loss that was very close to the experimental data.

Comparison of Specific Water Consumption per Unit of Biomass for Algal Culture Systems

The above studies clearly indicate a higher water evaporative loss in the RAB systems as compared to the control pond. In general, the total water use for a given algal culture system was attributed to two factors: water fed to the culture system for maintaining the continuous culture and the water loss due to evaporation, expressed as:

$$W = D + V_{loss-vol} \tag{17}$$

where W is the water used per unit of liquid volume and D is the dilution rate. Figure 6A shows the water evaporative loss of the different culture systems. The trough-based RAB system resulted in a much higher water loss due to the large surface area of biofilm relative to small volume of liquid. Figure 6B shows the trend of water use with the different algal culture systems had a same trend for water loss due to the same dilution rate being used for the different systems.

In addition to water use, the biomass production rate is another criterion to evaluate the efficiency of an algal culture system. For the RAB systems, the biomass produced was attributed to two sources, the attached biomass in the biofilm and the suspended cells in the liquid reservoir, the total biomass productivity per unit of culture volume (P_{total}) can be determined as,

$$P_{total} = \frac{P_{surface} \times S + P_{pond} \times A}{V} \tag{18}$$

Based on Eq. 18, the value of P_{total} the different algal culture systems were compared. For the control raceway pond, $P_{surface}$ was zero since no biofilm existed in this system, while the trough-based RAB system, P_{pond} was set to zero since the suspended cells in the trough were negligible. Figure 6C shows that the RAB systems, particularly the trough-based RAB systems, resulted in much higher biomass productivity per unit of water than the control raceway pond.

Based on the above analyses, we introduce the specific water consumption per unit of biomass produced (η) as a new criteria to evaluate the RAB system in terms of water use and biomass production. Here, η is defined as the amount of water needed to produce each unit of biomass, expressed as:

$$\eta = \frac{W}{P} \qquad (19)$$

Based on Eq. 19, the specific water consumption per unit of biomass produced of the different culture systems was compared. As shown in Figure 6D, the RAB systems, particularly the trough-based RAB systems, have much lower specific water consumption per unit of biomass produced than the open pond. For example, the trough-based RAB system (0.91-m tall) just needs 0.32 L to produce each gram of biomass as compare to the control system which requires 4.10 L to produce the same amount of biomass (Figure 6D).

Discussion

The RAB system has proved an efficient system to grow microalgae with multiple advantages in comparison to suspension-based culture systems. By increasing surface area vertically, the cells are more efficient at capturing light and conducting CO_2/O_2 gas exchange (Gross and Wen, 2014). The unique geometry of the RAB system also allows for drastic improvement in space utilization compared to raceway ponds. In raceway ponds, space can only be used in two-dimensions because the depth of the pond must be shallow due to light penetration limitations. On the contrary, the RAB system can grow microalgae vertically; this three-dimensional growth greatly enhances the efficiency of space utilization. This is specifically advantageous when cultivation is done in areas where space is limited. For example, algae have been recognized as an effective method for

removing nutrients from municipal wastewater (Christenson and Sims, 2012). However, many of the wastewater treatment plants are located in urban areas where space is too limited and valuable to implement traditional open ponds. The RAB system can provide wastewater treatment plants with the ability to grow microalgae at a very high productivity level using minimal land resources.

The raceway-based RAB system was able to produce 300% more biomass than a standard open pond (Gross and Wen, 2014). In this study, the trough-based RAB systems resulted in even higher (5-10 folds) biomass productivity levels compared to the open pond. This is due to the reduced footprint by the unique design of the trough as the liquid reservoir (Figure 1C). In addition, when the height of the attachment materials increased so did biomass productivity. This is consistent with our previous findings in which productivity increased as the attachment surface area increased for a given footprint (Gross and Wen, 2014).

However, we found the footprint biomass productivity of the trough-based RAB system did not proportionally increase with the height of the attachment materials. As a matter of fact, the surface biomass productivity (biomass per unit of attachment surface area) of the trough-based RAB decreased as the height of the RAB system increased (Figure 2A). It is believed that shading of the adjacent belt played an important role in this decrease. Indeed, in this work, the trough-based RAB system was implemented in such a way that the taller belt had a decreased proportion of the biofilm exposed to direct sunlight because of shading from nearby belts. In future studies, the geometry and spacing of the RAB belts needs to be carefully designed to minimize the shading effect so productivity can be maximized.

In addition to improved biomass production, the RAB system also demonstrated a drastically different biomass composition compared to the suspended algae. The decreased protein and increased carbohydrate contents in the biofilm cells in the raceway-based RAB were likely

due to the presence of extracellular polymeric substance (EPS) in the biofilm. It has been reported that EPS is produced by microorganisms in order to help the cells attach on solid surfaces (Wang et al., 2014). As a result, the polysaccharides, a major component of EPS, would contribute to the high carbohydrate content in the attached cells.

When comparing the biomass composition of the trough-based RAB, it was found that the ash content of biomass increased with height of the belt (Table I). The reason may be due to an increased amount of EPS produced in the taller RAB belts as a result of increased stress on the biofilm cells. In general, EPS contains negatively charged functional groups which have the ability to bind inorganic ions from the growth medium which could have contributed to the increased ash content (Tian et al., 2006). Further studies are required to confirm this hypothesis.

The RAB design has been challenged because of the perceived high level of water evaporation which makes the system undesirable for large-scale operation, particularly in an arid climate. We acknowledge this concern, as water is an additional cost and not always in unlimited supply. In this work, both the model prediction and experimental results indicate the RAB system has higher water loss compared to the open pond (Figures 4B and 5B). However, the evaluation of algal culture efficiency needs to be based on both the water loss and the corresponding biomass produced. As shown in this work, the specific water consumption per unit of biomass (Eq. 19) of the RAB systems, particularly the trough-based RAB, was actually much higher than for the open pond (Figure 6D), indicating the RAB systems can in fact save a significant amount of water compared to the raceway pond. These results clearly indicate that although the water loss in the RAB systems is high, the systems were capable of producing much more biomass, which makes the RAB systems more efficient at using water than the open ponds.

Conclusions

This work reported the operation of pilot-scale RAB culture systems for long-term (9 months) attached growth of the microalga *Chlorella vulgaris*. The trough-based RAB achieved a maximum surface biomass productivity of 5.5 g m^{-2} day^{-1} and footprint biomass productivity of 46.8 g m^{-2} day^{-1}. On average, the raceway-based RAB and the trough-based RAB outperformed the control raceway pond by 309% and 697%, respectively. Although the RAB systems had a higher total water loss than the control raceway pond, the biomass productivity from the RAB systems was much higher than the control raceway pond, which in turn resulted in low specific water consumption per unit of biomass. Collectively, the RAB system shows promise as an alternative culture system compared to suspension-based cultures.

Nomenclature:

A	Surface area of algal culture system (pond + biofilm), m^2
A_{pond}	Surface area of the raceway or trough, m^2
A_s	Surface area of the attachment materials (biofilm), m^2
C	Biomass concentration in the liquid, g L^{-1}
$C_{p(w)}$	Heat capacity of water, 4.18×10^3 J kg^{-1} K^{-1}
$C_{p(a)}$	Heat capacity of air, 1.006×10^3 J kg^{-1} K^{-1}
D	Dilution rate of the algal culture, L L^{-1} day^{-1}
f	Mutual shading factor caused by the adjacent belts in the RAB system
HRT	Hydraulic retention time, day
H_s	Solar irradiance, W m^{-2}
h	Convective heat transfer coefficient, W m^{-2} K^{-1}
k_a	Air thermal conductivity, 0.0257 W m^{-1} K^{-1}
L	Length of the attachment materials and/or the raceway pond, m
m_{loss}	Water evaporative loss (mass based), kg day^{-1}
Nu	Nusselt number

P_{total} Biomass (suspended + attached) productivity per unit of culture volume, g L^{-1} day^{-1}

P_{pond} Suspended biomass productivity per unit of pond area, g m^2 day^{-1}

$P_{surface}$ Attached biomass productivity per unit of attachment surface area, g m^2 day^{-1}

$P_{footprint}$Attached biomass productivity per unit of the footprint area, g m^2 day^{-1}

Pr Prandtl number, 0.71

$Q_{rad,air}$ Heat flux caused by the radiations of air, kJ day^{-1}

$Q_{rad,solar:}$Heat flux caused by the solar radiations, kJ day^{-1}

$Q_{rad,water:}$ Heat flux caused by the radiations of water, kJ day^{-1}

Q_{cond} Heat flux due to conduction, kJ day^{-1}

Q_{conv} Heat flux due to convection, kJ day^{-1}

Q_{evap} Heat flux due to evaporation, kJ day^{-1}

Re Reynolds number

t Cell culture time, day

T_a Ambient air temperature, °C

T_w Water temperature, °C

u Velocity of air on the surface of pond or liquid film of the RAB belt, m s^{-1}

V Total volume of culture liquid, (2 m^3 for raceway pond, 0.15 m^3 for trough)

$V_{loss-area}$ Volumetric water loss per unit of algal culture footprint area, L m^{-2} day^{-1}

$V_{loss-vol}$ Volumetric water loss per unit of algal culture volume, L L^{-1} day^{-1}

W Water used per unit of algal culture volume, L L^{-1} day^{-1}

Y Biomass yield per unit of attachment surface area, g/m^2

ε_a Air emissivity, 0.8

ε_w Water emissivity, 0.97

η Specific water consumption per biomass, L g^{-1}

λ Latent heat of water vaporization, 2.45×10^6 J kg^{-1}

ρ_a Density of air, 1.15~1.20 kg m^{-3}

ρ_ω Density of water, 9.98×10^2 kg m^{-3}

σ Stephan Boltzman constant, 5.67×10^{-8} W m^{-2} K^{-4}

μ_a Air viscosity 18.60×10^{-6} N s m^{-2}

Acknowledgement

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Table I. Proximate analyses of algal biomass harvested from control raceway pond, raceway-based RAB system, and the trough-based RAB system (with 0.91 m tall attachment materials).

Composition	Algal culture systems		
	Trough-based RAB	Raceway-based RAB	Control Pond
Ash (%DW)	9.21±3.1	12.4±3.2	28.3± 4.1
Chemical composition (% ash-free DW)			
Carbohydrate	30.68±3.0	64.2±2.9	24.2± 2.2
Lipid	19.48±0.9	7.6±0.8	12.8±1.1
Protein	49.82±0.1	28.2±0.5	63.0±0.3

Table II. Proximate analyses (including fatty acid compositions) of algal biomass harvested from the trough-based RAB systems with different heights of attachment materials.

Composition	Trough-based RAB system with different heights			
	0.91 m tall	1.22 m tall	1.52 m tall	1.83 m tall
Ash (%DW)	9.21±3.1	14.41±3.0	18.89±3.3	21.59±3.6
Chemical composition (% ash-free DW)				
Carbohydrate	30.68±3.0	32.67±3.2	34.78±2.9	40.11±2.5
Lipid	19.48±0.9	18.12±1.1	16.38±1.3	18.04±0.8
Protein	49.82±0.1	49.2±0.2	48.82±0.1	41.84±.01
Fatty acid (% TFA)				
C16:0	21±0.1	17±0.2	17±0.1	17±0.5
C16:1	2±0.2	6±0.1	6±0.2	4±0.1
C18:0	2±0.5	3±0.2	2±0.2	3±0.1
C18:1	29±0.8	29±0.8	29±0.3	34±0.9
C18:2	21±0.9	21±0.2	21±0.1	19±0.5
C18:3	25±0.5	25±0.3	25±0.2	23±0.4
TFA (% ash-free DW)	3.8±0.2	5.3±0.3	5.4±0.2	6.2±0.5
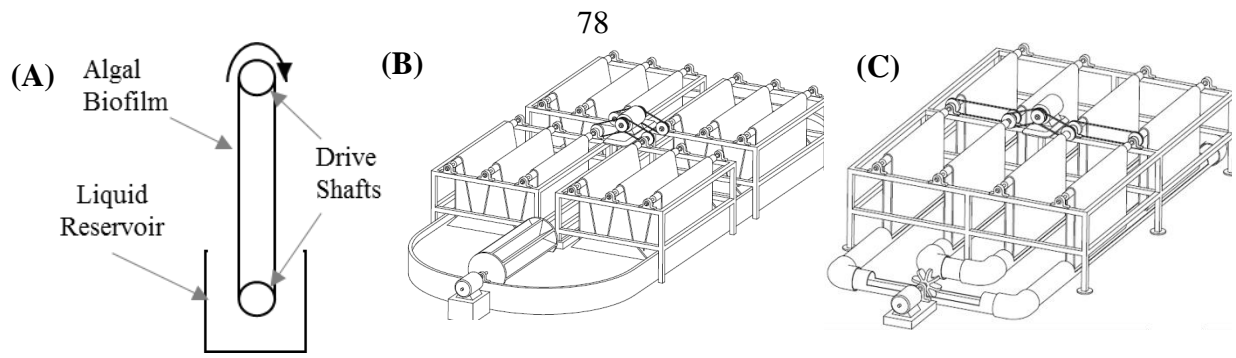
Figure 1. (A) Basic schematic of RAB **(B)** Schematic of raceway-based RAB **(C)** Schematic of pilot-scale trough-based RAB.
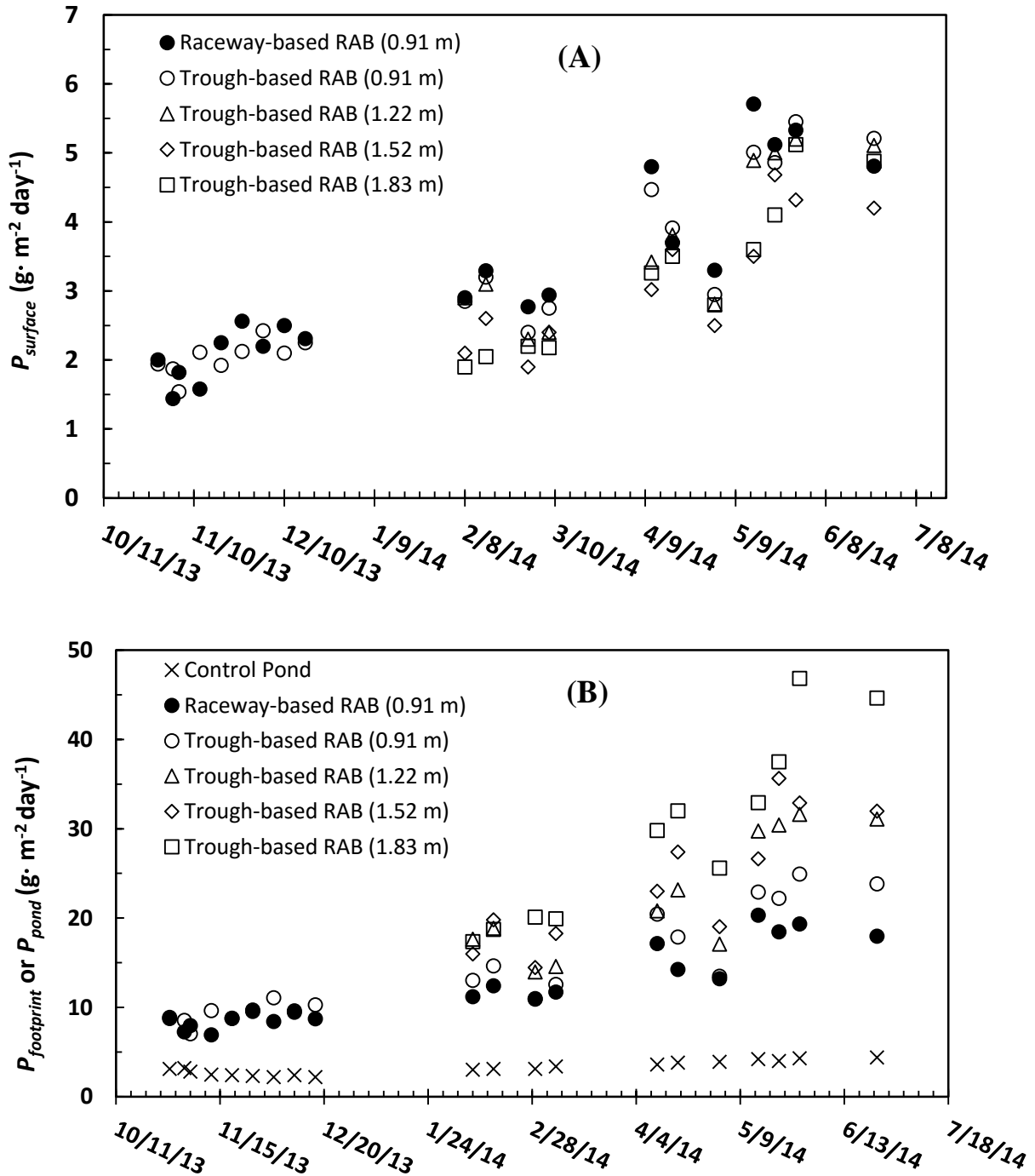
Figure 2. (A) Surface biomass productivity ($P_{surface}$, Eq. 2) of different RAB system; and **(B)** Footprint biomass productivity ($P_{footprint}$, Eq. 3) of different RAB systems and biomass productivity of the control pond (P_{pond}, Eq. 1).
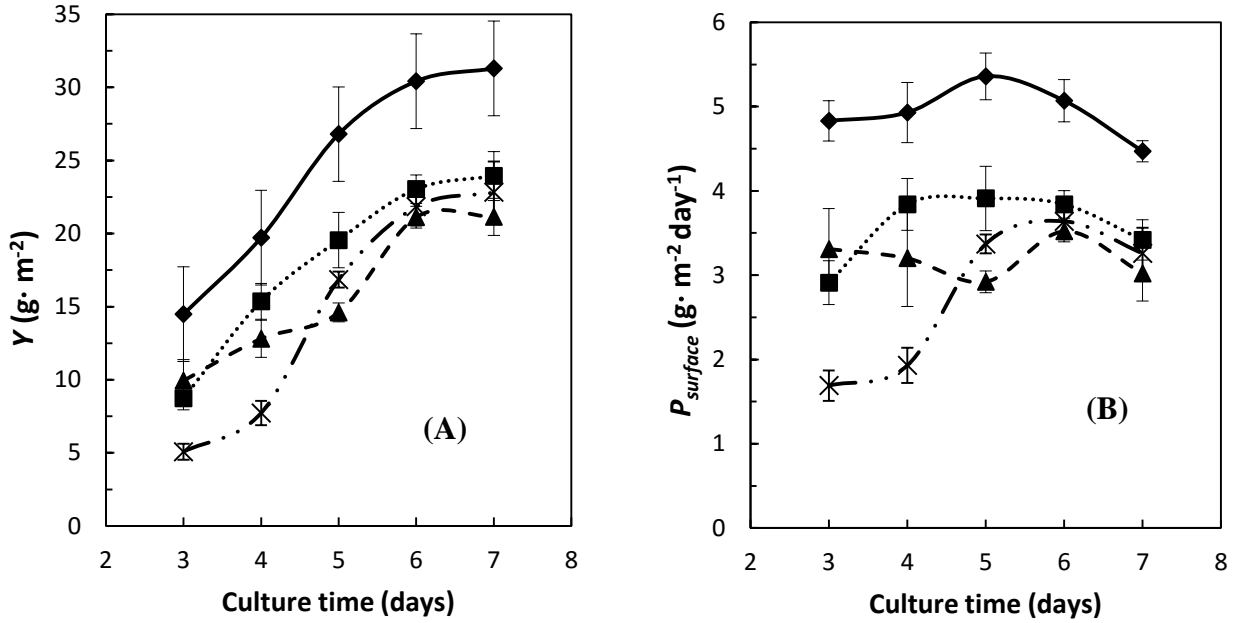
Figure 3. (A) The biomass yield per un it of attachment area (*Y*); and **(B)** Surface biomass productivity (*P_surface*, Eq. 2) of the trough-based RAB systems at different culture time. Symbols for the different belt heights: ♦, 0.91 m; ■, 1.22 m; ▲, 1.52 m; ×, 1.83 m. (data are means of three replicates and error bars show standard deviations).
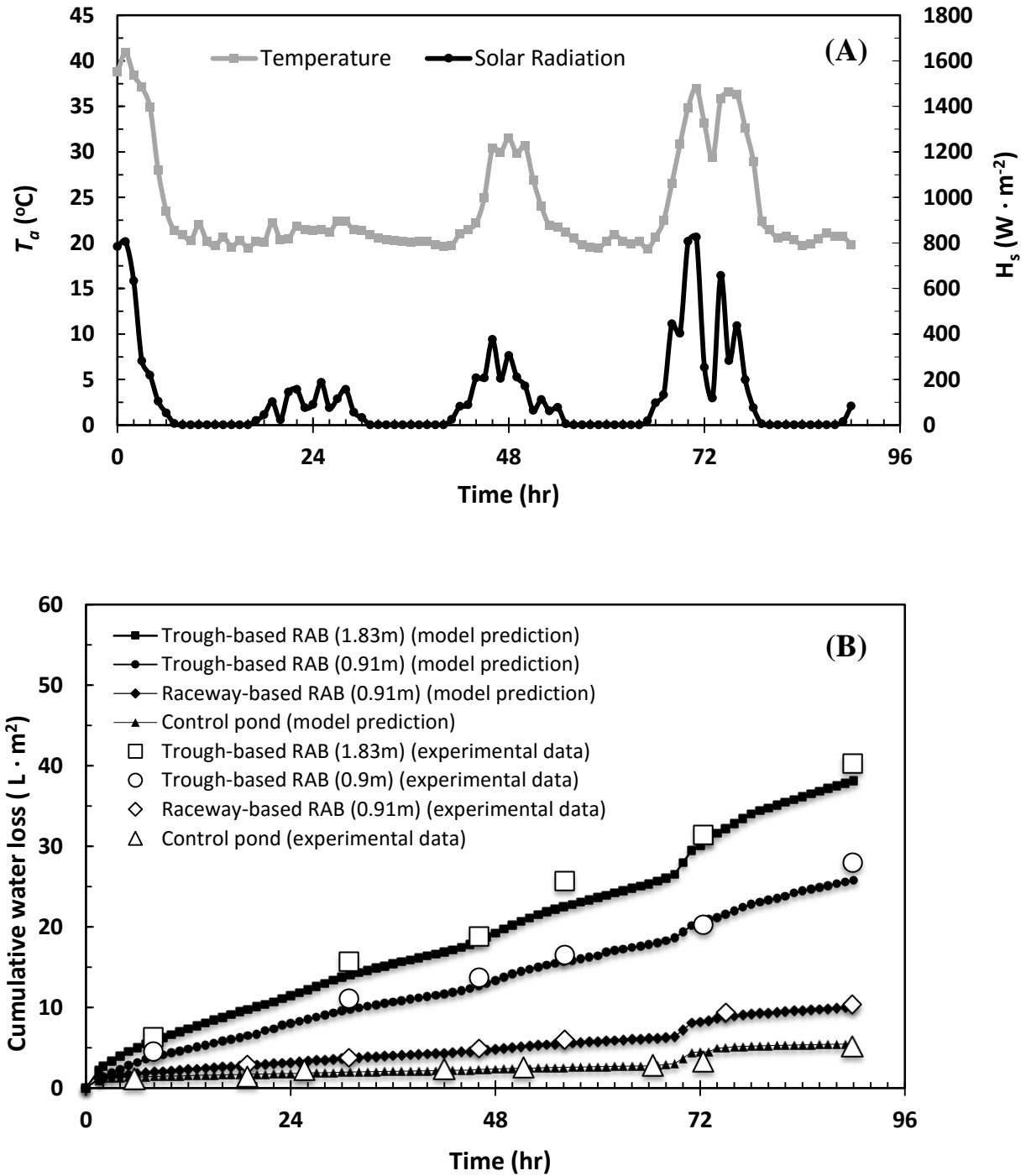
Figure 4. **(A)** Ambient air temperature (T_a) and solar irradiance (H_s) during the test period (July 7-10, 2014) **(B)** Modeled and experimental cumulative water evaporative loss per footprint area (i.e, $V_{loss\text{-}area}$, Eq. 15, multiplying time) of different algal culture systems.
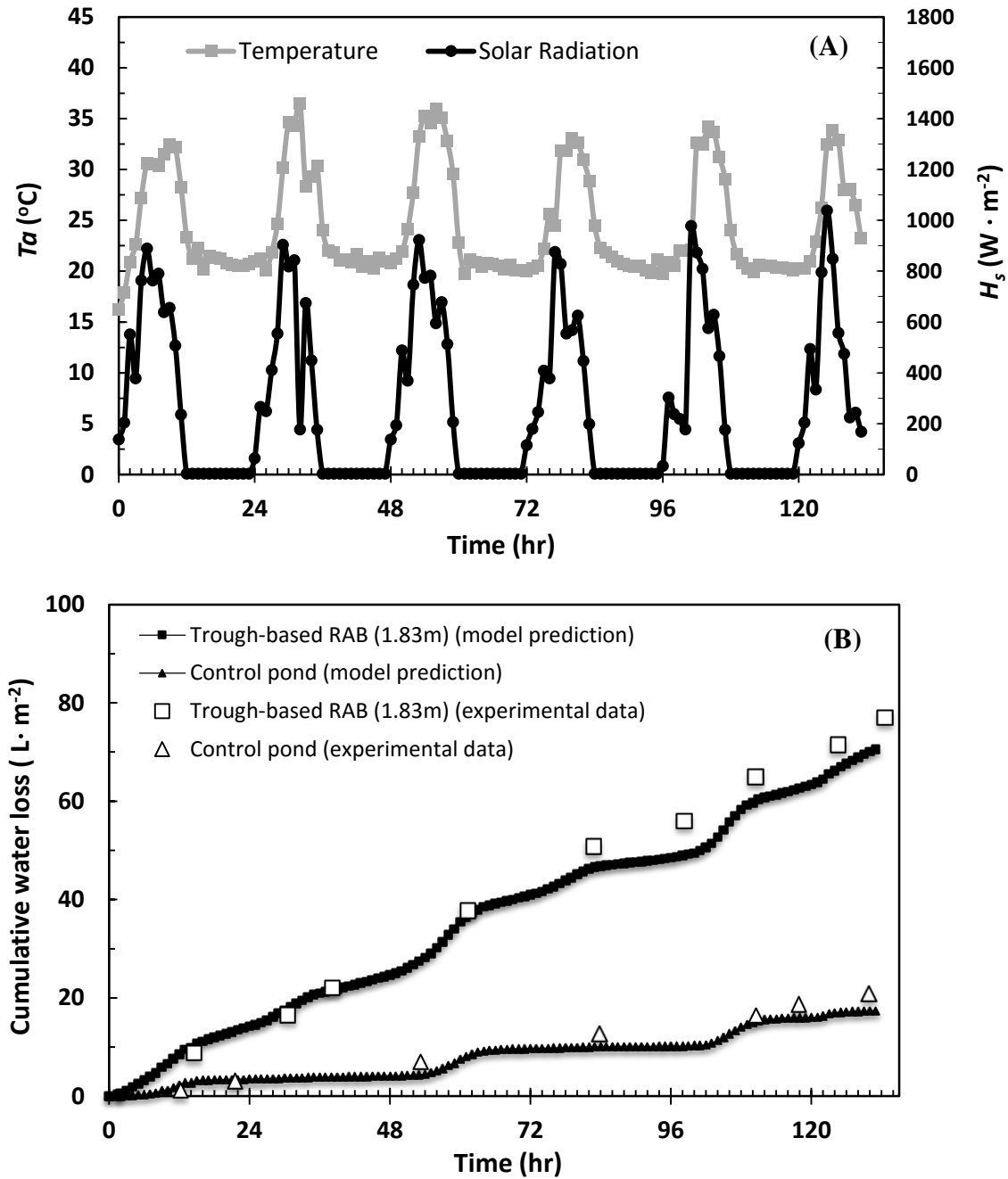
Figure 5. **(A)** Ambient air temperature (T_a) and solar irradiance (H_s) during the test period (September 16-21, 2014) **(B)** Modeled and experimental cumulative water evaporative loss per footprint area (i.e, $V_{loss-area}$, Eq. 15, multiplying time) of different algal culture systems.
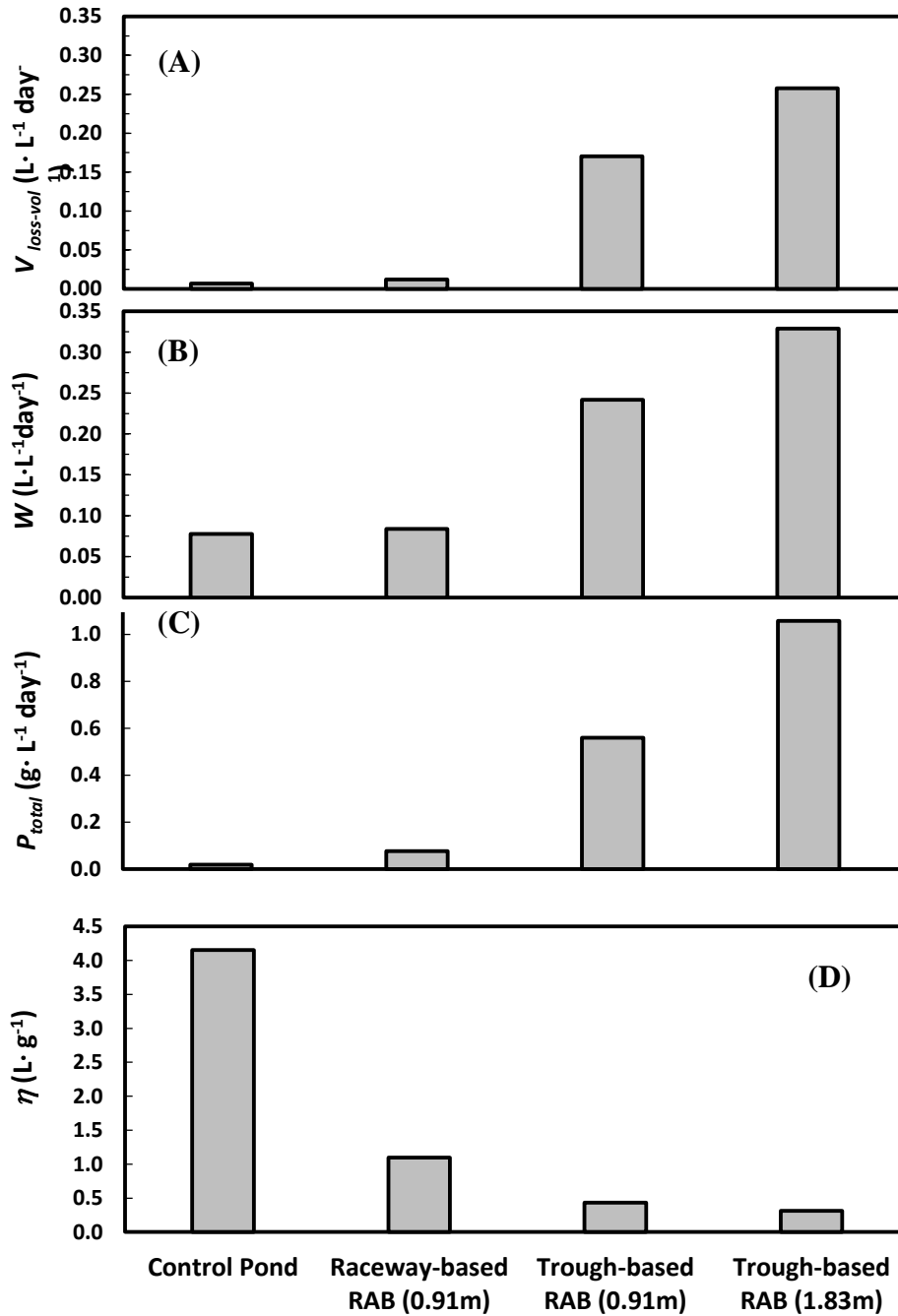
Figure 6. (A) water loss per unit of liquid volume ($V_{loss-vol}$, Eq. 16) **(B)** water used per unit of liquid volume (W, Eq. 17) **(C)** biomass productivity per unit of the liquid volume (P_{total}, Eq. 18) **(D)** Specific water consumption per unit of biomass produced (η, Eq. 19) (data are based weather conditions and corresponding algal culture results during July 10-14, 2014).

CHAPTER 5. EFFECT OF SURFACE PHYSICO-CHEMICAL PROPERTIES AND SURFACE TEXTURES ON THE INITIAL COLONIZATION AND ATTACHED GROWTH IN ALGAL BIOFILMS

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Abstract

Background: Algal biofilm reactors represent a promising cultivation system that can economically produce biomass without the need for expensive harvesting operations. A critical component of algal biofilm systems is the material used for attachment. This research reports a comprehensive study of the effects of material surface physico-chemical properties, the surface texture, and their interactions on the initial colonization and the long-term attached growth in algal biofilm systems. A total of 28 materials with a smooth surface were tested for initial cell colonization and it was found that the tetradecane contact angle of the materials had a good correlation with cell attachment. The effects of surface texture were evaluated using mesh materials (nylon, polypropylene, high density polyethylene, polyester, aluminum, and stainless steel) with openings ranging from 0.05–6.40 mm.

Results: The mesh materials with an opening of 0.5 mm resulted in the highest attachment. The interaction of surface physico-chemical properties and surface texture, and their co-effects on the cell attachment was quantitatively described using a second order polynomial regression. The long-term algal attached growth for the different materials showed a trend similar to that found in initial colonization.

Conclusion: Collectively, nylon and polypropylene mesh with 0.50–1.25 mm openings resulted in the best initial colonization and long-term attached growth, with a 28–30 g/m^2 biomass yield and 4.0–4.3 $g/m^2 \cdot day$ biomass productivity being achieved on a pilot-scale revolving algal biofilm system.

Background

Microalgae have been researched for production of a variety of fuels, feeds, and chemicals. It also has been used to mitigate various pollutants found in municipal wastewater [1, 2] agricultural effluents [3], and animal housing air with high levels of ammonia and CO_2 [4]. Current cultivation systems such as raceway ponds and photobioreactors require costly and energy intensive methods to harvest the suspended microscopic algae cells from liquid. For example, Davis et al. [5] reported that harvesting alone contributes 21% of capital costs in an open pond system.

Biofilm-based algal culture systems have proven effective in reducing expensive algae harvesting operations [6]. In biofilm systems, algae are attached on the surface of a material and are easily harvested via scraping. The harvested biomass has a water content similar to post-

centrifuged biomass (80–90% moisture) [7, 8, 9]. In addition to the benefit of easy harvesting, algal biofilms also have the features of minimizing light limitation and enhancing CO_2 mass transfer. The solids retention time of the cells is also increased due to the separation of the algal cells and liquid medium, which is a benefit in water treatment applications [6].

Various algal biofilm reactors have been developed in past decades and have drawn renewed interest in recent years. A comprehensive review of biofilm-based algal cultivation systems, including the reactor design configurations, the pros and cons of the systems, and the factors affecting biofilm growth performance has been provided in a recent review [6]. In terms of the mechanism of algae-material attachment, various theories and hypotheses have been proposed such as hydrophobic interactions [10], acid-base interactions [11, 12], and surface energy [13]. The effect of material on the algal attachment and attached growth is a rather complicated process. On one hand, the material surface physico-chemical properties such as hydrophobicity [14], surface energy [13], and dispersive surface energy [15] play an important role for the initial algal colonization, but this highly depends on the materials and model strain used. On the other hand, the different micro-patterns (texture) of attachment material surface affect cell recruitment and retention as well. The materials with an appropriate surface texture provide a "shelter" for the attached cells; as a result, the sloughing of the attached cells can be significantly reduced. Indeed, it has been reported that altering the surface can drastically increase algal attachment [16, 17, 18, 19].

It should be noted that most of the previous studies focused on initial colonization of the algal cells to the fresh material surface. Once the colony is established, the long-term sustained attached growth on the material is rarely reported. In addition, the other surface physico-chemical properties and surface texture of the materials have been reported individually; the

interactions between these two properties and their combined effect on the cell attachment and growth are not been well understood.

Selection of an appropriate attachment material is important in the development of an algal biofilm system. However, only a limited number of materials have been studied. In this work, a comprehensive study of 28 smooth materials with different surface physico-chemical properties and 6 textured materials with various levels of surface texture were investigated for their roles in cell attachment and growth, with the aim to provide insight into the cell attachment mechanism. Additionally, this work will assist in determining a promising attachment material that can be implemented for commercial algal biofilm growth systems.

Results

Algal Attachment as a Function of Material Surface Physico-chemical Properties

The surface physico-chemical properties, including water, glycerol, and tetradecane contact angle, as well as the free surface energy of the materials, were analyzed. As shown in Table 1, the liquid contact angle varied widely among different materials, with the surface energies, except aluminum and brass, in the range of the 20–60 mJ/m^2. The data was consistent with the previous reports [20].

Once the material surface physico-chemical properties were determined, the attachment of algal cells to the materials was determined. As shown in Figure 1, polylactic acid, neoprene, and latex demonstrated the best attachment in stationary conditions. The cell attachment in the rocking condition, however, did not show the same trend and no clear relationship was found between the stationary and rocking tests.

Cell attachment was further correlated with the contact angles and surface energy in order to provide a quantitative relationship between cell attachment and surface physico-chemical properties. Since the surface energy calculation involves three liquid contact angles (water-, glycerol-, and tetradecane-based) (see Materials and Methods section), the correlation was performed with each of these contact angles. No obvious quantitative correlations were observed between cell attachment and surface energy ($R^2 = 0.03$), water contact angle ($R^2 = 0.03$), and glycerol contact angle ($R^2 = 0.06$). However, a relatively strong correlation ($R^2 = 0.68$) between algal attachment and tetradecane contact angle was observed.

Algal Attachment as a Function of Materials Surface Texture

Six materials were used to test the effects of surface texture on algal attachment. A wide range of attachment performance (good, modest, and poor attachment, Figure 1), which was observed during the smooth surface test, was the criterion for selecting these six materials for inclusion. As shown in Figure 2, for all six materials tested, the smooth surface of the material resulted in the lowest level of cell attachment compared to the textured surfaces. Under the rocking conditions (Figure 2B), the best algal attachment occurred on a mesh with a 0.5 mm opening for stainless steel, aluminum, polyester, nylon, and polypropylene. The high density polyethylene (HDPE) mesh with a 0.5 mm opening was not available, and the best attachment was obtained with a 1.25–2.5 mm opening. For polypropylene, the mesh with 0.05, 0.5, and 1.25 mm openings resulted in a similar attachment (Figure 2B).

Following the quantitative determination of cell attachment, the effect of surface texture on cell attachment (under rocking conditions) was further evaluated using Scanning Electron Microscopy (SEM) observation. Two materials (aluminum and nylon) were selected for SEM

observation because nylon resulted in good attachment and aluminum resulted in poor attachment (Figure 2B). As shown in Figure 3, nylon attached more cells than the aluminum for the same size of mesh opening; while the mesh with 0.5 mm opening displayed the most biofilm for these same materials. Such observations were in agreement with the quantitative cell attachment results shown in Figure 2B. When SEM image magnification was increased (Figures 3I and 3J) the algal cell aggregates formed can be seen clearly and they exceed the mesh pore size. It is believed the inability of algal aggregates to fit into the mesh pores could be the reason for decreased cell attachment at the 0.05mm mesh opening (Figure 2B).

Co-effect of Surface Physico-chemical Properties and Texture on Algal Attachment

The previous results show that the cell attachment varied with surface physicochemical properties and the surface texture of the attachment material. In this section, the interactions and combined effects of these two parameters on the cell attachment were evaluated through a series of statistically-based analyses.

We first used a Tukey's Honestly Significant Difference (HSD) test to rank cell attachment among all of the 32 combinations of the materials and the textures. Under stationary conditions, 29 material-texture combinations showed no significant difference for cell attachment out of 32 combinations. Only the polypropylene with a 0.5 mm mesh opening showed significantly higher attachment than the stainless steel and high-density polyethylene with smooth surfaces (data not shown). For the rocking test, however, a wide range of cell attachment was observed with different materials and surface textures (Table 2). Among the 32 material-texture combinations, six combinations (nylon 0.50 mm mesh; polypropylene 1.25 mm, 0.5 mm, and 0.05 mm meshes; and polyester 0.05 and 0.5 mm meshes) led to significantly ($p <$

0.05) higher attachment than the other combinations (Table 2). To evaluate the individual contribution of materials and their surface texture on algal attachment, the cell attachment was further plotted as a function of surface texture (Figure 4), or as a function of different materials (Figure 5) using a box chart. As shown in Figure 4, cell attachment reached the highest level with a mesh opening of 0.5 mm and was lowest in the smooth materials. The variation of attachment under stationary conditions (Figure 4A) was less than that of the rocking condition (Figure 4B). Figure 5 shows the attachment material does not significantly ($p > 0.05$) affect algal attachment under stationary growth conditions (Figure 5A). However, under rocking conditions algal attachment is drastically affected by the material used (Figure 5B). Polypropylene, nylon, and polyester exhibited significantly higher ($p > 0.05$) attachment than stainless steel, aluminum, and HDPE (Figure 5B).

We further attempted to describe a quantitative relationship of algal attachment as a function the materials and the surface texture. Here, the tetradecane contact angle (θ_{te}) was used to quantitatively represent the material surface physico-chemical properties because of its high correlation to algal attachment ($R^2=0.68$). We also introduce Wenzel's number (r) as the quantitative representation of the surface texture [19], i.e.,

$$r = \frac{a}{A} \qquad (1)$$

where a is the actual surface area of the material of a rough surface and A is the geometry of the projected area. A second order polynomial model was used to correlate algal attachment with θ_{te} and r based on the experimental data obtained in Table 2. It should be noted that the data with $r < 1.60$ and those with $r \geq 1.60$ were respectively correlated in order to gain a better correlation. Table 3 lists the estimates of the coefficients and associated p-values obtained from the second order polynomial regression.

A 3-D response surface (Figure 6) was plotted based on the coefficients for the second polynomial model in Table 3. As shown in the figure, with an increase in Wenzel's number the algal attachment increases until it reaches 1.5–1.6 (corresponding to 0.5 mm mesh size). The change in cell attachment with tetradecane contact angles, however, was not as significant as the change in the Wenzel's numbers. The optimal tetradecane contact angle varying with the Wenzel's numbers indicates an interaction between the material and the surface texture.

Long-term Cell Attached Growth on Different Materials

Following the laboratory-scale testing, the materials exhibiting the best attachment (nylon and polypropylene with various surface textures) were further tested on a pilot-scale RAB system to evaluate long-term cell attached growth as a function of different materials. Cells were incubated on a RAB system for the first 7 days for initial attachment, and then subjected to 5 cycles of repeated harvesting and re-growth at 7 days/cycle for a total of 35 days of attached growth. As shown in Figure 7A, the initial cell attachment on the materials was lower than the biomass yield during the attached growth stage. However, the overall biomass yield for the attached growth stage was correlated with the initial attachment biomass yield for each material. Figure 7B shows that biomass productivity has the same trend as biomass yield. The optimal surface textures for the attached growth stage were 0.5–1.25 mm openings for both the nylon and polypropylene, which was similar to the optimal surface texture for the initial attachment stage (Figure 2).

We also used cotton duct material as the control attachment material as this material resulted in good attachment in our previous studies [21]. It was found that nylon and polypropylene sheets with 0.5, 1.25, and 2.5 mm openings showed better algal attached growth performance than cotton duct. Collectively, Figure 7 shows that nylon materials with a 0.5–1.25

mm mesh opening were the best material-texture combination for the attached growth of algae, with a yield of 31 g/m^2 and a productivity of 4.5 g m^{-2} day^{-1} which is 87% higher than previously reported for cotton duct (Figure 7).

Discussion

The mechanisms for cell attachment have been studied in algal biofilm systems. The surfaces physico-chemical properties of the materials have been reported to play significant roles in cell attachment. For example, Genin et al., [13] found that polar surface energy had a correlation (R^2= 0.69) with algal attachment based on a consortium of freshwater algae and six materials. Ozkan and Berberoglu [12] reported that acid-base interactions are the dominant mechanism for algal attachment and hydrophobic algae tend to form biofilms better than hydrophilic algae. Other factors such as hydrophobic interactions [16] and dispersive surface energy [15] have also been reported as affecting algal attachment.

In this work, we used free surface energy and contact angles as the parameters to represent the materials physico-chemical surface properties and their implication on cell attachment. To evaluate the effect of materials surface physico-chemical properties without the interference of surface texture, we first used the material with smooth surface for the cell attachment study. Our results indicate poor correlations of cell attachment with the surface energy, water contact angle, and glycerol contact angle. However, a reasonable correlation (R^2=0.68) was found between cell attachment and tetradecane contact angle, indicating tetradecane contact angle may be an appropriate parameter to predict cell attachment. The results reported here are somewhat different from a previous report that surface energy [13] has good

correlation with cell attachment. The reason may be due to the differing culture conditions and algal species used. Also, a very comprehensive group of materials was tested in our work while only a limited number of materials were used in the previous study.

In addition to the surface physico-chemical properties, material surface texture also affected the algal attachment. Previous research has shown that algal attachment increased with increased surface texture. For example, Sekar et al., [16] observed an enhanced algal attachment on metals that had been sanded with different grits of sand paper. Cao et al., [18] created a dimpled surface of steel materials (6–8 µm in diameter and 2–3 µm in depth) which resulted in higher cell attachment than a smooth surface. Sathananthan et al. [22] reported that a V-shape groove pattern with the same size scale as the algal cells resulted in higher biomass productivity than the smooth materials. Cui et al. [19] studied the effect of three different patterns (ridge, pillar, and groove) on cell attachment and concluded that attachment was preferred when the pattern size was close to the cell diameter.

In this work, we altered the surface texture by attaching a mesh sheet to a smooth surface of the material; the resulting square pore texture significantly increased cell attachment. However, the trend of cell attachment with pore size observed in this work is different from that reported previously [19]. For example, our results indicate that a mesh opening of 0.5 mm is optimal for algal attachment; the size less than this value (e.g., 0.05 mm opening) exhibited lower attachment. On the contrary, Cui et al., [19] reported that algal attachment increased with decreasing pore size until the opening was equal to or smaller than the algal cells. Based on this conclusion, the 0.05 mm opening, which is larger than the cell size used (*Chlorella sp*. 10-20 µm), should have given higher cell attachment than the 0.5 mm opening. The reason for this difference is that the algae in our study appeared to form flocks, which were not easily

"accommodated" by the 0.05 mm openings, while the Cui et al. [19] study used single cells. The SEM imaging also confirmed this hypothesis, i.e., the cells formed flocks during growth which would not easily fit into the 0.05 mesh opening size. The impact of algal cell flocking in biofilm systems needs to be carefully considered in future attachment materials development.

It should be noted that previous research on algal attachment has been done under either stationary liquid conditions [19, 15] or flowing liquid conditions [22, 13]. In this study, to provide the same baseline comparison, both stationary and flowing (rocking) conditions were used to investigate cell attachment. Our results indicate that cell attachment in stationary conditions was quite different from that obtained in rocking conditions. This may be due to the shear force applied to the algal biofilm under the rocking conditions as compared to the stationary condition which does not generate shear force. As liquid flow always exists in algal biofilm systems, we believe the rocking condition is more appropriate to mimic the true conditions in algal biofilm cultures. Therefore, future materials development should be investigated in rocking or similar systems with liquid flow instead of stationary conditions.

The above discussion shows that both surface physico-chemical properties and surface texture of the materials play important roles in algal attachment. In general, surface physico-chemical properties determine the thermodynamics of cell attachment, i.e., whether the cell can attached to the materials surface. The surface texture, however, determines the local hydrodynamic conditions the algal biofilm encounters, i.e., whether the attached algal cells can be "sheltered" from the shear stress and avoid sloughing off of the material surface. The ultimate algal attachment performance is the outcome of the interaction of the surface physico-chemical properties and surface texture. However, studies on the combined effects of these two group parameters on cell attachment are still very rare; it was also unclear what the relative importance

of these two parameters is for cell attachment. To fill these gaps, we performed a thorough investigation of the interaction of these two groups of parameters and their roles on cell attachment. In particular, this co-effect was quantified with a second-order polynomial.

Another issue worth noting is the difference between the initial cell attachment to fresh material and cell attached growth once the colony is established. The former is a usually a rapid process while the latter needs to be investigated in long-term cultivation. In the previous studies, these two concepts were not clearly defined and were intermingled. In this work, the research performed in first three Result sections focused on initial cell attachment, while the research in last Result section focused on long-term attached growth. The results show that similar to the initial cell attachment test, the cell attached growth also depends on both the material surface physico-chemical properties and the surface textures.

The pilot-scale attached growth experiments demonstrate that materials with an appropriate combination of surface physico-chemical properties and surface texture can lead to not only good short-term initial cell attachment, but also superior long-term attached growth. The optimal material/texture combinations were nylon and polypropylene mesh sheet with 0.5–1.25 mm openings. In our previous research, we used cotton duct sheet as the attachment material. Although good attachment was reported with the cotton duct sheet, this material tended to deteriorate after soaking in the liquid for 2–3 months [9] On the contrary, the nylon- and polypropylene-based materials with an appropriate surface texture are economical and resistant to degradation, and therefore, more applicable for commercial implementation.

Conclusions

This research reports a comprehensive study of the effects of material surface physico-chemical properties and texture on the initial colonization and the long-term attached growth in algal biofilm systems. The two properties and their interactions play important roles in both the initial colonization and sustained attached growth. The tetradecane contact angle and the Wenzel's number for the materials were good parameters to correlate algal attachment. Collectively, it was found that polypropylene and nylon mesh with 0.5–1.25 mm openings were the best materials for initial cell attachment and long-term attached growth, with a biomass yield of 31 g/m^2 and a productivity of 4.5 g m^{-2} day^{-1} achieved in a pilot-scale revolving algal biofilm system.

Materials and Methods

Algal Strain and Subculture

The microalgal cultures were taken from a raceway pond (2,000 L) in the Algal Production Facility at the Iowa State University Biocentury Research Farm in Boone, Iowa, USA. The pond was initially inoculated with *Chlorella vulgaris* (UTEX #265) and has been operated for a year to establish a stable algal community. The culture conditions for the open pond were described previously [8]. The algae culture collected from the raceway pond was maintained in a flat panel reactor (16-L) prior to use as the seed for the attachment experiment. The flat panel rector was illuminated under natural sunlight and the temperature maintained between 20–25°C. The medium used for culture maintenance was Bolds Basal Medium.

Algal Cell Attachment on Different Materials

A total of 28 materials with a smooth surface were tested for cell attachment (Table 1). The

materials can be categorized as metal, plastic, and rubber. The selection of the materials was

based on their nature of being readily available, non/slowly-degradable, and low cost. Each type

of material was cut into three replicate square pieces (10cm×10cm) and attached to the bottom of

transparent Plexiglas chambers. Each chamber had a dimension of 70 cm × 25 cm × 20 cm, and

thus could handle 12 pieces of materials at one time. The material pieces were randomly placed

in different locations of the chamber. Three liters of algal culture with a cell density of 1 g/L was

added to each chamber. The chambers were incubated at 20°C under continuous 110–120 μmol

s^{-1} m^{-2} of illumination for 7 days. The cell attachment was tested under either stationary or

rocking conditions. To create a rocking motion, the chambers were placed on a rocking shaker

with smooth gentle rocking at 15° from the horizontal plane at 20 cycles per minute.

To determine cell colonization on the materials, the chamber was lifted vertically to

remove the settled, but unattached, algae from the material. The attachment materials were

removed from the chamber. The cell colonization on each different material was evaluated with a

Likert scale to determine the percentage of attached cells on the materials surface [23]. For each

material, three trained researchers independently determined the percentage of colonization by

visual observation and these were then averaged.

Six materials were selected for further study to determine the effects of surface texture on

cell attachment. These materials were two metals (aluminum and stainless steel) and four plastics

(polyester, high density polyethylene, nylon, and polypropylene). To create different surface

textures, commercially available mesh sheets with different pore sizes (0.05 mm, 0.5 mm, 1.25

mm, 2.5 mm, and 6.4 mm openings) were adhered to the same material with a smooth surface.

The polyester sheet with 6.4 mm opening; high density polyethylene sheet with 0.05mm and 0.5 mm openings; and nylon sheet with 6.4 mm opening were not available. The mesh sheets were cut into 25 cm × 25 cm squares and attached to the bottom of the chambers, and incubated at 20°C under continuous 110–120 µmol s^{-1} m^{-2} of illumination for 7 days. The tests were performed under stationary and rocking conditions. Cell attachment on the materials with different textures was quantified by scrapping the biomass and measuring the cell dry weight.

Evaluation of Material Surface Physico-chemical Properties

Sessile drop tests were used to determine the liquid contact angle of the materials [24]. In short, 5µL of three reference liquids, distilled water, glycerol, and tetradecane were pipetted onto the surface of the material and a picture was taken using a Nikon D800 camera with an AF-S Nikkor 35mm F/1.4G lens. The images were analyzed using imageJ and a Java plug-in, DropSnake 2.1. This software used a global model of a reference drop and obtained contact angles reflecting the whole drop profile.

The contact angles were then used to determine the surface energies of the materials using Young's equation, i.e.,

$$\gamma_S = \gamma_L \cos\theta + \gamma_{SL} \tag{2}$$

where γ_S is the surface free energy of the solid material, γ_L is the surface energy of the liquid, θ is the contact angle, and γ_{SL} is the interfacial energy between solid and liquid. A Van Oss-Chaudhury-Good thermodynamic approach [25] was used to determine γ_{SL}. In brief, γ_{SL} consists of polar ($\gamma_{SL}{}^P$) and non-polar ($\gamma_{SL}{}^{LW}$) components, i.e.,

$$\gamma_{SL} = \gamma_{SL}{}^P + \gamma_{SL}{}^{LW} \tag{3}$$

The values of $\gamma_{SL}{}^P$ and $\gamma_{SL}{}^{LW}$ can be calculated as [25],

$$\gamma_{SL}{}^P = 2\left(\sqrt{\gamma_S{}^+} - \sqrt{\gamma_L{}^+}\right)\cdot\left(\sqrt{\gamma_S{}^-} - \sqrt{\gamma_L{}^-}\right) \qquad (4)$$

$$\gamma_{SL}{}^{LW} = \left(\sqrt{\gamma_S{}^{LW}} - \sqrt{\gamma_L{}^{LW}}\right)^2 \qquad (5)$$

where $\gamma_S{}^+$ and $\gamma_S{}^-$ are the acid and base interactions of the solid, $\gamma_L{}^+$ and $\gamma_L{}^-$ are the acid and

base interactions of the liquid, and $\gamma_S{}^{LW}$ and $\gamma_L{}^{LW}$ are Lifshitz-van der Waals forces/interactions

for solid and liquid, respectively. The solid properties, $\gamma_S{}^+$, $\gamma_S{}^-$, and $\gamma_S{}^{LW}$, can be obtained

through the van Oss-Chaudhury-Good Equation, i.e.,

$$(1 + \cos\theta)\gamma_L = 2\left(\sqrt{\gamma_S{}^{LW}\gamma_L{}^{LW}} + \sqrt{\gamma_S{}^+\gamma_L{}^-} + \sqrt{\gamma_S{}^+\gamma_L{}^-}\right) \qquad (6)$$

As the values of $\gamma_L{}^+$, $\gamma_L{}^-$, and $\gamma_L{}^{LW}$ can be known through choosing an appropriate

liquid, the unknown variables from Eq. (6) are $\gamma_S{}^+$, $\gamma_S{}^-$, and $\gamma_S{}^{LW}$. By applying a non-polar

liquid (tetradecane) to Eq. (6), the equation becomes,

$$(1 + \cos\theta)\gamma_L = 2\left(\sqrt{\gamma_S{}^{LW}\gamma_L{}^{LW}}\right) \qquad (7)$$

From which the value of $\gamma_S{}^{LW}$ can be obtained. Then, applying two other polar liquids (water

and glycerol) and Eq. (6) twice, the two remaining unknowns, $\gamma_S{}^+$ and $\gamma_S{}^-$, can be solved.

Scanning Electron Microscopy

A Quanta FEG 250 SEM was used to image the algal attachment on the mesh materials in E-

SEM (environmental scanning electron microscopy) mode. The chamber was set at 3 Torr of

water vapor pressure to try to minimize drying. The SEM was operated at 20 kV accelerating

voltage with a working distance of 14 mm. FEI's gaseous secondary electron detector (GSED)

was used to collect a secondary electron image.

Cell Attached Growth on Different Materials at Pilot-scale

Polypropylene and nylon demonstrated the best algal attachment and were selected for further testing to determine their performance for long-term algal attached growth using a pilot-scale revolving algal biofilm (RAB) system. The detailed design and operation of the RAB system was described in our previous publication [8]. In short, the RAB system consisted of vertical conveyor belts rotating though a standard raceway pond at a linear velocity of 4 cm/sec. For each material, four different levels of surface texture (0.05, 0.5, 1.25, and 2.5 mm mesh size) were used to support the attached algal growth for a total of 42 days of operation. During this period, the cells were harvested every 7 days; thus, there was a total of 6 harvest/regrowth cycles. During each harvest, attached cells were scraped from 1 ft^2 of the individual attachment material and then freeze dried to identify the dry weight.

Abbreviations

HSD: Tukey's Honestly Significant Difference

RAB: revolving algal biofilm

SEM: Scanning Electron Microscopy

θ: contact angle

θ_{te}: tetradecane contact angle

r: Wenzel's number

γ_S: surface free energy of the solid material

γ_L: surface energy of the liquid

γ_{SL}: interfacial energy between solid and liquid.

$\gamma_{SL}{}^P$: polar components of γ_{SL}

$\gamma_{SL}{}^{LW}$: non-polar of components of γ_{SL}

$\gamma_S{}^+$ and $\gamma_S{}^-$: acid and base interactions of the solid, respectively

$\gamma_L{}^+$ and $\gamma_L{}^-$: acid and base interactions of the liquid, respectively

$\gamma_S{}^{LW}$ and $\gamma_L{}^{LW}$: Lifshitz-van der Waals forces/interactions for solid and liquid, respectively.

Statistical Analysis

All the tests were performed in triplicate, with the results being presented as the mean ± SD or as a box chart. Three-way analysis of variance (ANOVA), Tukey's Honestly Significant Difference (HSD) test, and response surface were done by the software R [26].

Competing interests

Authors Z. Wen and M. Gross have equity interests and management roles in Gross-Wen Technologies, LLC. The terms of this arrangement have been reviewed and approved by Iowa State University in accordance with its conflict of interest policies. No non-financial conflicts of interests exist for any of the authors.

Author contributions

MG carried out the algal cultivation studies, participated SEM analysis, and drafted the manuscript. XZ participated in the design of the study and performed the statistical analysis. VW carried out the determination of the physio-chemcial properties of the attachment materials. ZW conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgments

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Table 1. Attachment materials (with smooth surface) and their surface physico-chemical properties using cell attachment tests.

Attachment Material	Liquid contact angle (º)			Surface energy (mJ/m²)
	Water	Glycerol	Tetradecane [1]	
Metals				
Aluminum	96.6	89.6	< 2	168.0
Brass	89.4	94.2	< 2	193.0
Stainless Steel	80.9	80.3	< 2	43.1
Plastics				
Acrylonitrile Butadiene Styrene (ABS)	84.7	78.2	23.4	36.7
Nylon	59.6	45.2	15.5	43.2
Polyethylene Terephthalate (PETG)	68.5	69.0	< 2	44.0
Chlorinated Polyvinyl Chloride (CPVC)	78.6	80.0	11.9	40.5
Derlin Acetal Resin	72.1	68.3	9.3	42.5
Polyester	82.7	74.2	6.3	41.0
Polylactic Acid (PLA)	80.8	88.1	24.6	38.7
Polycarbonate	86.1	77.6	< 2	46.0
Extruded Acrylic	76.8	61.7	6.2	44.7
Extruded Nylon	72.7	57.0	9.1	45.8
High Density Polyethylene (HDPE)	88.0	73.9	3.0	35.3
Polypropylene	91.5	81.0	16.6	32.6
Polystyrene	75.8	76.8	< 2	34.0
Low Density Polyethylene (LDPE)	89.1	60.4	< 2	33.6
Polyvinyl chloride (PVC)	88.3	77.5	< 2	33.0
Rexolite Polystyrene	51.8	140.3	< 2	38.3
Ultra-high-molecular-weight Polyethylene	84.2	63.1	< 2	37.2
Rubbers				
Buna-N Rubber	38.2	92.0	30.6	31.4
Neoprene Rubber	92.6	92.1	24.1	39.8
Noryl PPO	80.2	83.4	3.9	52.5
Gum Rubber	59.6	81.0	26.9	31.8
Butyl Rubber	92.9	93.0	33.8	29.9
Ethylene Propylene Diene Monomer (EPDM)	110.4	85.2	32.1	34.9
Epichlorohydrin (ECH) Rubber	55.5	58.6	< 2	43.2
Hypalon Rubber	71.2	83.5	16.6	57.9
Latex Rubber	92.9	124.2	28.2	36.3
Polyurethane	89.8	83.1	< 2	37.5
Santoprene Rubber	89.8	95.8	43.5	26.1
Styrene-butadiene (SBR) Rubber	84.2	89.0	23.8	44.9
Silicone Rubber	58.2	85.2	12.3	21.3

[1] Tetradecane contact angles with < 2° were below the measurement limit.

Table 2. Tukey's Honestly Significant Difference (HSD) test of cell attachment as a function of the materials and their surface textures under rocking conditions.

Ranking	Material	Surface texture (Mesh opening, mm)	Attachment (g/m²) [a]	Groups [b]
1	Nylon	0.50	91.16±10.13	A
2	Polypropylene	1.25	88.25±18.37	A
3	Polyester	0.50	82.59±26.38	AB
4	Polypropylene	0.50	82.34± 9.93	AB
5	Polypropylene	0.05	79.24± 0.97	AB
6	Polyester	0.05	67.37±16.70	ABC
7	Nylon	0.05	50.28±12.29	BCD
8	Aluminum	0.50	41.13±11.45	CDE
9	Stainless Steel	0.50	36.39± 9.49	CDEF
10	Polyester	2.50	34.64±11.06	DCEFG
11	Polyester	1.25	33.96±10.67	DEFG
12	Nylon	2.50	33.66±16.45	DEFG
13	Stainless Steel	1.25	33.20± 2.12	DEFG
14	High-density Polyethylene	2.50	25.39± 7.12	DEFG
15	Aluminum	1.25	24.73±14.03	DEFG
16	Nylon	1.25	22.43± 9.04	DEFG
17	Polypropylene	2.50	21.82± 3.98	DEFG
18	High-density Polyethylene	0.50	21.24± 8.63	DEFG
19	Stainless Steel	2.50	15.91±10.45	EFG
20	Aluminum	2.50	15.54± 6.19	EFG
21	Aluminum	0.05	14.64± 1.91	EFG
22	Polypropylene	6.40	14.23± 4.75	EFG
23	Polyester	Smooth	13.19±14.04	EFG
24	High-density Polyethylene	6.40	12.77± 1.94	EFG
25	Stainless Steel	0.05	12.72± 4.36	EFG
26	Aluminum	6.40	9.41± 2.10	EFG
27	Stainless Steel	6.40	6.46± 3.67	FG
28	High-density Polyethylene	Smooth	6.19± 5.19	FG
29	Aluminum	Smooth	4.07± 5.10	FG
30	Stainless Steel	Smooth	3.27± 1.89	G
31	Polypropylene	Smooth	2.40± 0.83	G
32	Nylon	Smooth	1.84± 1.15	G

[a] Data are mean ± standard deviations of three replicates.
[b] Attachment with at least one common letter are not significantly ($p > 0.05$) different.

Table 3. Estimates of coefficients of the variables in the second-order polynomial regression with different Wenzel's numbers.[a]

| Coefficient	Variables	$r < 1.60$		$r \geq 1.60$	
		Estimate	P-value	Estimate	P-value
α_0	Constant	156.2	0.08	104.6	0.15
α_1	θ_{te}	-2.2	0.43	19.4	< 0.05
α_2	r	275.3	< 0.05	65.4	0.13
α_{12}	$\theta_{te} \times r$	3.7	< 0.05	1.7	0.69
α_{11}	θ_{te}^{2}	-0.1	0.38	0.7	< 0.05
α_{22}	r^2	122.6	< 0.05	n.a.[b]	n.a.
Regression coefficient (R²)		0.57		0.75	
P-value		< 0.001		< 0.001	

[a] The equation $Y = \alpha_0 + \alpha_1\theta_{te} + \alpha_2 r + \alpha_{12}\theta_{te}r + \alpha_{11}\theta_{te}^{2} + \alpha_{22}r^2$ was used for correlation, where Y is cell attachment (g/m²), r is the Wenzel' number (dimensionless), and θ_{te} is tetradecane contact angle (degree °).

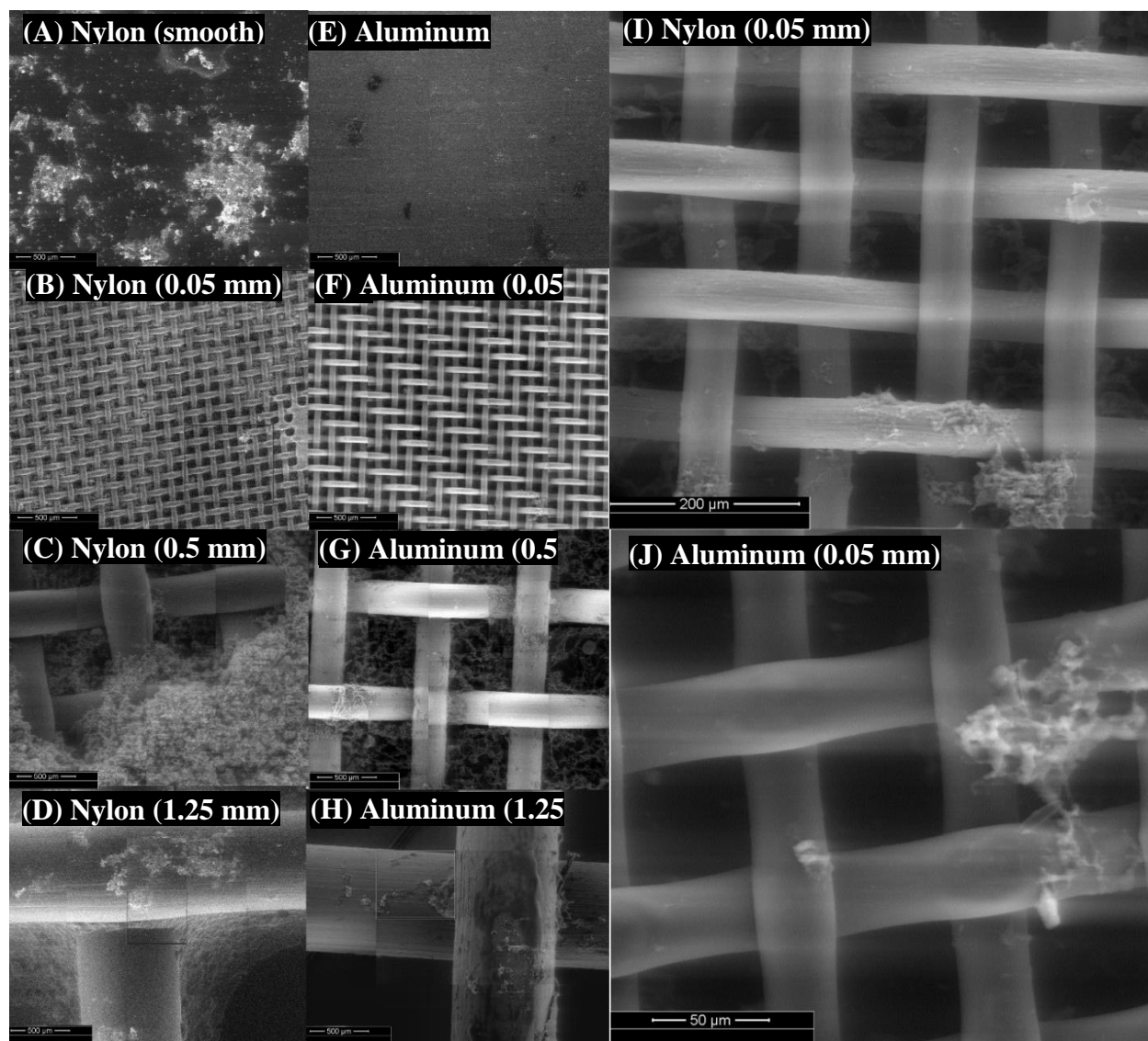[b] The estimate was not available.
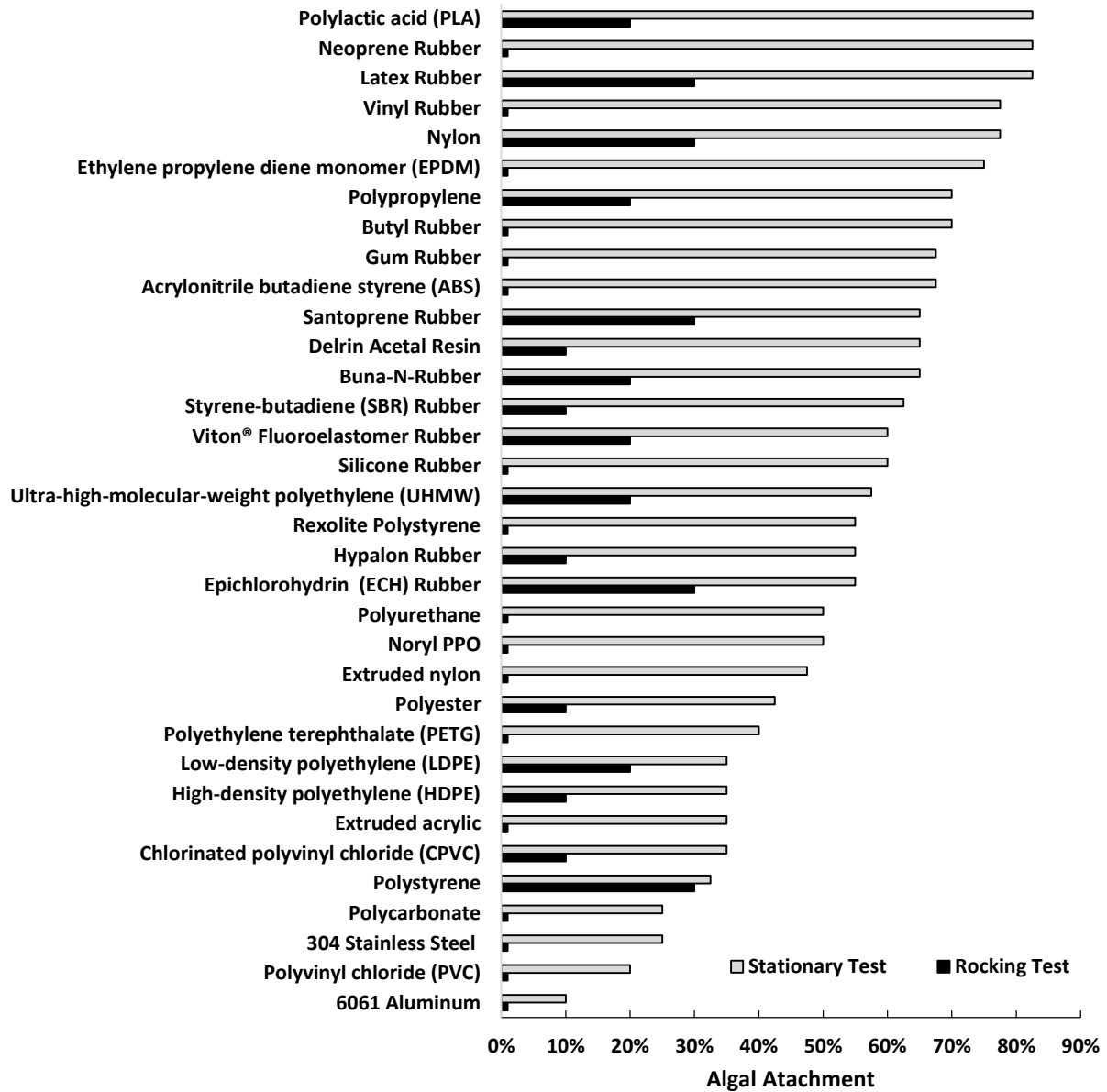
Figure 1. Algal colonization (represented as area covered by attached algae as a % of material surface) on different materials with smooth surface.
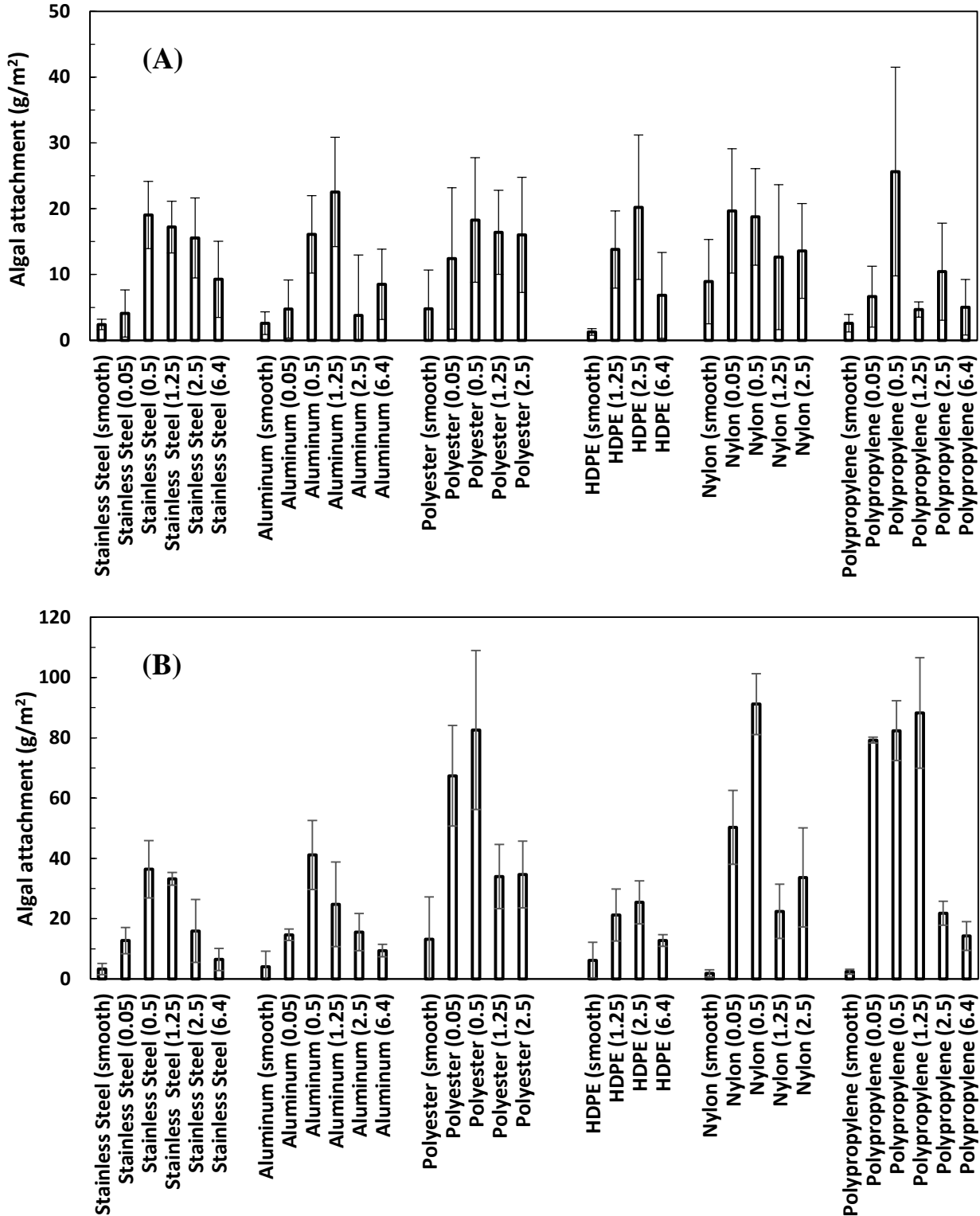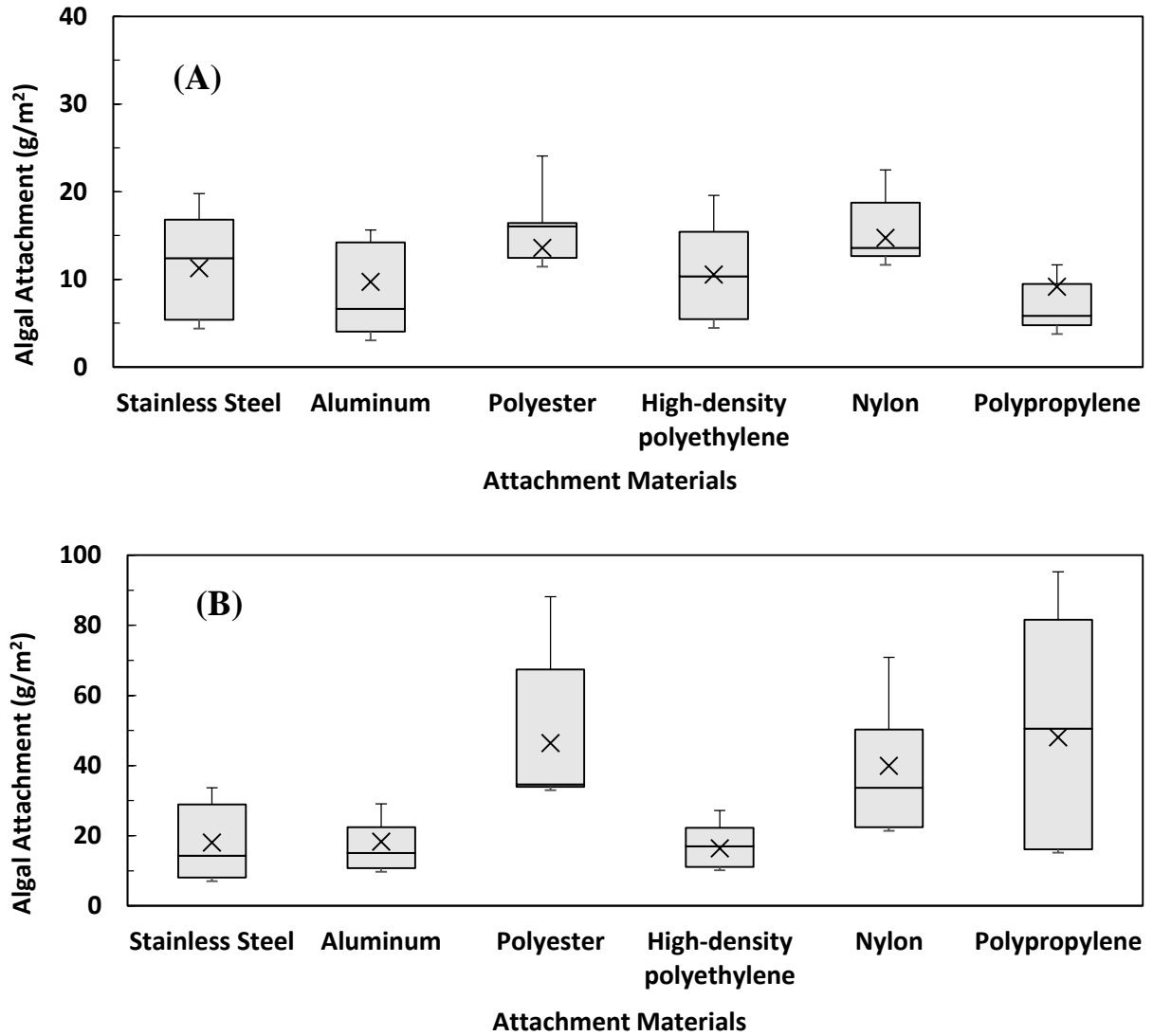
Figure 2. Algal attachment on materials with different textures at **(A)** stationary conditions and **(B)** rocking conditions. The figure in the parenthesis is the mesh opening size, mm.

Figure 3. SEM images of algal attachment on nylon and aluminum with different mesh size openings. **(A)** nylon with smooth surface, **(B)** nylon mesh with 0.05 mm opening, **(C)** nylon mesh with 0.05 mm opening, **(D)** nylon mesh with 1.25 mm opening, **(E)** aluminum with smooth surface, **(F)** aluminum mesh with 0.05 mm opening, **(G)**, aluminum mesh with 0.5 mm opening, **(H)** aluminum mesh with 1.25 mm opening, **(I)** nylon mesh with 0.05 mm opening with increased magnification, and **(J)** aluminum mesh with 0.05 mm mesh with increased magnification. The experiments were tested under rocking conditions.
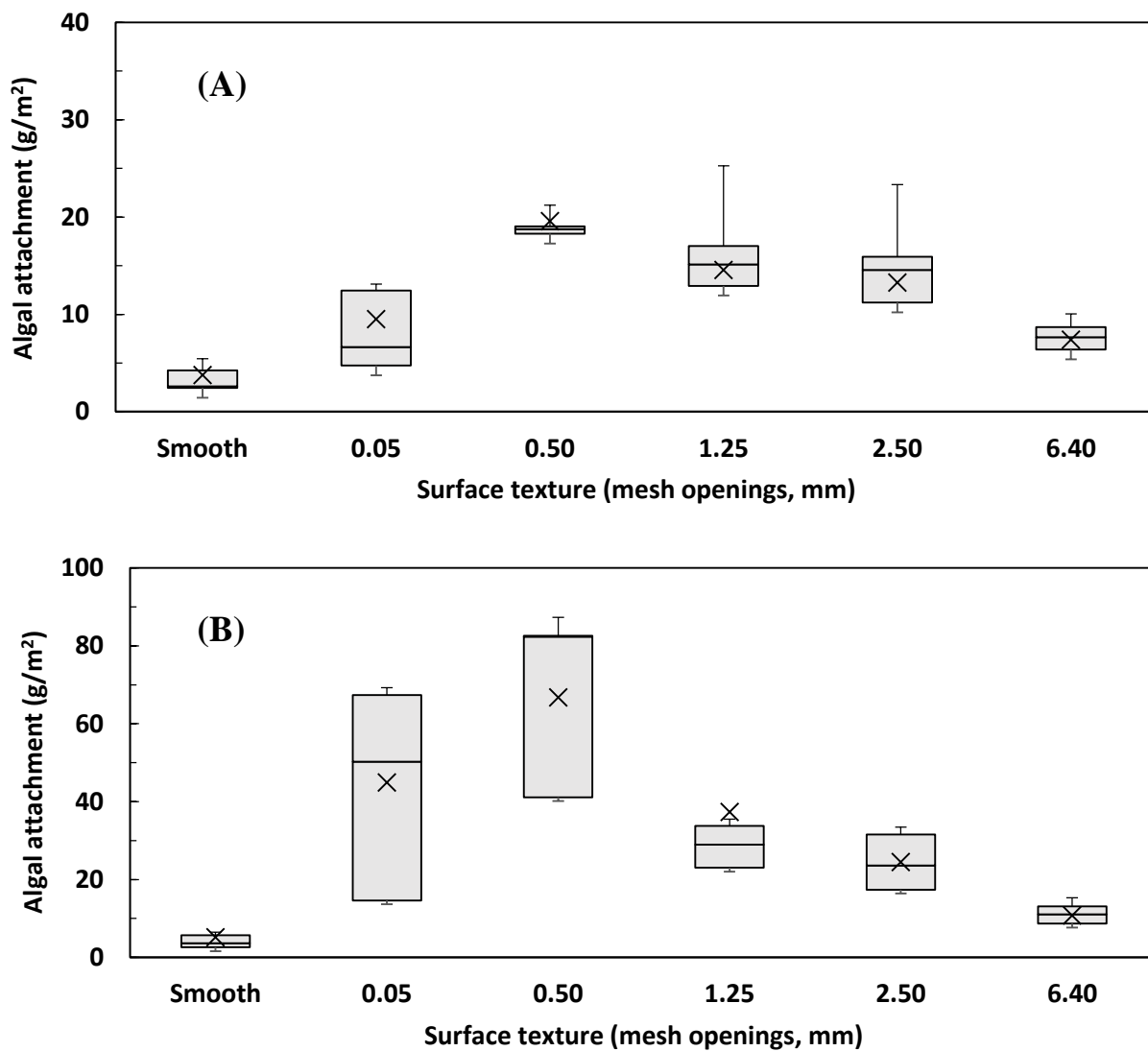
Figure 4. Algal attachment as a function of the material surface texture **(A)** stationary conditions and **(B)** rocking conditions. For each texture size, attachment on different materials (stainless steel, aluminum, polyester, HDPE, nylon, polypropylene) were grouped together to draw the box chart.

Figure 5. Algal attachment as a function of the materials **(A)** stationary conditions and **(B)** rocking conditions. For each material, attachment on different surface textures (smooth, 0.05, 0.5, 1.25, 2.50, 6.40 mm) were grouped together to draw the box chart.
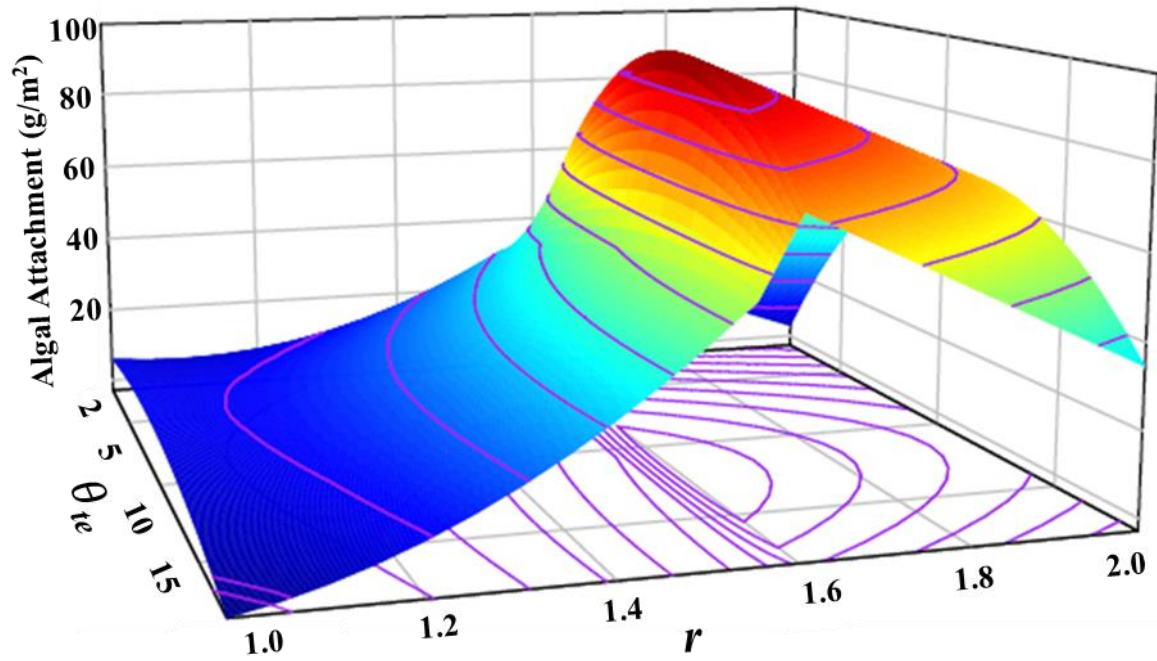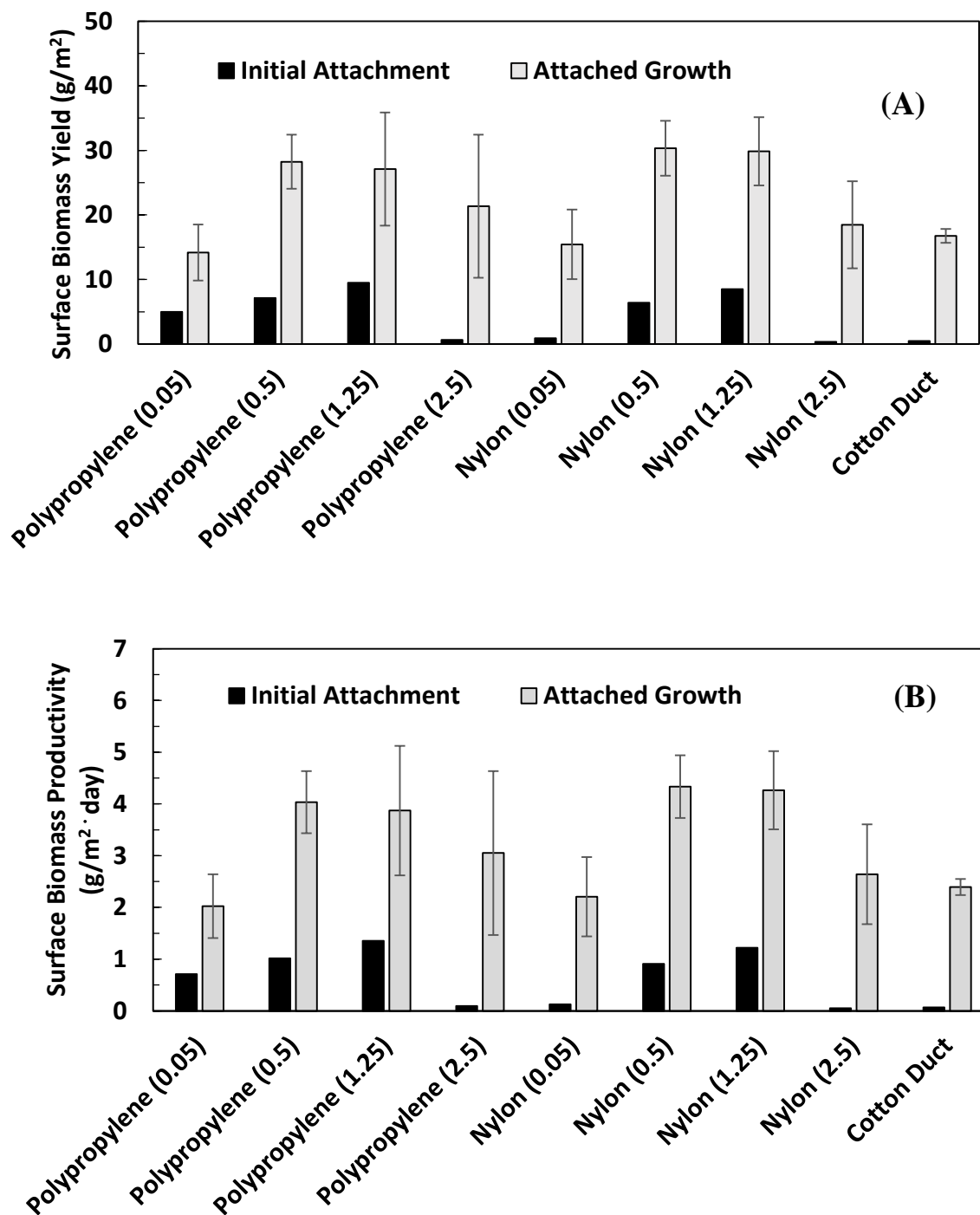
Figure 6

Figure 6. Response surface model of cell attachment as a function of material surface properties (tetradecane contact angle, θ_{te}) and surface texture (Wenzel's number, r).

Figure 7. Algal attached growth on materials with various mesh openings. **(A)** Biomass yield per surface area of attachment materials and **(B)** biomass productivity per surface area of attachment. Cells were incubated using a RAB system for 7 days for initial attachment, and then repeatedly harvested and re-grown for 5 cycles at 7 days/cycle with a total of 35 days attached growth. The figure in the parenthesis is the mesh opening size, mm.

CHAPTER 6. COMPARATIVE TECHNO-ECONOMIC ANALYSIS AND LIFE-CYCLE

ASSESSMENT OF MICROALGAL BIOMASS PRODUCTION IN A REVOLVING

ALGAL BIOFILM CULTIVATION SYSTEM AND A RACEWAY POND

A paper to be submitted to *Algal Research*

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1.0 Introduction

Microalgae represent a group of photosynthetic microorganisms that are capable of rapid

growth on minimal nutrients. Microalgae only require basic nutrients, CO_2 and sunlight to grow.

Furthermore it can be grown in areas with poor soil quality which are not suitable for terrestrial

agriculture. In addition to microalgae's rapid growth rate various species are capable of

producing a variety of biobased fuels, feeds, chemicals, and nutraceuticals. However questions

still remain on the best practices to sustainably and economically produce microalgae biomass.

One promising application of microalgae is its use in wastewater treatment, because it has shown

to rapidly accumulate nutrients such as nitrogen and phosphorus (Mata et al., 2010).

Recent assessments of current microalgae cultivation, harvest, and dewatering technologies

have shown large inputs of energy and materials. In a landmark study done by Benemann and

Oswald (1996), it was estimated that harvesting and dewatering of algae down to 80% moisture

would contribute to 33.2% of total capital costs in an open pond system. Additionally Molina

Grima et al. (2003) reported that water removal contributed up to 30% of total capital and

operating costs and Davis et al. (2011) reported that harvesting alone contributes to 21% of capital costs in an open pond system.

To address the high costs associated with harvesting a dewatering microalgae many biofilm based cultivation systems have been developed. In these biofilm based systems algae is allowed to attach on a surface of a material and is easily harvested via scraping. When harvested, the algal paste already has a water content similar to post-centrifuged algal biomass, (80-90%) so the expensive harvesting and dewatering steps can be avoided (Christianson and Sims, 2012; Gross et al., 2013).

In recent years our research group has developed a unique revolving algal biofilm (RAB) cultivation system for attached algal growth which has been shown to be effective at growing concentrated, easily harvested biomass. The specific details of the system are described in our previous publications (Gross et al. 2013, Gross and Wen, 2014). In short, it consists of a revolving belt that algae cells are attached on and when the biofilm gets to a certain thickness it is easily scrapped off. This unique design has shown several advantages that include simple harvest, high gas mass transfer rates, enhanced light utilization, and enhanced biomass productivity. In a yearlong pilot-scale study, we have demonstrated an average increased biomass productivity of 302% using the RAB system in comparison to a control raceway pond (Gross and Wen, 2014).

Although the RAB system appears to have several advantages compared to open pond culture there are still many areas that need to be evaluated, which include: (1) What is the optimal geometry of the belts? (2) What is the impact of growth in different climates? (3) What do the economics and life cycle look like? The main objective of this study is to address one of those key areas, what is the impact of the additional infrastructure and energy required to

produce microalgae on a continually rotating biofilm and because the algae is naturally

concentrated what is the impact of not having to purchase and operate capitally and energy

intensive harvesting and dewatering equipment.

In this paper a comparison between a raceway pond and the RAB cultivation system. In

previous research a yearlong pilot-study was conducted to evaluate productivity differences

between the RAB and raceway pond (Gross and Wen, 2014). This data will be used in this study

for algal productivity values. A raceway pond was selected to compare against the RAB system

because it is the most frequently used commercial algal cultivation method.

Both an LCA and a TEA of each system is reported at the scales of 8.5 m^2 (pilot-scale)

270 m^2 (greenhouse size) 1 hectare and 100 hectare at two different climates (Iowa and Arizona,

USA). In this study only cultivation, harvesting, and dewatering to 20% solids was considered.

If further downstream processing was considered it would detract from the motivation of the

study which was focused on evaluating the environmental impact and cost of producing biomass

in each of these systems.

2.0 Experimental approach

2.1 Goal and Scope

There was two main goals of this study. (1) Conduct an economic analysis of both the

raceway pond and RAB systems, treating municipal wastewater. (2) Evaluate and compare the

environmental impacts of both the raceway pond and RAB systems. When evaluating the

environmental impacts of each system both energy and materials consumed during cultivation,

harvest and dewatering are considered. Construction of the cultivation systems are not

considered in the environmental assessment due to the uncertainty of materials needed to construct either system (Figure 1A). In the TEA work construction of the RAB and raceway ponds were considered, in addition to cultivation and dewatering to 80% moisture (Figure 1B).
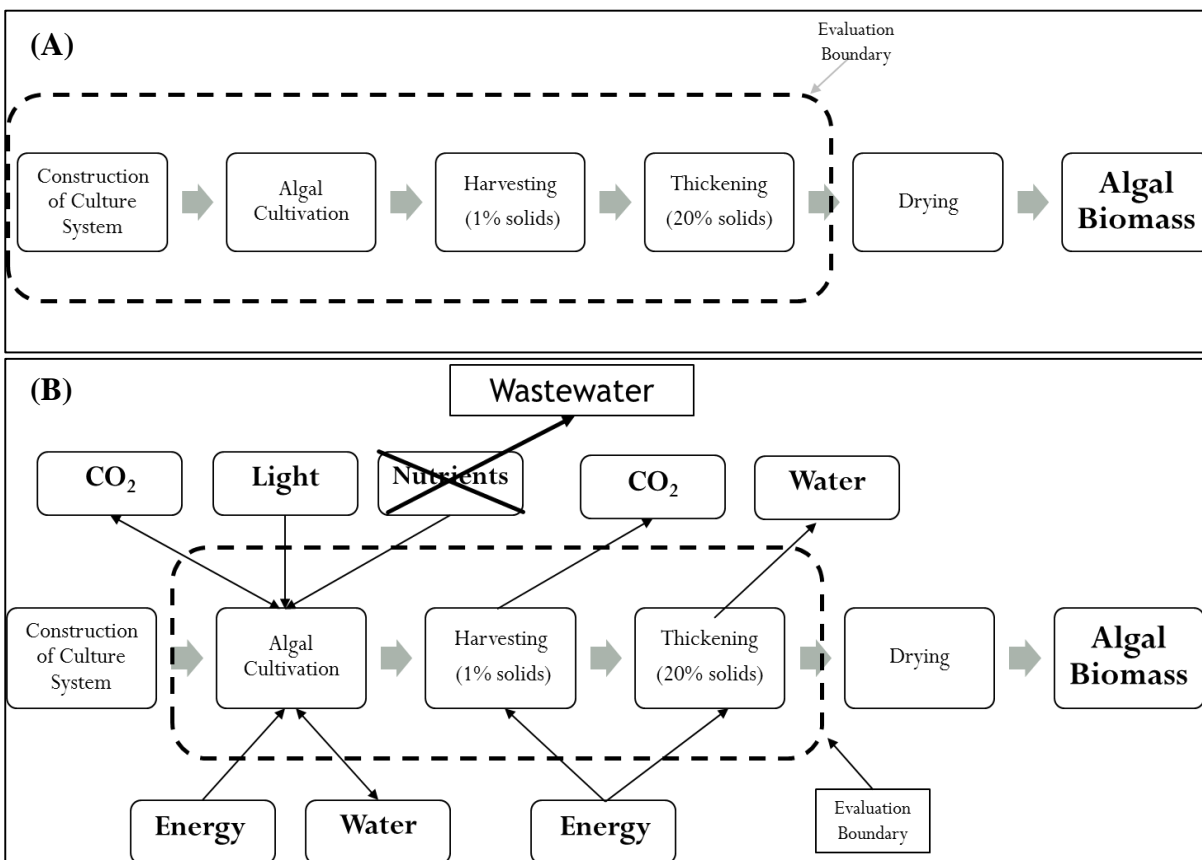
Figure 1. Evaluation boundaries for this study **(A)** TEA, **(B)** LCA

2.2 Functional Units

In the TEA study the functional unit will be cost (USD) per metric ton of algal biomass. In this study the functional unit for the LCA study will be based on energy, water and GHG emissions per metric ton of algal biomass.

In many other TEA/LCA studies the functional units are based on cost to produce biodiesel from algae. However in recent years hydrothermal conversion processes have been developed that utilize the whole cell to produce fuels. These hydrothermal processes have shown to be

more economically favorable in comparison to the traditional biodiesel from algae pathway. Do

to this transition, the quantity of biomass being produced is becoming more important than

producing a biomass high in oil. Additionally many other products such as fertilizers, and animal

feeds could be produced from algal biomass. Thus in this study the functional unit will be based

on dried algal biomass not biodiesel.

2.3 System descriptions and assumptions

In total, there are sixteen different scenarios presented in this study (Table 1). Two different

climates will be considered, Iowa and Arizona USA. These two climates were selected because

the pilot-scale research on the RAB was done in Iowa, and Iowa represents a non-ideal algal

growth environment, due to a moderate yearly light intensity. However, Arizona represents a

high light intensity environment that is ideal for algae production. Additionally each the RAB

and raceway pond will be evaluated at three different scales, 8.5 m^2, 270 m^2, 1, and 100 hectares.

These scales were selected because 8.5 m^2 represents the current pilot-scale system reported in

previous literature (Gross and Wen, 2014), and 270 m^2 represents the size of a commercial scale

greenhouse (30 x 96 ft) and 1, and 100 hectares represent potential commercial scale cultivation.

The algal productivity in the Iowa site analysis will be based on real data obtained from a

yearlong pilot-scale study done on side-by-side 8.5m^2 raceway based-RAB and raceway pond

systems. Based on data obtained from the ATP[3] harmonization study (http://atp3.org/wp-

content/uploads/2014/07/ATP3_BioWorldCongress2014.pdf). The algal productivity at the

AzCATI study site in Arizona, was approximately double the productivity that was achieved in

the Touchstone site that is located in Ohio. Iowa has a similar climate to that found in Ohio. So

in this study the assumption will be made that the productivity in Arizona is double that of the

productivity achieved in Iowa.

Table 1. Cultivation scenarios of this study

	Growth System	**Location**	**Size (m^2)**	**Volume (L)**
Scenario 1	Raceway Pond	Iowa	8.5	1,700
Scenario 2	Raceway Pond	Iowa	270	54,800
Scenario 3	Raceway Pond	Iowa	10000	2,032,000
Scenario 4	Raceway Pond	Iowa	1000000	203,200,000
Scenario 5	Raceway Pond	Arizona	8.5	1,700
Scenario 6	Raceway Pond	Arizona	270	54,800
Scenario 7	Raceway Pond	Arizona	10000	2,032,000
Scenario 8	Raceway Pond	Arizona	1000000	203,200,000
Scenario 9	Revolving algal biofilm	Iowa	8.5	1,700
Scenario 10	Revolving algal biofilm	Iowa	270	54,800
Scenario 11	Revolving algal biofilm	Iowa	10000	2,032,000
Scenario 12	Revolving algal biofilm	Iowa	1000000	203,200,000
Scenario 13	Revolving algal biofilm	Arizona	8.5	1,700
Scenario 14	Revolving algal biofilm	Arizona	270	54,800
Scenario 15	Revolving algal biofilm	Arizona	10000	2,032,000
Scenario 16	Revolving algal biofilm	Arizona	1000000	203,200,000

2.3.1 Raceway pond system

The raceway ponds will be operated at 0.209 m depth, with an average liquid flowing velocity of

20 cm/sec. The Iowa yearly average productivity of 5 g m^{-2} day^{-1}used for calculations was the

average productivity determined during a year-round study by Gross and Wen (2014). In this

study the capital and operational expenses were estimated based on Benemann and Oswald's

(1996) study that estimated these costs for a 100 hectare raceway pond facility. To scale down

from 100 hectares to the smaller scales the following equation was used:

$$(1) \qquad C_{p,s} = C_{p,b} \, (S_s/S_b)^n$$

Where $C_{p,s}$ is predicted capital cost of specified equipment, $C_{p,b}$ is known cost of baseline sized

equipment, S_s is size of specified (predicted) equipment, S_b is size of baseline equipment and n is

the scaling factor. For this work the scaling factor of 0.67 was used for all equipment other than paddlewheels (0.4) and greenhouse (0.8).

2.3.2 Revolving algal biofilm system

Each RAB system in this study retrofitted a raceway pond that was identical to the raceway pond used in this comparison study. The RAB reactors were 2 m tall and 2m wide and serpentine perpendicular to fluid flow in the raceway (Figure 2). The costs in this study were estimated by adding the same costs associated with the raceway pond then adding additional costs for the RAB frame, attachment belts, and power for rotation. The RAB system did not have cost inputs for the primary harvesting or dewatering down to 80% moisture because algae harvested from the RAB is already at that level.
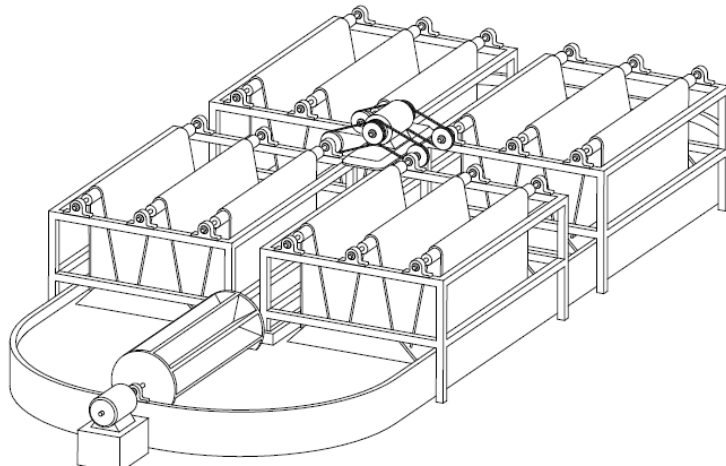
Figure 2. Raceway based-RAB schematic

2.3.3 Input data for algal biomass

In both the TEA and LCA in this study the algae biomass produced is only dried to 80% moisture, however the sale price for this algae is based on a dry bases. Algae for the most part is a very immature bioproduct and since it has largely not been sold at large volumes it is difficult

to put a price on the biomass, especially because the algae grown in this study will be grown on wastewater, which means the algal species grown will be largely dependent on wild species present in the wastewater. Due to this uncertainty it was decided we would sell the algae as organic fertilizer which has a value of $990/ton (http://www.alibaba.com/showroom/organic-fertilizer.html). Additionally the algae grown in this study will be using nutrients acquired from wastewater so an additional income stream will be from both N and P removal. Municipalities currently spend significant amounts of money for power and infrastructure to remove these nutrients. On average they spend $2,270/ton to remove N and $5,140/ton to remove P (Wang et al., 2010). We assume that these municipalities will pay these current rates to us for a more sustainable removal approach such as algal cultivation. In our in house analysis we have determined our wastewater grown algae consists of 10%, and 5% of N and P respectively. So the net income will be based on total algae plus N and P removed by the algae during growth.

3.0 Results and Discussion

3.1 TEA

This comparative TEA was conducted to evaluate two important metrics: (1) how much does it cost to produce 1 ton of algal biomass (2) how large does a cultivation facility need to be before it becomes profitable. These metrics will be important decisions in designing and implementing either of the two cultivation systems.

To evaluate the cost to produce a ton of algal biomass both capital expenses amortized over 20 years and operational costs were considered. Then the total yearly cost was divided by total yearly tonnage of algal biomass produced. This is shown in Table 2. It can be seen that at the

same size the RAB system outperforms that raceway pond in all scenarios, thus showing that the RAB system is more efficient at producing algal biomass in comparison to raceway pond systems. It should also be noted that the RAB system in Iowa also outperforms the raceway system in Arizona. This shows the potential for the RAB system to be implemented in northern climates with a decreased light intensity.

Table 2. Cost of algal biomass ($/ton) in different growing conditions

	Algae Production Cost ($/ton)			
	8.5 m^2 Raceway	270 m^2 Raceway	1 hectare Raceway	100 hectare raceway
Raceway-Iowa	$1,148,225.22	$135,131.10	$16,221.94	$1,101.94
Raceway-Arizona	$574,112.61	$67,565.55	$8,110.97	$550.97
RAB-Iowa	$112,303.14	$16,662.06	$2,968.88	$421.07
RAB-Arizona	$56,151.57	$8,331.03	$1,484.44	$210.53

The next metric of interest is to evaluate at what scale each cultivation system becomes profitable. To conduct this evaluation, the annual yearly costs were subtracted from annual income to identify annual profits for the raceway and RAB system in both the Iowa and Arizona climates. These profits were then plotted against their respective size of cultivation system to identify at what scale each cultivation system becomes profitable (Figure 3). It can be seen that the RAB system can be much smaller than the raceway system and be profitable. In an Iowa climate the RAB only needs to be 23,000 m^2 compared to the raceway that would need to be 290,000 m^2 before it was profitable. If each of these systems were located in a climate similar to Arizona the RAB reactor would only need to be 8,500 m^2 and while the raceway would need to be 80,000 m^2. This decrease in scale will be very important when considering the locations that that each cultivation system could be implemented. For wastewater treatment purposes in particular, land is very limited. This is because many municipal treatment facilities are in urban areas with very little area to expand.
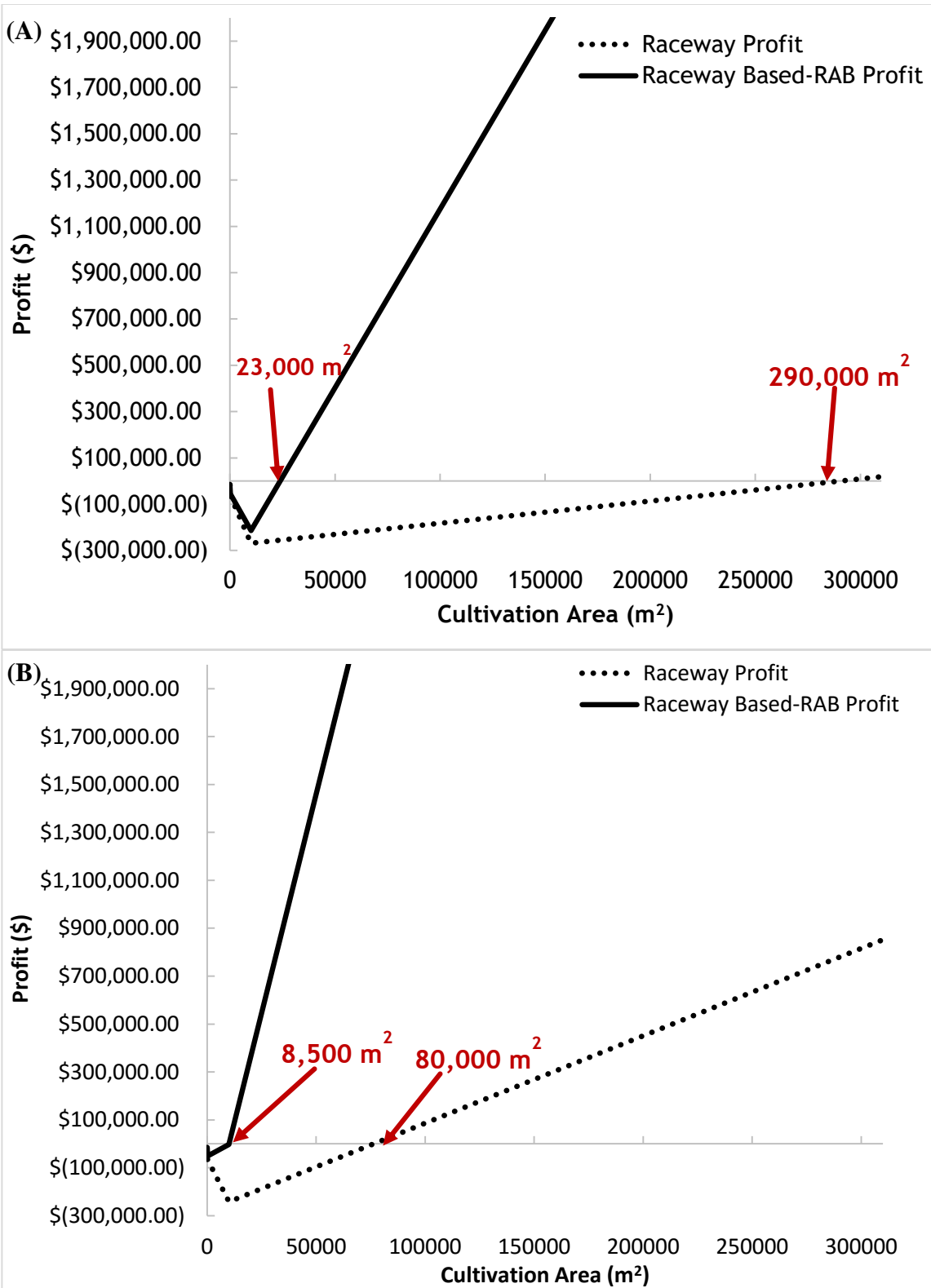
Figure 3. Scale each cultivation system becomes profitable **(A)** Iowa climate **(B)** Arizona climate

3.2 LCA

In this study a balance of energy, greenhouse gas (GHG) emissions, and water was conducted (Table 3). The net GHG was based on the total amount of GHG's released from electrical power generation for each system. The total electrical power was input into the GREET Model to evaluate the GHG's emitted from a typical US electrical mix. The other factor effecting net GHG emissions is the amount of CO_2 that the algae consume during growth. According to a publication by Fernandez, et al., (2012), 1.83 grams of CO2 is consumed per gram of algal biomass produced. Based on these two input/output of GHG emissions the net GHG's released by each system is shown in Table 3.

An energy balance was conducted to evaluate the net energy of each scenario. In this energy balance, the energy used to power each system was subtracted from the total energy available in the algal biomass produced. The energy density of algae is 19.82 kJ/g for algae that contains 20% lipid, 35% protein, 35% carbohydrate and 10% ash. The energy balance is shown in Table 3. It can be seen the RAB system is more efficient than the raceway system. This is because the RAB system does not require the energy intensive harvesting and dewatering operations needed in raceway systems.

Lastly water usage was evaluated in each system. The RAB system shows a less water usage in comparison to the raceway system. This is in agreement to our previous research which showed that the RAB system is more efficient at specific water use than a raceway system. For example the raceway pond requires approximately 2.64 L of water to produce 1 gram of biomass while the RAB only requires 0.72 L of water to produce a gram of biomass. However in this TEA/LCA study we are using wastewater for nutrients and wastewater will be at an unlimited supply in wastewater treatment so water usage is not an important issue.

Table 3. Comparison of GHG, energy, and water consumption in different cultivation scenarios

Cultivation System	Net GHG Emissions (tons/yr)	Net Energy (MJ/yr)	Net Water (1000 m3/yr)
Raceway 8.5 m^2 Iowa	19.80	-129,532.79	-0.04
Raceway 270 m^2 Iowa	-19.30	-396,726.11	-1.15
Raceway 1 hectare Iowa	-3,024.61	-1,445,690.71	-42.43
Raceway 100 hectare Iowa	-332,534.54	27,910,014.29	-4,243.13
Raceway 8.5 m^2 Arizona	16.97	-129,225.58	-0.07
Raceway 270 m^2 Arizona	-109.47	-386,958.82	-2.29
Raceway 1 hectare Arizona	-6,364.36	-1,083,975.71	-84.86
Raceway 100 hectare Arizona	-666,509.54	715,168,514.29	-8,650.50
RAB 8.5 m^2 Iowa	-11.98	-67,223.76	-0.04
RAB 270 m^2 Iowa	-609.29	-298,077.23	-1.30
RAB 1 hectare Iowa	-25,803.57	646,743.98	-48.17
RAB 100 hectare Iowa	-2,614,092.40	258,158,763.71	-4,816.54
RAB 8.5 m^2 Arizona	-36.13	-64,607.52	-0.08
RAB 270 m^2 Arizona	-1,283.28	-225,080.17	-2.60
RAB 1 hectare Arizona	-51,988.86	3,482,767.96	-96.33
RAB 100 hectare Arizona	-5,232,621.10	541,761,161.71	-9,633.09

4.0 Conclusions

In this study a comparative analysis of a raceway pond and revolving algal biofilm cultivation system was evaluated. The RAB system was able to grow algae at a lower cost and was shown to be profitable at a smaller scale than the raceway pond style of algal cultivation. Additionally the RAB system was projected to have lower GHG emissions, and better energy and water use efficiencies in comparison to a raceway system.

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CHAPTER 7. CONCLUSIONS

Algae is a promising biobased feedstock that can be converted into many valuable products. However large-scale cultivation continues to stall due to the high costs of growing and processing it into various products. In this dissertation a novel revolving algal biofilm (RAB) algal cultivation system is investigated. It was found that the RAB system has a significantly higher algal productivity than a raceway pond in side-by-side growth comparisons. Additionally it was found that the RAB system more efficiently uses water than raceway pond based cultivation. A review was written that outlines the important aspects of biofilm based cultivation. One important factor of biofilm based algal cultivation is the material which is used for algal attachment. Experimentation was conducted that evaluated both material surface physico-chemical and texture properties on algal attachment. A material and surface texture that promoted superior algal attachment was identified. The research conducted in this dissertation gives future researchers information about an alternative, biofilm based, method for algal cultivation.