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Algal Biofilm Production and Harvesting System for Wastewater Treatment with Biofuels By- Products

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ALGAL BIOFILM PRODUCTION AND HARVESTING SYSTEM FOR
WASTEWATER TREATMENT WITH BIOFUELS BY-PRODUCTS

by

Logan Christenson

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Biological Engineering

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2011

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ABSTRACT

Algal Biofilm Production and Harvesting System for
Wastewater Treatment with Biofuels By-Products

by

Logan Christenson, Master of Science

Utah State University, 2011

Major Professor: Dr. Ronald C. Sims
Department: Biological Engineering

Excess nitrogen and phosphorus in discharged wastewaters can lead to downstream eutrophication, ecosystem damage, and impaired water quality that may affect human health. Chemical-based and physical-based technologies are available to remove these nutrients; however, they often consume significant amounts of energy and chemicals, greatly increasing treatment costs. Algae are capable of removing these pollutants through biomass assimilation, and if harvested, can be utilized as a feedstock for biomethane or biodiesel production. Currently, difficulties in harvesting, concentrating, and dewatering algae have limited the development of an economically feasible treatment and production process. When algae are grown as surface-attached biofilms, the biomass is naturally concentrated and more easily harvested, leading to less expensive removal from treated water, and less expensive downstream processing for biofuel production. In this study, a novel algal biofilm production and harvesting system was designed, built, and tested. Key growth parameters were optimized in order to

maximize biomass production and nutrient uptake from wastewater. Compared to suspended algae systems, the attached algal biofilm design of this study led to increased biomass production and greater treatment of domestic wastewater. An efficient and inexpensive algal biofilm harvesting technique was also developed in order to obtain a concentrated biosolids product, resulting in improved water quality and a feedstock suitable for further processing in the production of biofuels.

(103 pages)

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CHAPTER 1

NEED FOR STUDY

1. Wastewater Remediation Challenges

Excess nitrogen (N) and phosphorus (P) in discharged wastewaters can lead to downstream eutrophication and ecosystem damage. The negative effects of such nutrient overloading include nuisance algae, low dissolved oxygen concentrations, substantial diurnal pH shifts, and cyanotoxin production (UDEQ, 2009). Chemical and physical based technologies are available to remove nutrients; however, they consume significant amounts of energy and chemicals, making them costly processes (Graham et al., 2009; Tchobanoglous and Burton, 1991). Chemical treatment often leads to secondary contamination of the sludge byproduct as well, creating additional problems of safe disposal (Hoffmann, 1998). The energy and cost required for tertiary treatment of wastewater remains a problem for industries and municipalities.

The City of Logan, located in northern Utah's Cache County, maintains a regional wastewater treatment facility consisting of 460 acres of aerated lagoons, 160 acres of polishing wetlands, and two storage ponds that give a total volume of 400 million gallons. The discharged effluent enters Cutler Reservoir, a recreational waterway protected for waterfowl, shorebirds, warm water game fish, and other wildlife. Water discharged from Cutler Reservoir also has an impact on the Bear River Migratory Bird Refuge, located near the Great Salt Lake. The current load of total phosphorus (TP) entering Cutler Reservoir is in excess of the determined loading capacity. As the largest

point source contributor of phosphorus, the Logan Regional WWTP is required to reduce effluent TP levels by 62% (UDEQ, 2009).

Biological wastewater treatment typically provides good bacterial growth and decomposition of organic carbon, but has little capacity to remove inorganic nutrients such as N and P (Guzzon et al., 2008). Heterotrophic bacteria typically become carbon limited before removing all soluble N and P, but because algae are autotrophic, they can overcome this limitation and assimilate the remaining nutrients (Stumm and Morgan, 1981). Compared to physical and chemical processes, algal treatment can potentially achieve nutrient removal in a less expensive and ecologically safer way with the added benefits of resource recovery and recycling (Oswald, 2003). However, acceptable nutrient levels in the effluent cannot be achieved without sufficient harvesting of the algal crop. Unfortunately, no current harvesting approach has proven to be simple and inexpensive enough for large scale use (Uduman et al., 2010).

2. Biofuel Feedstock Challenges

With growing concerns surrounding the continued use of fossil fuels, renewable biofuels have received a large amount of recent attention. In addition to wastewater treatment applications, algae are also a potential source of feedstock for biofuel production. Biofuels produced using oil crops and waste oils cannot meet the existing demand for fuel, and algae appear to be a more promising feedstock option (Chisti, 2007, 2008). Algae could provide substantially more biodiesel than existing oilseed crops while using far less water and land (Sheehan et al., 1998). In addition to biodiesel, algal sludge may also be fed to an anaerobic digester for methane production (Golueke et al., 1957).

Residual biomass from these processes can also be used as a fertilizer, soil amendment, or feed for fish or livestock (Roeselers et al., 2007). However, algal biofuel production has been handicapped by an inability to find a reliable and cost effective method of harvesting and processing the algae feedstock (Molina Grima et al., 2003).

3. Potential Benefits of Algal Biofilms

A biofilm has been defined as a layer of cells anchored to a substratum surface and embedded in an organic matrix of biological origin (Characklis and Wilderer, 1989). A matrix of extracellular polymeric substance (EPS) secreted by the microorganisms of the biofilm enhances the attachment of the cellular community. Biofilms are ubiquitous in nature and seem to constitute the preferred form of microbial life (Costerton et al., 1995).

In industry, biofilms are often considered a nuisance as they reduce heat transfer in heat exchangers and cooling towers, foul membranes, and contaminate food processing equipment (Qureshi et al., 2005). In the field of wastewater treatment, however, biofilms play a beneficial role.

Most research in using algae to reduce nutrient levels in wastewater or to produce biofuel feedstock has focused on suspended microalgae. Because of the harvesting challenges associated with algae grown in this form, there has recently been an increased interest in the use of immobilized or attached algal communities (Hoffmann, 1998).

When algae are grown as surface attached biofilms, the biomass is naturally concentrated and more easily harvested, leading to lower downstream processing costs. By producing algae in the form of a biofilm, costly concentration operations can be

avoided, and an easily harvestable source of biofuel feedstock can be provided (Roeselers et al., 2007). Notwithstanding these potential benefits, there is no consensus on the best method of growing and harvesting algal biofilms. Therefore, there is a need for further investigation to address the engineering design of algal biofilm systems.

References

- Characklis WG, Wilderer PA. Structure and function of biofilms: report of the Dahlem workshop on structure and function of biofilms. Berlin: Wiley; 1989.
- Chisti Y. Biodiesel from microalgae. *Biotechnol Adv* 2007; 25: 294-306.
- Chisti Y. Biodiesel from microalgae beats bioethanol. *Trends Biotechnol* 2008; 26: 126-131.
- Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. Microbial biofilms. *Annu Rev Microbiol* 1995; 49: 711-745.
- Golueke CG, Oswald WJ, Gotaas HB. Anaerobic digestion of algae. *Appl Microbiol* 1957; 5: 47-55.
- Graham LE, Graham J, Graham JM, Wilcox LW. *Algae*. San Francisco: Benjamin Cummings; 2009.
- Guzzon A, Bohn A, Diociaiuti M, Albertano P. Cultured phototrophic biofilms for phosphorus removal in wastewater treatment. *Water Res* 2008; 42: 4357-4367.
- Hoffmann JP. Wastewater treatment with suspended and nonsuspended algae. *J Phycol* 1998; 34: 757-763.
- Molina Grima E, Belarbi EH, Ación Fernández FG, Robles Medina A, Chisti Y. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnol Adv* 2003; 20: 491-515.
- Oswald WJ. My sixty years in applied algology. *J Appl Phycol* 2003; 15: 99-106.
- Qureshi N, Annous B, Ezeji T, Karcher P, Maddox I. Biofilm reactors for industrial bioconversion processes: employing potential of enhanced reaction rates. *Microbial Cell Factories* 2005; 4: 24.

- Roeselers G, Loosdrecht MCM van, Muyzer G. Phototrophic biofilms and their potential applications. *J Appl Phycol* 2007; 20: 227-235.
- Sheehan J, Dunahay T, Benemann J, Roessler P. A look back at the US department of energy's aquatic species program—biodiesel from algae. National Renewable Energy Laboratory (NREL) report: NREL. TP-580-24190. Golden, CO; 1998.
- Stumm W, Morgan JJ. Aquatic chemistry: an introduction emphasizing chemical equilibria in natural waters. Hoboken: John Wiley; 1981.
- Tchobanoglous G, Burton FL. Wastewater engineering: treatment, disposal, and reuse. New York City: McGraw-Hill; 1991.
- UDEQ. Middle bear river and cutler reservoir TMDLs - public draft. Salt Lake City, Utah: Utah Department of Environmental Quality, Division of Water Quality TMDL Section; 2009.
- Uduman N, Qi Y, Danquah MK, Forde GM, Hoadley A. Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. *J Renew Sustain Energy* 2010; 2: 012701.

CHAPTER 2
PRODUCTION AND HARVESTING OF MICROALGAE FOR
WASTEWATER TREATMENT, BIOFUELS,
AND BIOPRODUCTS

1. Introduction

With growing concerns surrounding the continued use of fossil fuels, renewable biofuels have received a large amount of recent attention. While biofuels produced using oil crops and waste oils cannot alone meet the existing demand for fuel, microalgae appear to be a more promising feedstock option (Chisti, 2007, 2008). Microalgae include microscopic eukaryotic algae as well as cyanobacteria (Acreman, 1994). Such algae could provide substantially more biodiesel than existing oilseed crops while using less water and land (Sheehan et al., 1998). Algae biomass may also be fed to an anaerobic digester for methane production (Golueke et al., 1957; Gunaseelan, 1997; Yen and Brune, 2007), or used to produce bioplastic materials (Chiellini et al., 2008). Residual biomass from these processes can potentially be used as a fertilizer, soil amendment, or feed for fish or livestock (Mulbry and Wilkie, 2001; Mulbry et al., 2005; Roeselers et al., 2008). However, the production of biofuels and bioproducts using algal biomass has been handicapped by an inability to find a reliable and cost effective method of producing and harvesting large quantities of algae feedstock.

In addition to biofuel and other bioproduct applications, large-scale methods of producing and harvesting algae have uses in wastewater treatment (Hoffmann, 1998; Oswald, 2003). Without proper treatment, excess nitrogen and phosphorus is discharged

wastewaters can lead to downstream eutrophication and ecosystem damage (Correll, 1998). The negative effects of such nutrient overloading of receiver systems include nuisance algae, low dissolved oxygen concentrations and fish kills, undesirable pH shifts, and cyanotoxin production. While chemical and physical based technologies are available to remove these nutrients, they consume significant amounts of energy and chemicals, making them costly processes (Tchobanoglous and Burton, 1991). Chemical treatment often leads to secondary contamination of the sludge byproduct as well, creating additional problems of safe disposal (Hoffmann, 1998). The energy and cost required for tertiary treatment of wastewater remains a problem for industries and municipalities.

Compared to physical and chemical treatment processes, algae based treatment can potentially achieve nutrient removal in a less expensive and ecologically safer way with the added benefits of resource recovery and recycling (Graham et al., 2009; Oswald, 2003). Common nitrogen removal methods such as bacterial nitrification/denitrification remove the majority of the nitrogen as N_2 gas, whereas algal treatment retains useful nitrogen compounds in the biomass. Notwithstanding these benefits, acceptable nutrient levels in the effluent cannot be achieved without sufficient production and harvesting of the algae crop. Unfortunately, no current approach has been demonstrated to be simple and inexpensive enough for economical large-scale use with algae.

The U.S. Department of Energy has recognized the potential synergy of wastewater treatment and biofuel production from algae, stating that “inevitably, wastewater treatment and recycling must be incorporated with algae biofuel production” (U.S. DOE, 2010). Because much of the infrastructure is already in place, algae-based

wastewater treatment can be deployed relatively soon. The use of wastewater can offset the cost of commercial fertilizers otherwise needed for the production of algae, and wastewater treatment revenues can offset algae production costs. It is apparent that overcoming the current challenges to the production and harvesting of algae will be beneficial for both wastewater treatment and for the production of biofuels and bioproducts.

Considering the benefits of cost-effective algae production and harvesting to both wastewater treatment and the production of biofuels and other bioproducts, this review has the following objectives:

1. Identify the major challenges to cost-effective production and harvesting of algae.
2. Compare the benefits and limitations of the different approaches to algae production, including open ponds, closed reactors, and immobilized systems.
3. Compare the benefits and limitations of algae harvesting approaches, including chemical, mechanical, biological, and electrical based harvesting.
4. Examine algae production and harvesting approaches in industry.
5. Identify research needs and potential solutions to the major challenges of production and harvesting of algae.

2. Major Challenges

The two major challenges to the implementation of an integrated algae system include the large-scale production of algae and the harvesting of algae in a way that allows for downstream processing to produce biofuels and other bioproducts of value.

The challenges with regard to large-scale production of algae include nutrient supply and

recycling, gas transfer and exchange, photosynthetically active radiation (PAR) delivery, culture integrity, environment control, land and water availability, and harvesting.

Algae growth requires the availability of primary nutrients and micronutrients, which can be costly if they need to be added in great amounts. When gas exchange is insufficient, the algae culture can become carbon limited, and the oxygen byproduct of photosynthesis can reach inhibitory levels (Carvalho et al., 2006). Delivery of light in the form of photosynthetically active radiation (PAR) can also be the limiting factor at high culture densities (Tredici and Zittelli, 1998; Zijffers et al., 2008). Depending on the characteristics of the microalgae culture, contamination can be difficult to avoid. Increasing control of the growth environment can enhance productivity but involves additional costs. Sufficient land and water must also be available. The most pressing challenge, however, lies not in the production of the algae crop, but in the harvesting and downstream processing of it in a manner suitable for the production of bioproducts (Molina Grima et al., 2003; Uduman et al., 2010). Each of the challenges identified above is addressed in the following subsections.

2.1. Nutrient Supply & Recycling

Growing algae requires consideration of three primary nutrients: carbon, nitrogen, and phosphorus. Micronutrients required in trace amounts include silica, calcium, magnesium, potassium, iron, manganese, sulfur, zinc, copper, and cobalt, although the supply of these essential micronutrients rarely limits algal growth when wastewater is used (Knud-Hansen et al., 1998). If not already available in the water source, the addition of commercial fertilizers can significantly increase production costs, making the price of

algae derived fuel cost prohibitive (U.S. DOE, 2010). For this reason, wastewater is an attractive resource for algae production.

Pittman et al. (2011) reviewed the potential of algal biofuel production and concluded that, based on current technologies, algae cultivation for biofuels without the use of wastewater is unlikely to be economically viable or provide a positive energy return. Lundquist et al. (2010) analyzed several different scenarios of algae-based wastewater treatment coupled with biofuel production and concluded that only those cases that emphasized wastewater treatment were able to produce cost competitive biofuels. They concluded that the near-term outcome for large scale algae biofuels production is not favorable without wastewater treatment as the primary goal.

Although available carbon can be the limiting factor, the atmosphere provides a near infinite, although slowly transferred, source of carbon dioxide. Nitrogen and phosphorus, therefore, are the two nutrients of most concern when analyzing a water source for potential algae growth. To prevent limitations by either, the molar ratio of the water supply must match the stoichiometric ratio of the algae biomass. This nitrogen to phosphorus ratio is often assumed to match the Redfield ratio of 16:1 (Stumm and Morgan, 1981). This ratio is not a universal biochemical optimum, but instead represents an average of species specific N to P ratios that vary from 8 to 45 (Klausmeier et al., 2004). This means that even when wastewater is used to supply nutrients, addition of nitrogen or phosphorus may be needed in order to reach the proper ratio. Table 1 shows N and P characteristics of domestic wastewater types (Tchobanoglous and Burton, 1991).

Table 1: Characterization of typical domestic wastewaters with respect to algal nutrients nitrogen and phosphorus. Adapted from Tchobanoglous and Burton (1991).

Wastewater Strength	Total Nitrogen (mg/l)	Total Phosphorus (mg/l)	N:P (molar ratio)^a
Weak	20	4	11
Medium	40	8	11
Strong	85	15	13

^a Average molar ratio for algae is 16:1 (Stumm and Morgan, 1981)

Nutrient starvation can also be intentionally designed into a process as a method of increasing the value of the algae biomass. Much of the focus of the Department of Energy's Aquatic Species Program was on enhancing lipid production within the cells through stress conditions such as nitrogen deficiency. This often led to higher lipid accumulation, but these gains were more than offset by the slower growth rates and did not lead to an overall increase in lipid production (Sheehan et al., 1998).

2.2. Gas Transfer & Exchange

Proper gas exchange for algae growth includes both sufficient transfer of carbon dioxide to the cells and sufficient removal of oxygen gas. Although some algae can be grown heterotrophically, an environmentally and economically viable process must make use of algae's autotrophic abilities by using inorganic carbon as the carbon source. The three principle forms of dissolved inorganic carbon (DIC) associated with algal growth exist in equilibrium as carbon dioxide, bicarbonate, and carbonate. Algae can directly utilize carbon dioxide and often bicarbonate, but generally not carbonate (Knud-Hansen et al., 1998; Round, 1984).

Open ponds can potentially be carbon limited due to mass transfer limitations. Azov (1982) recommended artificially maintaining high free carbon dioxide concentrations in outdoor algae cultures after finding that cultures at higher levels had 65–95% more dry weight than the control. Increases in lipid content have also been shown with carbon dioxide addition (Chiu et al., 2009; Griffiths, 2009). Simply bubbling carbon dioxide into the culture, however, may not be effective enough because bubble residence time is too short, and much ends up being lost to the atmosphere (Mata et al., 2010). In addition, high concentrations of carbon dioxide, such as from flue gas, are not always near enough to wastewater sources to justify the cost of transfer and use.

A challenge directly related to carbon dioxide supply is the removal of excess oxygen. Oxygen concentrations above air saturation begin to inhibit photosynthesis, and this byproduct must be removed in order to prevent photooxidative damage. For closed reactors especially, oxygen removal is considered one of the most difficult challenges to overcome (Carvalho et al., 2006).

Even when atmospheric carbon dioxide is the only available source, methods can be employed to increase transfer to the liquid phase. Both carbon dioxide transfer and oxygen release can be increased through the use of gas-liquid contactor reactors such as rotating biological contactors (RBCs) common in secondary wastewater treatment (Zeevalkink et al., 1979). Patwardhan (2003) reported that RBC systems show much higher gas transfer efficiency than surface aerators, diffuser aerators, or trickling filters. Putt (2007) showed that a wetted ramp contactor would increase the carbon uptake of a pond by a factor of 36 relative to a regular pond, although he concluded that this was still

not sufficient enough. Cost effectively delivering carbon dioxide while allowing adequate oxygen release remains a challenge.

2.3. PAR Delivery

PAR is different from other algae growth requirements in that it cannot be mixed. Full sunlight is often too intense for algae to utilize all available photons, and excess energy absorbed by cells is lost in the form of fluorescence or heat (Niyogi, 2003; Powles, 1984). Not only is this inefficient use of available light, but prolonged exposure to such high intensities can overpower the energy dissipating machinery of the cells resulting in photoinhibition and cell damage (Niyogi, 2003; Powles, 1984). In contrast, algae in deeper portions of a culture are often light limited because the majority of light has already been absorbed by the outermost layer of cells (Borowitzka, 1999). Thus, cultures often suffer from photoinhibition and photodeprivation simultaneously.

Increasing the utilization of PAR is usually dealt with by designing the reactor with high surface area to volume ratio and/or vigorous mixing to ensure all cells reside in the illuminated area for an appropriate length of time. Hu et al. (1996) and Hu and Richmond (1996) have shown high culture densities using well mixed flat panel reactors with high surface area to volume ratio. Degen et al. (2001) were able to show 1.7 times greater productivity simply by placing baffles in an air lift reactor to better manage the light/dark frequency of the culture.

2.4. Culture Integrity

In monocultures grown for nutritional supplements or other bioproducts, algal cultures are susceptible to contamination by less desirable strains unless additional means

of control are utilized (U.S. DOE, 2010). Monocultures of high lipid producing strains are likely to be outcompeted by faster growing species of microalgae or cyanobacteria (Vasudevan and Briggs, 2008). Carefully maintained monocultures are not found in wastewater treatment systems. When wastewater resources are used, naturally occurring mixed cultures of algae dominate. Although culture composition and growth conditions may be less manageable, lipid accumulation of mixed cultures in municipal wastewater has been shown to reach 11.3% (Woertz et al., 2009), and as high as 29% when grown with anaerobic digester effluent (Woertz et al., 2009). Griffiths (2009) reported a fatty acid methyl ester content of as high as 23.4% after in situ transesterification of a mixed culture grown in municipal wastewater.

2.5. Environment Control

Both biomass production and nutrient removal can be optimized if the important growth parameters such as temperature and pH are better controlled (Abu-Rezq et al., 1999). More control over the growth environment includes additional costs, however, such as with the use of closed reactors instead of open ponds (Shen et al., 2009). Concerning wastewater treatment ponds and lagoons, the large scales involved lessen available means of environmental control. Finding ways to achieve proper control of the growth environment without adding unreasonable costs remains a challenge.

2.6. Land & Water Availability

Large scale production of microalgae likely requires a large expanse of land with an available water source. Wastewater treatment facilities have plenty of nutrient rich water available, but may not have the necessary land, especially considering newer

membrane reactor facilities designed to leave a small footprint. Regardless, Sheehan et al. (1998) concluded that at least in the United States, land is definitely not a limitation, and although the technology faces many research and development hurdles, resource limitation is not a valid argument against further development.

According to the United States Environmental Protection Agency, there are more than 7,000 facultative lagoon systems in the United States (U.S. EPA, 2002). From the perspective of algae production, lagoon treatment facilities provide the combined benefits of land, water, and nutrient availability, with reduced need for preliminary site construction and infrastructure development. For these reasons, lagoons stand out as promising potential algae production facilities. One such facility is the Logan Regional Wastewater Treatment Plant, located in northern Utah. The plant consists of 460 acres of lagoons, and facility directors are dedicated to an algae-based approach to wastewater treatment with additional production of bioproducts (Griffiths, 2009).

2.7. Harvesting

Separating the algae from water remains a major hurdle to industrial scale processing partly because of the small size of the algal cells, with unicellular eukaryotic algae typically 3–30 micrometers (Molina Grima et al., 2003), and cyanobacteria as small as 0.2–2 micrometers (Chorus and Bartram, 1999). In addition, relatively dilute cultures of 200–600 mg/l are common (Uduman et al., 2010), and require that large volumes of water be processed. Recovery has been estimated to contribute 20–30% of the total cost of producing the biomass (Molina Grima et al., 2003). The initial harvesting step is not only costly, but also affects any later processes downstream.

Most wastewater treatment lagoons in the U.S. do not harvest algae (Salerno et al., 2009). Middlebrooks et al. (1974) reviewed several removal methods suitable for wastewater lagoons and recommended granular media filters for communities with smaller ponds. At plants that do remove algae, chemical coagulation followed by sedimentation or dissolved air flotation (DAF) is a common approach (Friedman et al., 1977), with DAF generally considered more effective than sedimentation in the treatment of algae rich waters (Teixeira and Rosa, 2006). Though effective at full scale, the addition of chemical coagulants transforms a potential resource into waste sludge that must be disposed of (Hoffmann, 1998). Lowering the cost of harvesting algae and harvesting in a way that allows for the creation of bioproducts remains a challenge.

2.8. Summary of Major Challenges

Several challenges remain in the development of a large-scale algae production and harvesting system. The use of existing wastewater lagoons can resolve many of the challenges discussed, including nutrient supply and recycling as well as land and water availability, but of the thousands of existing lagoons, few harvest algae (Salerno et al., 2009), and those that do favor processes involving chemical coagulants (Friedman et al., 1977; Hoffmann, 1998; Teixeira and Rosa, 2006). Other than preliminary research at Utah State University (Griffiths, 2009) and California Polytechnic State University (Woertz et al., 2009), little has been done to produce biofuels and bioproducts from algae grown in wastewater.

3. Algae Production Methods

Suspended cultures, including open ponds and closed reactors, and immobilized cultures, including matrix-immobilized systems and biofilms, are addressed in the following sections. Table 2 compares open ponds, closed reactors, and biofilm systems against scalability and operating parameters.

Table 2: Benefits and limitations of design approaches for algae production.

Design	Culture Density (g l ⁻¹)	Gas Exchange	Scalability	Culture Control
Raceway Pond	0.25–1 ^a	Low	High	Low
Tubular Reactor	1.5–1.7 ^b	Very low	Medium	High
Biofilm System	70 ^c	High	High	Low

^a U.S. DOE (2010); Shen et al. (2009)

^b Norsker et al. (2011); Shen et al. (2009)

^c Biofilm of 7% solids as reported by Johnson and Wen (2010)

3.1. Suspended Cultures

The greatest amount of information on how to treat wastewater with algae pertains to suspended algae systems comprised of naturally occurring mixed cultures. Most methods of producing algae for the purpose of biofuels are also based on suspended algae. Table 3 shows biomass productivity and wastewater nutrient removal by suspended culture designs.

Table 3: Algae biomass production and wastewater nutrient removal in suspended systems

Design	Nutrient loading^a (mg l ⁻¹ day ⁻¹)	Nutrient Removal	Biomass Production (g m ⁻² day ⁻¹)	Scale	Reference
Raceway Pond	P: 1.2-7.5	P: 96%	10–20	Pilot and demonstration	Hoffmann (1998), Shen et al. (2009), Lundquist et al. (2010)
Tubular Reactor	N: 17.3 P: 1.4	N: 99% P: 86%	20–45	Pilot and demonstration	Chisti (2007), González et al. (2008), Shen et al. (2009)

^a Soluble/dissolved forms of N and P

3.1.1. Open Ponds

The most common large scale production systems in practice are high rate algal ponds, also known as HRAPs or raceway ponds. In use since the 1950's, raceway ponds are open, shallow ponds with a paddle wheel to provide circulation of the algae and nutrients. Raceways are relatively inexpensive to build and operate, but often suffer low productivity due to contamination, poor mixing, dark zones, and inefficient use of CO₂ (Chisti, 2007; Mata et al., 2010). Raceway ponds should theoretically have production levels of 50–60 g m⁻² day⁻¹, and single day productivities at this level have been reported (Sheehan et al., 1998), but in practice, productivities of even 10–20 g m⁻² day⁻¹ are difficult to achieve (Shen et al., 2009). The high evaporation rate of open ponds is most often seen as a limitation, but it also helps somewhat with temperature regulation through evaporative cooling (U.S. DOE, 2010). A major conclusion of cost analysis studies conducted by the U.S. Department of Energy's Aquatic Species Program was that there is little prospect for alternatives to the open pond system given the requirements for low cost of fuel (Sheehan et al., 1998).

3.1.2. Closed Reactors

Tubular photobioreactors are the only type of closed systems used at large scale (Chisti, 2007). Vertical, horizontal, and helical designs are common, although helical designs are considered the easiest to scale up (Carvalho et al., 2006). Compared to open ponds, tubular photobioreactors can give better pH and temperature control, better protection against culture contamination, better mixing, less evaporative loss, and higher cell densities (Mata et al., 2010). Reported productivities generally range from 20–40 g m⁻² day⁻¹ (Shen et al., 2009). Despite these benefits, tubular reactors have not achieved significant use due to problems with toxic accumulation of oxygen, adverse pH and CO₂ gradients, overheating, bio-fouling, and high material and maintenance costs (Mata et al., 2010; Molina Grima et al., 1999). Oxygen removal is considered one of the most difficult problems to overcome, especially when considering scale up, as it effectively limits tube or panel length and forces a more complex or modular design (Carvalho et al., 2006).

3.2. Immobilized Cultures

Regardless of the specific advantages and disadvantages of raceways and tubular photobioreactors, both involve significant challenges of biomass recovery. Because of the harvesting challenges associated with suspended algae, there is growing interest in the use of immobilized or attached algal processes (Hoffmann, 1998). The U.S. Department of Energy reviewed immobilized algae designs, mostly focusing on the use of immobilization particles in a packed or fluidized bed reactor (U.S. DOE, 1985). Although they reported that the economics of such a scheme were prohibitive, they also concluded that the benefits of increased culture densities and lower water and land requirements of

immobilized algae systems could be realized through future design innovation (U.S. DOE, 1985).

3.2.1. Matrix-Immobilized Microalgae

Results from experiments with algae immobilized in carrageenan or alginate matrices have shown some potential benefits of immobilization, including efficient nutrient removal in wastewater applications (Chevalier et al., 2000). According to Hameed and Ebrahim (2007), results comparing growth rates of immobilized cultures and suspended cultures are mixed. Immobilization has also been shown to result in enhanced hydrocarbon production (Bailliez et al., 1985), as well as increased cellular pigment, lipid content, and lipid variety (de-Bashan et al., 2002). For these immobilization processes, however, such benefits are likely offset by the high cost of the immobilization matrix. Such designs have thus far been confined to the laboratory. At the scale necessary for wastewater treatment and biofuel production, the cost of the polymeric matrix becomes prohibitive (Hoffmann, 1998).

3.2.2. Algal Biofilms

Algal biofilms could play a large role in overcoming the major challenges to production and harvesting of microalgae. The wastewater treatment industry is already accustomed to large scale biofilm processes (Wuertz et al., 2003), and according to Middlebrooks et al. (1974), if enough surface area is provided, algae biofilm growth can be more than suspended growth. A scalable algal biofilm system could be integrated into the treatment process, thereby achieving the dual benefits of inexpensive nutrient supply and treated water. Surface attached algal biofilms can offer the same increased culture

density and lower land and water requirements of matrix-immobilized cultures (U.S. DOE, 1985) without the associated costs of the matrix. Compared to suspended cultures, an algal biofilm system can better integrate production, harvesting, and dewatering operations, potentially leading to a more streamlined process with reduced downstream processing costs.

Biofilm formation occurs due to the concentration of cations, proteins, and organic molecules on submerged surfaces relative to the bulk aqueous environment, creating a favorable location for microbial growth. Microbes colonizing a surface then secrete extracellular polymeric substance (EPS) composed of polysaccharides, proteins, nucleic acids, and phospholipids (Qureshi et al., 2005).

Algae biofilms are likely to be benefited by bacteria present in wastewater. Hodoki (2005) showed that attached algae increased significantly when more bacteria were present on all substrata tested, and Holmes (1986) saw that attachment of unialgal cultures with bacterial contaminants was one to two orders of magnitude higher than without bacteria. Both investigators theorized that entrapment by attached bacteria is the major cause of early algal migration.

Much of the research on algae biofilms has been associated with limnological studies involving periphyton monitoring, often utilizing artificial streams lined with Styrofoam (Bothwell, 1983; Sperling and Grunewald, 1969). In the wastewater treatment field, bacterial biofilm based reactors including trickling filters and rotating biological contactors have been used successfully at large scales (Wuertz et al., 2003). Some research has been done to optimize algae growth with these designs or incorporate them into an algae growth process. Integrating a trickling filter after a raceway was shown to

aid in algae harvesting after the algae became entrapped in the biofilm of the filter (Hoffmann, 1998). Torpey et al. (1971) used artificially illuminated rotating aluminum disks to grow algae for removal of nitrogen and phosphorus, and Przytocka-Jusiak et al. (1984) used rotating Styrofoam disks to grow algae for ammonia removal; however, neither study attempted to harvest the algae or maximize production.

Cao et al. (2009) envisioned a floating conveyer belt system of dimpled metal sheets for continuous algae attachment and harvesting. They qualitatively showed that more algae attached to a textured steel surface than to a smooth steel surface. Johnson and Wen (2010) compared the performance of an attached culture to a suspended culture grown under the same conditions and reported greater yields from the attached culture and the same lipid content. The attached culture was grown on a section of submerged polystyrene operated using a rocking motion.

Another design, the Algal Turf Scrubber, consists of a plastic mesh for filamentous algae attachment with intermittent wave surges. It has been reported to have a biomass production of 15–27 g m⁻²day⁻¹ (Adey et al., 1993). Several other studies with this design have shown good nutrient uptake and biomass productivity that typically ranges from 5–20 g m⁻²day⁻¹ (Mulbry et al., 2005; Mulbry and Wilkie, 2001; Wilkie and Mulbry, 2002). The filamentous algae grown on the Algal Turf Scrubber has low fatty acid content, however, reducing its value as a biofuel feedstock (Mulbry et al., 2008). Table 4 summarizes algal biofilm designs with respect to nutrient loading and removal, biomass productivity, and scale.

Table 4: Algae biomass production and wastewater nutrient removal in algal biofilm systems

Design	Nutrient loading ^a (mg l ⁻¹ day ⁻¹)	Nutrient Removal	Biomass Production (g m ⁻² day ⁻¹)	Scale	Reference
PVC Brushes	TN: 5.5 TP: 1.7	TN: 87% TP: 98%	not reported	Lab	Wei et al. (2008)
Rotating Styrofoam disks	N: 45-180 P: 1.7-3.3	N: 100%	2.2	Lab	Przytocka-Jusiak et al. (1984)
Rotating Aluminum Disks	N: 312	N: 60%	not reported	Bench	Torpey et al. (1971)
Polycarbonate flow lanes	P: 1.2	P: 100%	2.9	Lab	Guzzon et al. (2008)
Algal Turf Scrubber	TN: 160–1030 TP: 80–160	TN: 36–92% TP: 51–93%	5.3–5.5	Bench	Wilkie and Mulbry (2002)
Polystyrene rocker system	N: 30.9 P: 1.8	N: 100% P: 70%	2.59	Lab	Johnson and Wen (2010)

^a Soluble/dissolved forms of N and P unless specified as Total N (TN) and Total P (TP)

4. Algae Harvesting Methods

Current harvesting methods include chemical based, mechanical based, and to a lesser extent, electrical based operations, with various combinations or sequences of these methods also common (Bernhardt and Clasen, 1991; Danquah et al., 2009; Kumar et al., 1981). Biological based methods are also being investigated as a cost reducing means of harvesting. There is no proven single best method of harvesting microalgae (Shelef et al., 1984).

4.1. Chemical Based

Because of the small size of algae cells, chemical flocculation is often performed as a pretreatment to increase the particle size before using another method such as flotation to harvest the algae. Electrolytes and synthetic polymers are typically added to coagulate (neutralize charge) and flocculate the cells, respectively (Bernhardt and Clasen, 1991). Because of the +3 charge of the aluminum and ferric cations, aluminum sulfate and ferric chloride are often used for charge neutralization. When considering downstream processes to produce bioproducts from algae, the use of metal salts for coagulation and flocculation is cautioned. Aluminum and sulphate have been shown to inhibit the specific methanogenic activity of methanogenic and acetogenic bacteria fed wastewater sludge (Cabirol et al., 2003). Land application of aluminum treated sludge can increase heavy metal uptake and cause phosphorus deficiencies in plants (Bugbee and Frink, 1985).

Natural polymers that do not involve the same concerns of secondary pollution may also be used as flocculants, although these are less studied. Divakaran and Sivasankara Pillai (2002) saw successful flocculation and settling of algae by adding chitosan. Cationic starch has also been identified as an effective flocculating agent (Pal et al., 2005), and has been shown to flocculate freshwater microalgae in jar test experiments (Vandamme et al., 2009).

4.2. Mechanical Based

Centrifugation is perhaps the most rapid and reliable method of recovering suspended algae. Centrifugal forces are utilized to separate based on density differences.

According to Shelef et al. (1984), nozzle type disc centrifuges are easily cleaned and sterilized and are suitable for all types of microalgae, but the high investment and operating costs must also be considered. The U.S. Department of Energy has concluded that at the current level of centrifugation technology, this method is cost-prohibitive for any large scale use (U.S. DOE, 2010).

Low-cost filtration methods are often used to harvest filamentous algae strains (Vonshak and Richmond, 1988). Wood (1987) described a high rate algae pond system to select for more easily harvested filamentous algae by microscreening to retain larger cells and washing out smaller non-filamentous algae. Other researchers, however, have not been able to confirm dominance of these species (Hoffmann, 1998), and for applications in biofuels, filamentous algae are less useful due to their low lipid content (Mulbry et al., 2008). For smaller suspended algae, tangential flow filtration is considered to be more feasible than dead-end filtration, but membrane fouling and replacement are significant costs (Uduman et al., 2010), and power requirements are high (Danquah et al., 2009).

Sedimentation is a low cost harvesting option that can typically give concentrations of 1.5% solids (Uduman et al., 2010), but because of the fluctuating density of algae cells, reliability is also low (Shen et al., 2009). At settling rates of 0.1–2.6 cm h⁻¹, sedimentation is relatively slow, and much of the biomass may deteriorate during the settling time (Greenwell et al., 2010).

Dissolved air flotation (DAF) is a method commonly used in wastewater treatment sludge removal (Friedman et al., 1977). In algae rich waters, DAF is usually preferred over sedimentation methods (Teixeira and Rosa, 2006). The major advantage of

DAF is that it has been proven at large scales, but the use of flocculants can be a problem in downstream processing of the algae (Hoffmann, 1998; Greenwell et al., 2010).

Designs that use attached algae biofilms also mechanically harvest the algae. Filamentous algae grown on a turf scrubber could be vacuumed (Jensen, 1996) or scraped (Adey, 1982, 1998). Johnson and Wen (2010) used simple scraping to harvest a *Chlorella* biofilm that had a solids concentration of 6.3%. At such concentrations, any additional harvesting or concentrating operation is likely unnecessary.

Table 5 compares the most common mechanical harvesting methods for algae with regard to benefits, limitations, solids recovery, and solids concentrations.

Table 5: Comparison of mechanical harvesting methods for algae. Adapted from Shelef et al. (1984), Shen et al. (2009), Greenwell et al. (2010), and Uduman et al. (2010)

Method	Solids Concentration After Harvesting	Recovery	Scale	Major Benefits	Major Limitations
Centrifugation	12–22%	>90%	Bench	Reliable, High solids conc.	Energy intensive, High cost
Tangential filtration	5–27%	70–90%	Bench	Reliable, High solids conc.	Membrane fouling, High cost
Gravity sedimentation	0.5–3%	10–90%	Pilot	Low cost	Slow, Unreliable
Dissolved air flotation	3–6%	50–90%	Pilot	Proven at large scale	Flocculants usually required

4.3. Electrical Based

Separation methods based on electrophoresis of the algae cells have also been attempted. Because of the negative charge of algae cells, they can be concentrated by movement in an electric field (Kumar et al., 1981). The major benefit of approaches

based on these principles is that no chemical addition is required; however, the high power requirements and electrode costs do not make for an appealing harvesting method, especially for large-scale applications (Uduman et al., 2010).

4.4. Biological Based

Algae are known to sometimes flocculate spontaneously without chemical addition (Sukenik and Shelef, 1984). Exploiting and controlling this ability could significantly reduce harvesting costs. Although the terms are used somewhat interchangeably, autoflocculation and bioflocculation describe different phenomena.

Autoflocculation occurs at high pH levels caused by consumption of dissolved carbon dioxide. Increasing pH causes supersaturation of calcium and phosphate ions. If an excess of calcium ions are present, the calcium phosphate precipitate will be positively charged. Algae cells serve as a solid support for the precipitant and charge neutralization is accomplished (Lavoie and de la Noüe, 1987). Autoflocculation may not be possible in all waters. Sukenik and Shelef (1984) determined that optimum concentrations for calcium phosphate precipitation and autoflocculation at a pH of 8.5–9 were 3.1–6.2 mg l⁻¹ phosphate and 60–100 mg l⁻¹ calcium. Nurdogan and Oswald (1995) overcame such a limitation by adding lime to a raceway pond. This alone brought nitrogen, phosphorus, and algae removal to above 90%.

The term bioflocculation is usually meant to describe flocculation caused by secreted biopolymers. Sedimentation of phytoplankton blooms has been positively correlated with an increase in EPS concentrations (Bhaskar and Bhosle, 2005). Passow and Alldredge (1995) reported that a controlled diatom bloom underwent mass

flocculation soon after a sudden increase in the amount of cells enclosed by biopolymer. EPS produced by algae biofilms in a trickling filter enhanced solids flocculation in a later clarifier operation (Shipin et al., 1999). EPS production has been reported to be maximal at the end of the growth phase (Bhaskar and Bhosle, 2005; Staats et al., 1999), although light and temperature conditions also affect bioflocculation (Wolfstein and Stal, 2002).

Another biological approach is microbial flocculation of algae. Lee et al. (2008) added flocculating microbes to an algae culture. After feeding 0.1 g l^{-1} acetate, glucose, or glycerin and mixing for 24 hours, they achieved 90% recovery and a concentration factor of 226. Oh et al. (2001) reported better efficiency using a flocculant from soil microbes than with aluminum sulfate or polyacrylamide for harvesting *Chlorella vulgaris*.

Another biological based approach to harvesting involves the use of planktivorous fish such as tilapia. The Controlled Eutrophication Process starts with raceway ponds to grow algae. The algae are then batch fed to caged fish, and the fish droppings and any sedimented algae are brought to the surface on an inclined conveyer belt to be fed to an anaerobic digester (Brune et al., 2007). Rectenwald and Drenner (2000) described a similar process of passing nutrient rich water through porous screens to grow periphyton. Excrement from tilapia feeding on the algae is collected in a sediment trap. Reductions in total phosphorus and total nitrogen of 82% and 23%, respectively, were observed.

5. Approaches to Algae Production and Harvesting in Industry

Because of the high commercial potential of algae based biofuels and algae based wastewater treatment, research and development of algae production and harvesting

technologies is being conducted by private companies and industries. Many of the needed innovations can be solved through collaborations between academia, algae production companies, the wastewater treatment industry, and users of algae-based technologies including municipalities and industries. Table 6 lists algae production and harvesting designs and processes, along with scale of application, associated companies, and involvement with wastewater treatment. Table 6 is grouped according to production approach and ordered according to scale. It is not intended to rank or endorse the companies in any way.

Table 6: Companies involved in algae production and/or harvesting

Production Approach	Harvesting Approach	Company	Scale^a	Reference
Open Ponds				
raceway ponds	foam fractionation, cavitation bubble disruption	Kai Bioenergy	not disclosed	Larach (2010)
floatable pond ^b	pond lifted out of water	Blue Marble Energy	no longer producing algae	Stephens et al. (2009a, 2009b)
open ponds	flocculation and DAF	Honeywell's UOP	bench	Marker et al. (2009)
two stage process: CSTR feeds an unlighted PFR ^b	vacuum belt	Algae to Energy (A2E)	pilot	Shepherd (2010)
two stage process: CSTR to PFR	flocculation then settling or DAF	General Atomics	small pilot (6,000 gal pond), developing a 40 acre site	Dunlop and Hazlebeck (2010), Hazlebeck and Dunlop (2008, 2010),
raceway ponds	autoflocculation, centrifugation	Seambiotic	pilot (1/2 acre site)	Weiss (2008)
raceway ponds	flocculation then settling or DAF	Aurora Algae	pilot (1 acre site)	Vick and Fleischer (2009), Vick (2010), Weissman et al. (2010), Weismann and Radaelli (2010)
clay raceway ponds followed by starvation pond	gravity settling followed by other	Aquatic Energy	pilot (2 acre site)	Demaris et al. (2009)

two stage process: closed reactors to open ponds	gravity settling followed by centrifugation	HR Biopetroleum	pilot (6 acre site)	Huntley and Redalje (2010)
raceway ponds ^d	conveyer belt or skimmer	PetroAlgae	demonstration (40 acre site)	Javan et al. (2010)
raceway ponds	cell-viable extraction	Phycal	demonstration (40 acre site)	Swanson et al. (2010), Lane et al. (2010)
open ponds ^b	planktivorous fish	LiveFuels	demonstration (45 acre site)	Wu et al. (2010a, 2010b)
raceway ponds for <i>Spirulina</i>	filtration	Cyanotech	full (90 acre site)	Jensen and Reichl (1997)
CEP (raceway ponds) ^c	inclined conveyer belt for fish feces	Kent BioEnergy	full (160 acre site)	Brune et al. (2007), Schwartz et al. (2010)

Closed Reactors

tubular reactors ^b	not specified	A2BE Carbon Capture	bench	Sears (2007)
NASA's OMEGA system ^c	forward osmosis	Algae Systems	bench	Trent et al. (2010)
flat panel or tubular reactors ^b	3rd party	Bionavitas	bench	Wilkerson et al. (2009), Wilkerson and Watters (2009),
closed reactors with internal light rods ^b	cavitation bubble disruption then skimming	Origin Oil	reactor= bench; extraction method= pilot (300 gal min ⁻¹)	Eckelberry and Eckelberry (2009)
tubular reactors	centrifuge with textured walls	Scipio Biofuels	bench	Wells and Snyder (2010)
tubular reactors	not specified	Sunrise Ridge Algae	bench	Whitton (2008)
helical tubular reactors	not specified	Texas Clean Fuels	bench	Gal (2009)
corrugated panel reactor ^e	not specified	Joule Unlimited	bench	Devroe et al. (2009, 2010), Van Walsem et al. (2010)
closed greenhouses ^e	no (secreted ethanol)	Algenol	pilot	Woods et al. (2010)
bag reactors with light delivery rods ^e	induced flocculation	Sapphire Energy	pilot	Fang et al. (2010), Mendez et al. (2009a, 2009b, 2010a, 2010b), Olaizola (2010)
tubular reactors ^b	whirlpool concentrator then centrifuge	Solix Biofuels	pilot (2 acre site)	Willson et al. (2008, 2009)

Hybrid Designs

covered raceway ponds	concentrate to 10-20% slurry	Genifuel	not disclosed	Oyler (2008a, 2008b, 2010)
covered ponds	Evodos centrifuge	MBD Energy	small pilot (1,000 gal pond)	Boele (2010)
Rapid Algae Farms (covered ponds) ^b	capillary extraction belt	Algaeventure Systems	pilot	Youngs and Cook (2010)
Simgae System (covered furrows) ^b	flocculation	Diversified Energy	demonstration (40 acre site)	Keeler et al. (2010)

Biofilm Reactors

biofilms on polyester sheets	sprayed with water jets	Greenshift	pilot	Bayless et al. (2003)
biofilms in open channels ^c	sprayed with water jets	SBAE Industries	pilot	Vanhoutte and Vanhoutte (2009)
biofilms on baffled rotating contactors ^c	collect sheared biofilms	Algaewheel	pilot (100,000 gal day ⁻¹)	Limaco (2010)
turf scrubber for filamentous algae ^c	vaccum or mechanically scrape turf	Aquafiber Technologies	full (7.5 MGD)	Jensen (1996)
turf scrubber for filamentous algae ^c	mechanically scrape turf	Hydromentia	full (up to 30 MGD)	Adey (1982, 1998)

Other

not specified ^c	no (secreted fatty acids and alcohols)	Synthetic Genomics	bench	Roessler et al. (2009, 2010)
heterotrophic fermentation	not specified	Solazyme	demonstration scale fermentation	Dillon (2008)

^a According to information available on company website

^b Possible applications to wastewater treatment mentioned

^c Demonstrated wastewater treatment or specifically intended for wastewater treatment

^d Duckweed product is not technically microalgae

^e Genetically modified algae

For the purposes of this section, scale is defined as laboratory if volumes of less than 10 gallons are used, bench at 10 to 1,000 gallons, pilot for several thousand gallons or a site of 0.5 to 10 acres, demonstration for a site of 20 to 80 acres or a flow of approximately 1 million gallons per day (MGD), and full for a site greater than 80 acres or if flow is several MGD.

5.1. Reactor Designs for Algae Production

Several companies are seeking to increase algae production through reactor design. Most reactors fall under the category of open ponds or closed reactors, though some are best described as a hybrid combination of the two. Hybrid designs attempt to balance the benefits of low cost open ponds with the control of a closed system. This is usually accomplished by placing a cover over an open pond or channel. A smaller number of designs are for algal biofilm attached growth.

5.1.1. Open Ponds

Many of the companies that have been longest involved in the mass production of algae grow and harvest the filamentous cyanobacteria *Spirulina* as a nutraceutical product in clean, non-wastewater systems. Earthrise Nutritionals and Cyanotech are two companies using open raceway ponds to grow *Spirulina* (Jensen and Reichl, 1997). Because of the filamentous morphology of *Spirulina*, harvesting through simple filtration methods is effective (Vonshak and Richmond, 1988).

Petroalgae is one company using the open pond approach, although the end product is not technically microalgae. The company is listed as assignee on a patent application describing a central seed area with several final ponds radiating from the central area (Howard et al., 2008). The application states that wedge shaped ponds are useful for growing algae continuously because the inoculum can be added at the point of the wedge so that as the culture moves toward the wide section, there is greater surface area for sunlight and multiplying cells. No application to wastewater treatment is mentioned. Despite the company name, it appears that Petroalgae is currently producing

duckweed, not microalgae. Javan et al. (2010) describe a paddlewheel-mixed raceway for growing *Lemna*. Harvesting is accomplished by lowering a conveyor belt or surface skimmer into the raceway before transporting the *Lemna* to a screw auger.

Kai Bioenergy is another company using the open raceway pond approach. Foam fractionation is used to concentrate cells before they are lysed by cavitation bubble collapse (Larach, 2010). There is no mention of any wastewater treatment applications.

Seambiotic is an Israeli company growing algae in outdoor raceway ponds near power plants. Concentrated CO₂ from flue gasses is fed to the raceway ponds (Weiss, 2008). Wastewater treatment applications are not discussed.

General Atomics has several patents related to algae cultivation. Dunlop and Hazlebeck (2010) explain the use of submerged horizontal bars in a growth channel to produce vortices in the passing liquid. This is intended to improve vertical mixing for better light distribution through the culture. Wastewater treatment is not discussed.

Blue Marble is attempting to specialize in the anaerobic digestion of algae and other biomass to produce biomethane and ammonia fertilizer (Stephens, 2010), although earlier patent applications describe a production and harvesting device. The device is made from a micron mesh liner attached to a buoyant frame floated on an open body of water. The liner is intended to allow water and nutrients in without letting cells out. The buoyancy of the frame is controlled by adjusting the amounts of water and air in the frame tubing. After sufficient algae growth, the buoyancy can be increased to lift the entire apparatus out of the water for collection of the culture (Stephens et al., 2009a). A related application describes the potential of using the floating pond reactors to remove

undesirable components such as nitrogen and phosphorus from water (Stephens et al., 2009b).

5.1.2. Closed Reactors

Solix Biofuels and A2BE Carbon Capture are assignees on a patent application describing a closed reactor system with a rotatable internal transparent insulator (Sears, 2007). The insulator can be placed between the bulk of the reactor and the air, leaving thermal contact with the ground, or the insulator can be placed between the bulk of the reactor and the ground, leaving thermal contact with the surrounding air. The reactor design also contains a harvesting chamber where fluid motion maintains a whirlpool to pre-concentrate the algae before it is passed through a roller press. Wastewater treatment is not directly discussed, but the patent application does mention that the algae can be largely fed by industrial, agricultural, and municipal waste products. Solix has other designs including floatable vertical tubular reactors for improved thermal regulation (Willson et al., 2008), and a tubular reactor that incorporates gas permeable membranes into sections of the tubes to improve O₂ release (Willson et al., 2009).

Sunrise Ridge Algae also claims to have a low cost tubular reactor design made of flexible materials that can be rolled out on site and mixed by air sparging (Whitton, 2008). This particular patent application does not discuss any uses for wastewater treatment, but like Petroalgae, the company's recent focus appears to be on wastewater treatment using duckweed.

Algae Systems is a company that has licensed NASA's Offshore Membrane Enclosure for Growing Algae (OMEGA) system in addition to purchasing intellectual

property from Greenfuel Technologies (no longer in operation). The OMEGA system consists of flexible plastic bags that are at least partially permeable to CO₂ and O₂. The bags are filled with domestic wastewater and placed in seawater. The idea is for the reactors to automatically dewater as the treated wastewater leaves through forward osmosis (Trent et al., 2010). Greenfuel Technologies had a portfolio containing several reactor designs; one describing a closed reactor designed to float on a pond for better thermal regulation (Berzin et al., 2009), and another describing a modified air lift bioreactor (Berzin and Wu, 2007).

Origin Oil is also designing reactors that can better distribute light throughout the culture. A perforated rod is placed in the middle of the reactor. Nutrients and CO₂ are delivered through the perforations. Light is channeled through the rod to transparent paddles connected to the ends. The rod and paddles also act as a static mixer. Cell disruption is achieved using cavitation (Eckelberry and Eckelberry, 2009). A related patent application mentions that, although not an exemplary use, the light arrays could be incorporated into wastewater aeration tanks (Shigematsu and Eckelberry, 2009). The company has announced that it has filed a patent application for an attached algae system for wastewater treatment, but the application is not yet published.

Bionavitas is a company attempting to overcome the challenge of PAR delivery by collecting solar radiation and delivering it to a plurality of optical waveguides spaced within the reactor to more efficiently distribute the light (Wilkerson et al., 2009; Wilkerson and Watters, 2009). The patent applications discuss the possibility of using wastewater effluent as part of the nutrient supply system to the reactor.

Texas Clean Fuels uses a basic helical tubular reactor design. A transparent cylinder is used as the core to which the tube is wrapped around so that light can reach both sides of the cylinder (Gal, 2009). The patent application does not mention wastewater treatment.

5.1.3. Hybrid Designs

Diversified Energy Corp. has created the Simgae system for producing algae. The approach is to make the setup and operation of algae reactors as simple as possible by designing them so much of the work can be done using typical farm equipment. Furrows are lined with plastic, filled with media, and covered (Keeler et al., 2010). Harvesting can be done at the end of the furrows after sufficient growth has occurred. According to the patent application, at least a portion of the fertilizer solution fed to the furrows may come from dairy farms and wastewater treatment facilities.

Genifuel Corporation's reactor design is also a hybrid system. Oyler (2008a) describes a covered paddlewheel mixed raceway with continuous gas injection to keep a positive pressure in the chamber to prevent inflow and contamination from the outer environment. Wastewater treatment is not discussed.

5.1.4. Biofilm Reactors

Except where a genetically modified culture or other monoculture is intended, most algae production designs could be tailored to handle wastewater as a nutrient source. There are several approaches, however, that are specifically intended to be incorporated into wastewater treatment, and these are most often biofilm based designs such as those discussed in this section.

Hydromentia has rights to the Algal Turf Scrubber. Filamentous algae grow on a plastic mesh in a spillway as wastewater or other nutrient rich water surges over the surface (Adey, 1982, 1998). Mature turf can be harvested by pulling a scraper behind an ATV (Stewart and Zivojnovich, 2003). Aquafiber Technologies Corporation uses a similar approach with a vacuum harvester to obtain the mature turf (Jensen, 1996). Algaewheel Technologies uses a modified rotating biological contactor design to grow algae and treat wastewater. The contactors are much smaller and are baffled so that air jets can rotate them. The interior of each contactor is filled with polystyrene balls to support bacterial growth while algae biofilms grow on the outer baffles in a symbiotic relationship (Limcaco, 2010).

SBAE Industries, from the Netherlands, is another one of the few companies working on biofilm based algae production. Vanhoutte and Vanhoutte (2009b) describe a conveyer belt system where a growth substratum is partially submerged in wastewater. A continuous operation can be developed by starting growth at a point farthest from a central collection area and allowing a certain amount of time for growth before reaching the harvesting area. SBAE's Diaforce system consists of sections of growth substrata placed in an open channel with wastewater flowing through. As biofilms become established, sections are removed and taken to a harvesting area where the biofilm is removed by spraying with water jets. The biomass is then recovered after settling (Vanhoutte and Vanhoutte, 2009a).

Greenshift Corp. has rights to a gas treatment reactor made of vertical polyester panels for attached algae growth. Optical waveguides can be placed between each panel to distribute light to each side. Harvesting is done by increasing the pressure of the water

delivery system to spray the biofilms off the panels (Bayless et al., 2003). GS Clean Tech, a subsidiary of Greenshift, is the assignee on an application that describes the use of a similar system in conjunction with an ethanol production plant (Winsness et al., 2007). This biofilm reactor was designed to treat waste gas streams, and it would not be easily adapted to wastewater treatment facilities.

5.2. Harvester Designs & Harvesting Processes

To overcome the challenge of harvesting suspended algae, industry researchers are looking for improvements to harvester designs and/or processes. Some companies are attempting to improve mechanical harvesters or create new ones while others are focusing on biological based harvesting. A few companies are attempting to bypass the algae separation step altogether.

5.2.1. Mechanical Harvesters

Algae to Energy, or A2E, uses what it calls the Shepherd Harvester for algae separation (Shepherd, 2010). The harvester uses a continuous belt that moves through the algae culture and a vacuum system. As the belt moves, any algae collected on the belt is harvested by the vacuum system before the belt passes through the culture again. The patent application does not directly discuss use of the harvester in wastewater treatment plants, but the need to incorporate large scale algae cultivation into existing infrastructure such as sewage treatment facilities is mentioned.

Algaeventure Systems, Inc. also uses a continuous belt harvester based on capillary extraction (Youngs and Cook, 2010). The design uses a primary belt to collect algae and a secondary capillary belt made of a super absorbent polymer. The secondary

belt is in contact with the bottom portion of the primary belt so that water is pulled through the algae and primary belt into the secondary belt. The dried biomass on the primary belt is collected and the secondary belt is compressed to drain water before it contacts the primary belt again. The patent application does not discuss the use of the harvester in wastewater treatment, but the company does discuss the potential use of wastewater in covered ponds called Rapid Algae Farms on their website. General Atomics has also awarded a purchase order to Algaeventure Systems for their harvesting device.

MBD Energy is an Australian company using coal plant wastewater and covered raceway ponds for algae production. The company is collaborating with Evodos, a Dutch company, and using their separators. The Evodos separator is a centrifuge that allows for easier removal of solids after concentration. The inner assembly is made of curved but flexible disks. This inner assembly can be removed and rotated so that the curved disks become straight and solids become unwedged (Boele, 2010).

Scipio Biofuels grows algae in closed tubular reactors. Their continuous harvester is basically a low speed centrifuge. A circular chamber with a textured side wall rotates to force cells against the side wall. Because flocs or larger cells cannot pass over the rough surface as readily as smaller cells, they remain against the wall. A skimmer blade then continually passes along the wall to remove these flocs (Wells and Snyder, 2010). The patent application does not discuss any wastewater treatment capabilities.

5.2.2. Biological Based Harvesting

Kent Bioenergy has rights to the Controlled Eutrophication Process developed at Clemson that was described in section 4.4 of this document. The company is also the assignee on a patent application describing a sequence of decanting operations to select for a culture more disposed to flocculate and settle. Flow in a raceway pond is stopped and a settling period elapses. An upper layer of water is then removed along with any algae in it. The removed volume is then replaced and the process is repeated until sediment-ready algae sufficiently dominate the culture (Schwartz et al., 2010). The patent application specifically describes the use of such a process for wastewater treatment, and the technique was demonstrated in two treatment ponds measuring 80 ft² each.

Live Fuels Inc. is another company utilizing fish as a means of harvesting algae. The planktivorous fish, such as tilapia, are harvested for oil and fishmeal (Wu et al., 2010a). A series of foam fractionation units may be used to pre-concentrate the algae as well (Wu et al., 2010b). Regarding wastewater treatment, the patent applications briefly mention the possibility of using agricultural, industrial, or municipal wastewater in the system. Live Fuels also has a separate Patent Cooperation Treaty application describing the use of transgenic fish in this process (Stephen and Morgenthaler, 2010).

For Sapphire Energy, Mendez et al. (2009a) describe algae genetically modified to enable controlled flocculation and simpler harvesting. The algae are modified to express a ligand or receptor molecule such as an antibody or antigen. The molecule can be attached to the cell wall or secreted. For example, a culture expressing an antibody could be mixed with a separate culture expressing the corresponding antigen to induce

flocculation. Expression of a ligand/receptor pair could be sequentially induced to initiate flocculation.

5.2.3. *Bypassing the Harvesting Step*

Phycal is trying to bypass much of the harvesting and dewatering process by performing non-destructive oil extraction of live cells. Phycal's process involves mixing a portion of a *Nannochloropsis* culture with a lipid extracting solvent such as dodecane for approximately five minutes while sonicating the cells at 40 kHz for two seconds (Swanson et al., 2010). This process also aids in reducing levels of predators and unwanted species (Lane et al., 2010). The possibility of using wastewater is not discussed, and it would likely be difficult if monocultures of *Nannochloropsis* are intended.

Algenol Biofuels Inc. is also attempting to bypass the biomass harvesting step altogether by using genetically modified algae or cyanobacteria capable of secreting ethanol. Such a culture would be enclosed in greenhouse where evaporated water and ethanol would condense on the ceiling and travel to a collection trough (Woods et al., 2010).

Synthetic Genomics, partnered with ExxonMobil, is using genetic engineering approaches to create algae for fuel production. Roessler et al. (2009) describe genetically modified algae or cyanobacteria capable of secreting fatty acids into the growth media. The fatty acids can then be collected by liquid-liquid extraction or chromatography. Another patent application deals not with fatty acids, but with secreted branched chain alcohols (Roessler et al., 2010).

5.3. Process Design

Approaches to improved process design often have the objectives of greater culture control, increased cellular lipid content, or cost reduction through nutrient and gas recycle and the utilization of waste streams.

5.3.1. Culture Control

Aquatic Energy claims to maintain culture selectivity simply by matching the residence time of their clay lined raceway ponds to the doubling time of their target organism (Demaris et al., 2009). No applications to wastewater treatment are discussed in the patent application.

Aurora Algae, previously called Aurora Biofuels, has patent literature describing the use of mutant pale-green *Nannochloropsis* with low chlorophyll content so light can reach deeper into the culture. 2-hydroxy-5-oxoproline is also added to enhance growth (Vick and Fleischer, 2009). To maintain selectivity, glyphosate herbicide can be added to glyphosate resistant *Nannochloropsis* cultures (Vick, 2010). They also report that *Nannochloropsis* will better dominate a lower salinity environment and recover more quickly from disinfectant exposure than invasive strains (Weissman and Radaelli, 2010), and that ozone shock can be used for the same purpose (Weissman et al., 2010). None of the documents discuss any potential wastewater treatment applications, and the company's focus on monocultures of *Nannochloropsis* would be incompatible with the mixed culture constraint of a wastewater treatment lagoon.

Cellana is an algae biofuels company originally created as a joint venture between HR Biopetroleum and Royal Dutch Shell, though it is now owned solely by HR

Biopetroleum. HR Biopetroleum is the assignee on a patent that describes a continuously operated system of closed reactors used to inoculate batch operated open ponds (Huntley and Redalje, 2010). The idea is to prevent contamination of the open ponds by ensuring the inoculum from the closed reactors is enough to give the preferred organism an advantage. There is no indication that Cellana is looking to apply their technology to wastewater treatment.

5.3.2. Lipid Accumulation

Aquatic Energy uses an additional production stage after sufficient growth has been achieved in clay lined raceways. After the raceways, cells enter a secondary stress pond for nitrogen starvation and lipid accumulation for 48 hours before being harvested (Demaris et al., 2009).

For Genifuel Corporation, Oyler (2008b) describes a two-stage process for producing algae with high lipid content consisting of a first stage of autotrophic conditions to produce the biomass and a second stage of heterotrophic conditions to increase lipid content.

Before the live extraction described in section 5.2.3, Phycal's process increases lipid production by inhibiting nitrate uptake, either through the addition of chlorate or by inducing the production of a nitrate reductase inhibitor in a genetically engineered culture (Swanson et al., 2010).

5.3.3. Nutrient & Gas Recycle

GS Clean Tech's patent application for a vertical sheet biofilm system described in section 5.1.4 also describes the use of CO₂ from an ethanol production plant to grow

algae. The algae biomass is then added to the original feedstock of the ethanol plant, thus recycling the CO₂ byproduct of ethanol fermentation (Winsness et al., 2007).

For General Atomics, Hazlebeck and Dunlop (2008) have described a gas-liquid contactor reactor used to scrub CO₂ before feeding the solution to an algae culture. Greenfuel Technologies also had an application describing a gas-liquid contactor to scrub flue gas before feeding the liquid to a photobioreactor (Wu et al., 2007). Another General Atomics patent describes the recycling of nutrients back to a growth chamber after cell lysing and transesterification steps. More specifically, the non-oil fraction of lysed cell matter from the lysing step is combined with the glycerin byproduct of the transesterification step before being fed to a chemostat for algae growth (Hazlebeck and Dunlop, 2010).

Genifuel Corporation has a patent application describing the gasification of wet biomass before recycling the CO₂ and nutrients back to a growing chamber (Oyler, 2010). Honeywell's UOP is the assignee on a patent application describing the capture of CO₂ from a biodiesel production process. The CO₂ is then fed to an algae culture to produce more biomass for the process (Marker et al., 2009).

5.4. Genetic Manipulation

Algenol Biofuels and Synthetic Genomics are using genetic engineering approaches to enable secretion of ethanol, fatty acids, or alcohols as described in section 5.2.3. In section 5.2.2, the Sapphire Energy method of controllable flocculation using genetically modified algae is described.

Sapphire Energy has several other patent applications related to the genetic manipulation of algae. Mendez et al. (2009b) describe the construction of synthetic chloroplasts and Mendez et al. (2010a) describe the increased expression of fatty acid synthesizing enzymes in algae. On the production side, Olaizola (2010) describes the use of transparent rods with floats on top and weights on the bottom in an algae pond or reactor. Any light that enters the rods is delivered to the darker portions of the culture below the surface. Sapphire also has patent cooperation treaty applications for genetically modified herbicide resistant algae (Fang et al., 2010), and genetically modified salt tolerant algae (Mendez et al., 2010b).

Joule Unlimited is focused on creating enhanced algae through genetic engineering. Devroe et al. (2010) describe the upregulating, downregulating, or knocking out of specified genes in order to potentially give enhanced light utilization, carbon fixation, NADH and NADPH production, thermotolerance, pH tolerance, salt tolerance, flue gas tolerance, nutrient independence, and near infrared absorbance. Devroe et al. (2009) disclose mechanisms to confer photosynthetic properties to a heterotrophic organism with better understood techniques for genetic manipulation and industrial processing such as *Escherichia coli*. Joule's reactors are modified flat panel closed reactors with corrugated panels to act as static mixers for increased fluid turbulence (Van Walsem et al., 2010).

Solazyme is best known for producing algae under heterotrophic conditions using fermentation technology, but a patent application from earlier work describes genetic alterations to algae to downregulate production of light harvesting pigments so more light can pass the top layer of cells and reach the bulk of the culture (Dillon, 2008).

5.5. Summary of Approaches in Industry

Figure 1 shows the number of algae production companies reviewed in this paper operating at bench, pilot, demonstration, and full scale, as well as the proportion of those companies that have discussed the potential of integrating wastewater treatment resources or have designed for and/or demonstrated it. Although several companies have moved from bench scale to small pilot operations, the majority of companies operating at bench or pilot scale have not displayed an interest in using wastewater resources or integrating wastewater treatment into their production approaches. Although few companies are operating beyond the small pilot scale, there is more wastewater integration with companies at demonstration and full scale operation, likely because cost effective scale-up beyond small pilot plants necessitates the use of such available resources.

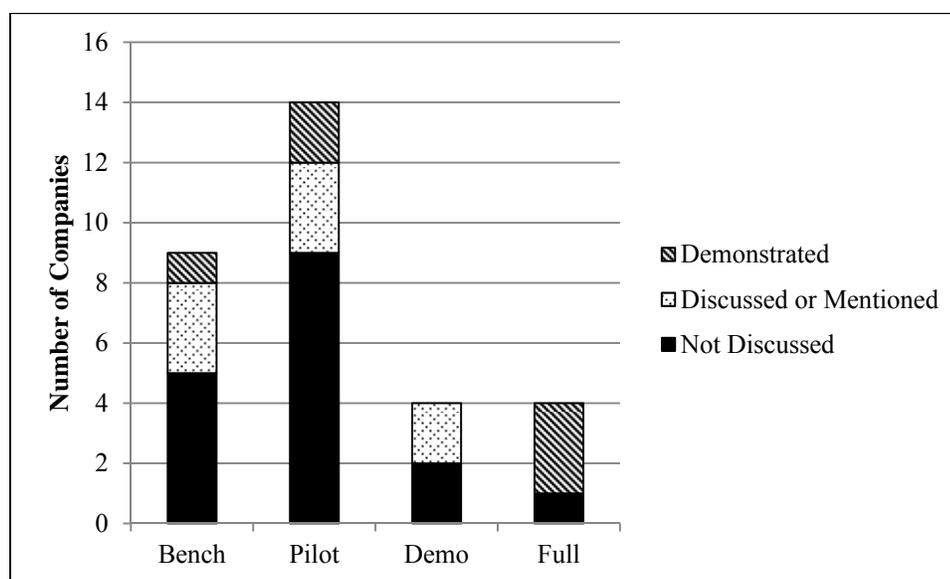


Figure 1: Scale of algae production companies and involvement with wastewater treatment.

Figure 2 shows the number of companies reviewed in this paper operating at different scales and the proportion of those companies using open ponds, closed reactors, hybrid designs, or biofilm reactors. The majority of companies using a closed reactor approach are operating at bench scale, and no closed reactor approach is operating beyond the small pilot scale. This is likely due to the difficulty in scaling up closed reactors relative to other production approaches. At pilot, demonstration, and full scales, the most common approach is open ponds, although at full scale, the attached algal turf scrubber represents two of the four operations.

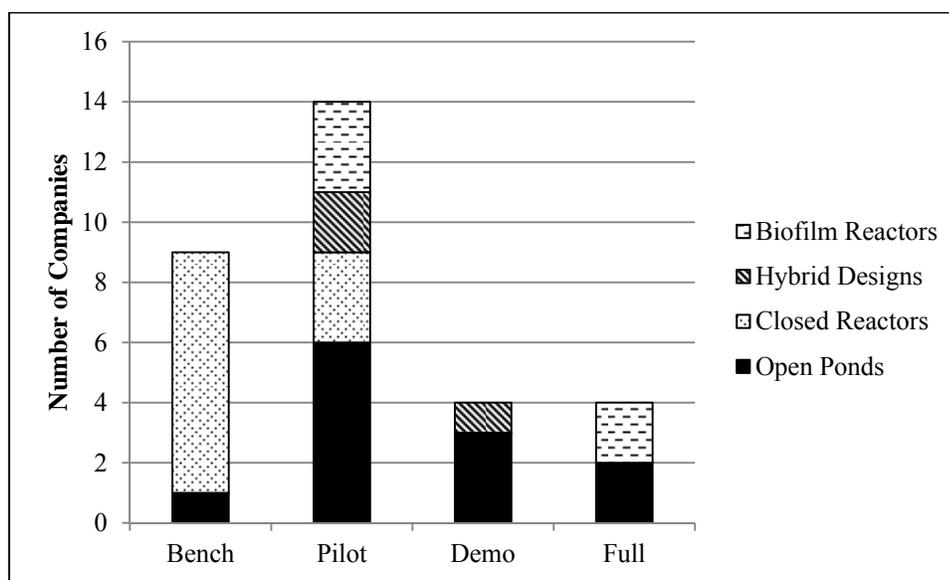


Figure 2: Scale of algae production companies and production approach.

6. Conclusions

Much of the research addressing algae production and harvesting is currently confined to the laboratory. Although many companies have moved from laboratory and bench scales to small pilot scale, but the major challenges of nutrient supply, land and

water availability, PAR delivery, gas exchange, environment control, and culture integrity have limited further scale-up, and the number of studies and companies operating at demonstration and full scale is limited.

Overcoming current challenges to the production and harvesting of algae will benefit both the biofuel and wastewater treatment fields. It appears, however, that this collaborative potential has not been realized, as those testing algae production systems have not often integrated their research with the wastewater industry's need for algae production technologies. Using wastewater as a resource and combining wastewater treatment with the production of algae based bioproducts can overcome several of the major challenges identified herein. Additionally, the existing infrastructure of wastewater treatment facilities can be utilized for managed algae production, thereby reducing capital costs and scalability challenges. Despite these benefits, only a few preliminary studies have been conducted to produce biofuels and bioproducts from algae grown in wastewater.

The separate operations that result in an algae biosolids product cannot be considered to be mutually exclusive. An upstream choice concerning nutrient source, reactor design, or reactor operation will affect downstream harvesting and dewatering alternatives and constraints. Conversely, the choice of a particular harvesting or dewatering method will dictate what upstream conditions must be met. The use of a biofilm based system could more effectively and efficiently integrate production, harvesting, and dewatering operations; however, there is little information on the use of such a design outside of the laboratory. Considering all the approaches reviewed, algae biofilm based production and harvesting methods are the least understood and the least

attempted, despite the potential benefits with regard to productivity, yield, harvesting, and bioproducts. Indeed, there is need for improving biofilm designs to optimize algae biomass production since any large scale systems in use today are designed for wastewater treatment only. Genetic engineering approaches could also solve many of the present challenges, but until there is more development on the technical and regulatory side, scalable biofilm based systems warrant further investigation.

References

- Abu-Rezq TS, Al-Musallam L, Al-Shimmari J, Dias P. Optimum production conditions for different high-quality marine algae. *Hydrobiologia* 1999; 403: 97–107.
- Acreman J. Algae and cyanobacteria: isolation, culture and long-term maintenance. *J Ind Microbiol* 1994; 13: 193-194.
- Adey WH, Luckett C, Jensen K. Phosphorus removal from natural waters using controlled algal production. *Restor Ecol* 1993; 1: 29-39.
- Adey WH. Algal turf scrubber. United States patent US 4333263. 1982.
- Adey WH. Algal turf water purification method. United States patent US 5851398. 1998.
- Azov Y. Effect of pH on inorganic carbon uptake in algal cultures. *Appl Environ Microbiol* 1982; 43: 1300.
- Bailliez C, Largeau C, Casadevall E. Growth and hydrocarbon production of *Botryococcus braunii* immobilized in calcium alginate gel. *Appl Microbiol Biotechnol* 1985; 23: 99-105.
- Bayless DJ, Vis-Chiasson ML, Kremer GG. Enhanced practical photosynthetic CO₂ mitigation. United States patent US 6667171. 2003.
- Bernhardt H, Clasen J. Flocculation of micro-organisms. *Aqua- J Water Supply: Res Technol* 1991; 40: 76–87.
- Berzin I, de Luis J, Fair D, Parrish J, Richtman K, Chen F, et al. Photobioreactor systems positioned on bodies of water. United States patent application US 20090130706. 2009.

- Berzin I, Wu X. Photobioreactor and process for biomass production and mitigation of pollutants in flue gases. Patent cooperation treaty application WO 2007011343. 2007.
- Bhaskar PV, Bhosle NB. Microbial extracellular polymeric substances in marine biogeochemical processes. *Curr Sci* 2005; 88: 45–53.
- Boele HA. Separating device. European Patent EP 2014346. 2010.
- Borowitzka MA. Commercial production of microalgae: ponds, tanks, and fermenters. *Prog Ind Microbiol* 1999; 35: 313–321.
- Bothwell ML. All-weather troughs for periphyton studies. *Water Res* 1983; 17: 1735–1741.
- Brune DE, Collier JA, Schwedler TE, Eversole AG. Controlled eutrophication system and process. United States patent US 7258790. 2007.
- Bugbee GJ, Frink CR. Alum sludge as a soil amendment: effects on soil properties and plant growth. New Haven, (CT): The Connecticut Agricultural Experiment Station; 1985 Nov. Bulletin: 827.
- Cabirol N, Barragán EJ, Durán A, Noyola A. Effect of aluminium and sulphate on anaerobic digestion of sludge from wastewater enhanced primary treatment. *Water Sci. Technol* 2003; 48: 235-240.
- Cao J, Yuan W, Pei ZJ, Davis T, Cui Y, Beltran M. A preliminary study of the effect of surface texture on algae cell attachment for a mechanical-biological energy manufacturing system. *J Manuf Sci Eng* 2009; 131: 064505-4.
- Carvalho A, Meireles L, Malcata F. Microalgal Reactors: A review of enclosed system designs and performances. *Biotechnol Prog* 2006; 22: 1490-1506.
- Chevalier P, Proulx D, Lessard P, Vincent WF, De La Noüe J. Nitrogen and phosphorus removal by high latitude mat-forming cyanobacteria for potential use in tertiary wastewater treatment. *J Appl Phycol* 2000; 12: 105–112.
- Chiellini E, Cinelli P, Ilieva VI, Martera M. Biodegradable thermoplastic composites based on polyvinyl alcohol and algae. *Biomacromolecules* 2008; 9: 1007-1013.
- Chisti Y. Biodiesel from microalgae. *Biotechnol Adv* 2007; 25: 294-306.
- Chisti Y. Biodiesel from microalgae beats bioethanol. *Trends Biotechnol* 2008; 26: 126-131.

- Chiu S, Kao C, Tsai M, Ong S, Chen C, Lin C. Lipid accumulation and CO₂ utilization of *Nannochloropsis oculata* in response to CO₂ aeration. *Bioresour Technol* 2009; 100: 833-838.
- Chorus I, Bartram J. Toxic cyanobacteria in water: a guide to their public health consequences, monitoring, and management. London: Taylor & Francis; 1999.
- Correll DL. Role of phosphorus in the eutrophication of receiving waters: a review. *J Environ Qual* 1998; 27: 261–266.
- Danquah MK, Ang L, Uduman N, Moheimani N, Forde GM. Dewatering of microalgal culture for biodiesel production: exploring polymer flocculation and tangential flow filtration. *J Chem Technol Biotechnol* 2009; 84: 1078-1083.
- de-Bashan LE, Bashan Y, Moreno M, Lebsky VK, Bustillos JJ. Increased pigment and lipid content, lipid variety, and cell and population size of the microalgae *Chlorella* spp. when co-immobilized in alginate beads with the microalgae-growth-promoting bacterium *Azospirillum brasilense*. *Can J Microbiol* 2002; 48: 514–521.
- Degen J, Uebele A, Retze A, Schmid-Staiger U, Trösch W. A novel airlift photobioreactor with baffles for improved light utilization through the flashing light effect. *J Biotechnol* 2001; 92: 89-94.
- Demaris PB, Ravikumar R, Vandevivere P. Algal culture production, harvesting, and processing. Patent cooperation treaty application WO 2009094440. 2009.
- Devroe EJ, Berry DA, Afeyan NB, Robertson DE, Skraly FA, Ridley CP. Engineered light-harvesting organisms. United States patent application US 20090191599. 2009.
- Devroe EJ, Kosuri S, Berry DA, Afeyan NB, Skraly FA, Robertson DE, et al. Hyperphotosynthetic organisms. United States patent US 7785861. 2010.
- Dillon H. Light utilization alteration of photosynthetic microorganisms. United States patent application US 20080124756. 2008.
- Divakaran R, Sivasankara Pillai VN. Flocculation of algae using chitosan. *Journal of Appl Phycol* 2002; 14: 419–422.
- Dunlop EH, Hazlebeck DA. High photoefficiency microalgae bioreactors. United States patent US 7763457. 2010.
- Eckelberry N, Eckelberry TR. Algae growth system for oil production. United States patent application US 20090029445. 2009.

- Fang S, Poon Y, Mendez M. Genetically engineered herbicide resistant algae. Patent cooperation treaty application WO 2010078156. 2010.
- Friedman AA, Peaks DA, Nichols RL. Algae separation from oxidation pond effluents. *J (Water Pollut Control Fed)* 1977; 49: 111-119.
- Gal JL. Micro-organism production system and method. United States patent application US 20090023199. 2009.
- Golueke CG, Oswald WJ, Gotaas HB. Anaerobic Digestion of Algae. *Appl Microbiol* 1957; 5: 47-55.
- González C, Marciniak J, Villaverde S, León C, García PA, Muñoz R. Efficient nutrient removal from swine manure in a tubular biofilm photo-bioreactor using algae-bacteria consortia. *Water Sci Technol* 2008; 58: 95-102.
- Graham LE, Graham J, Graham JM, Wilcox LW. *Algae*. San Francisco: Benjamin Cummings; 2009.
- Greenwell HC, Laurens LML, Shields RJ, Lovitt RW, Flynn KJ. Placing microalgae on the biofuels priority list: a review of the technological challenges. *J R Soc Interface* 2010; 7: 703 -726.
- Griffiths E. Removal and utilization of wastewater nutrients for algae biomass and biofuels. All Graduate Theses and Dissertations 2009; Paper 631. Available from: <http://digitalcommons.usu.edu/etd/631>
- Gunaseelan VN. Anaerobic digestion of biomass for methane production: a review. *Biomass Bioeng* 1997; 13: 83-114.
- Guzzon A, Bohn A, Diociaiuti M, Albertano P. Cultured phototrophic biofilms for phosphorus removal in wastewater treatment. *Water Res* 2008; 42: 4357–4367.
- Hameed MS, Ebrahim OH. Biotechnological potential uses of immobilized algae. *J Agric Biol* 2007; 9: 183–192.
- Hazlebeck DA, Dunlop EH. Photosynthetic carbon dioxide sequestration and pollution abatement. United States patent application US 20080086938. 2008.
- Hazlebeck DA, Dunlop EH. Photosynthetic oil production with high carbon dioxide utilization. United States patent US 7662616. 2010.
- Hodoki Y. Bacteria biofilm encourages algal immigration onto substrata in lotic systems. *Hydrobiologia* 2005; 539: 27-34.

- Hoffmann JP. Wastewater treatment with suspended and nonsuspended algae. *J Phycol* 1998; 34: 757-763.
- Holmes PE. Bacterial enhancement of vinyl fouling by algae. *Appl Environ Microbiol* 1986; 52: 1391-1393.
- Howard EE, Alianell GA, Riding TJ, Barile PJ, Foster TR. Systems and methods for large-scale production and harvesting of oil-rich algae. United States patent application US 20080096267. 2008.
- Hu Q, Guterman H, Richmond A. A flat inclined modular photobioreactor for outdoor mass cultivation of photoautotrophs. *Biotechnol Bioeng* 1996; 51: 51–60.
- Hu Q, Richmond A. Productivity and photosynthetic efficiency of *Spirulina platensis* as affected by light intensity, algal density and rate of mixing in a flat plate photobioreactor. *J Appl Phycol* 1996; 8: 139-145.
- Huntley ME, Redalje DG. Continuous-batch hybrid process for production of oil and other useful products from photosynthetic microbes. United States patent US 7770322. 2010.
- Javan A, Grieco W, Cumba HJ, Weaver H, Lovas B. Cultivation, harvesting, and processing of floating aquatic species with high growth rates. Patent cooperation treaty application WO 2010123943. 2010.
- Jensen KR. Apparatus for water purification by culturing and harvesting attached algal communities. United States patent US 5527456. 1996.
- Jensen G, Reichl EH. Integrated microalgae production and electricity cogeneration. United States patent US 5659977. 1997.
- Johnson MB, Wen Z. Development of an attached microalgal growth system for biofuel production. *Appl Microbiol Biotechnol* 2010; 85: 525-534.
- Keeler CC, Stephenson JD, Schenk SW, Cloud GB, Naradikian S, Irwin SW. Algae production systems and associated methods. United States patent application US 20100170149. 2010.
- Klausmeier CA, Litchman E, Daufresne T, Levin SA. Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. *Nature* 2004; 429: 171-174.
- Knud-Hansen CF, McElwee K, Baker J, Clair D. Pond fertilization: ecological approach and practical application. Pond Dynamics/Aquaculture Collaborative Research Support Program, Oregon State University; 1998.

- Kumar H, Yadava P, Gaur J. Electrical flocculation of the unicellular green alga *Chlorella vulgaris* Beijerinck. *Aquat Bot* 1981; 11: 187-195.
- Lane CD, Swanson AK, Brister AB, Allnutt FCT. Biofuel production from algae. Patent cooperation treaty application WO 2010132414. 2010.
- Larach M. Continuous cultivation, harvesting, and oil extraction of photosynthetic cultures. Patent cooperation treaty application WO 2010017243. 2010.
- Lavoie A, de la Noüe J. Harvesting of *Scenedesmus obliquus* in wastewaters: auto or bioflocculation? *Biotechnol Bioeng* 1987; 30: 852-859.
- Lee AK, Lewis DM, Ashman PJ. Microbial flocculation, a potentially low-cost harvesting technique for marine microalgae for the production of biodiesel. *J Appl Phycol* 2008; 21: 559-567.
- Limcaco CA. System and method for biological wastewater treatment and for using the byproduct thereof. United States patent US 7736508. 2010.
- Lundquist TJ, Woertz IC, Quinn NWT, Benemann JR. A realistic technology and engineering assessment of algae biofuel production. Berkeley, CA: Energy Biosciences Institute; 2010.
- Marker TL, Ellis GD, Gosling CD, Rispoli GF, D'Addario EN. Integrated process of algae cultivation and production of diesel fuel from biorenewable feedstocks. United States patent application US 20090077864. 2009.
- Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: a review. *Renew Sustain Energy Rev* 2010; 14: 217-232.
- Mendez M, Behnke C, Poon Y, Lee P. Induction of flocculation in photosynthetic organisms. Patent cooperation treaty application WO 2009158658. 2009a.
- Mendez M, Fang S, Richard S. Engineering salt tolerance in photosynthetic microorganisms. Patent cooperation treaty application WO 2010105095. 2010b.
- Mendez M, O'Neill B, Burkart M, Behnke C, Lieberman S, Bielinski V, et al. Production of fatty acids by genetically modified photosynthetic microorganisms. Patent cooperation treaty application WO 2010019813. 2010a.
- Mendez M, O'Neill B, Mikkelsen K. System for capturing and modifying large pieces of genomic DNA and constructing organisms with synthetic chloroplasts. United States patent application US 20090123977. 2009b.

- Middlebrooks EJ, Porcella DB, Gearheart RA, Marshall GR, Reynolds JH, Grenney WJ. Techniques for algae removal from wastewater stabilization ponds. *J (Water Pollut Control Fed)* 1974; 46: 2676-2695.
- Molina Grima E, Belarbi E-, Acién Fernández FG, Robles Medina A, Chisti Y. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnol Adv* 2003; 20: 491-515.
- Molina Grima E, Fernández FGA, García Camacho F, Chisti Y. Photobioreactors: light regime, mass transfer, and scaleup. *J Biotechnol* 1999; 70: 231-247.
- Mulbry W, Westhead EK, Pizarro C, Sikora L. Recycling of manure nutrients: use of algal biomass from dairy manure treatment as a slow release fertilizer. *Bioresour Technol* 2005; 96: 451–458.
- Mulbry W, Kondrad S, Buyer J. Treatment of dairy and swine manure effluents using freshwater algae: fatty acid content and composition of algal biomass at different manure loading rates. *J Appl Phycol* 2008; 20: 1079-1085.
- Mulbry WW, Wilkie AC. Growth of benthic freshwater algae on dairy manures. *J Appl Phycol* 2001; 13: 301–306.
- Niyogi KK. Photoprotection revisited: genetic and molecular approaches. *Annu Rev Plant Physiol Plant Mol Biol* 2003; 50: 333.
- Norsker N, Barbosa MJ, Vermuë MH, Wijffels RH. Microalgal production – a close look at the economics. *Biotechnol Adv* 2011; 29: 24-27.
- Nurdogan Y, Oswald WJ. Enhanced nutrient removal in high-rate ponds. *Water Sci Technol* 1995; 31: 33-43.
- Oh HM, Lee SJ, Park MH, Kim HS, Kim HC, Yoon JH, et al. Harvesting of *Chlorella vulgaris* using a bioflocculant from *Paenibacillus* sp. AM49. *Biotechnol Lett* 2001; 23: 1229–1234.
- Olaizola M. Light transport in a bioreactor system. Patent cooperation treaty application WO 2010077638. 2010.
- Oswald WJ. My sixty years in applied algology. *J Appl Phycol* 2003; 15: 99–106.
- Oyler JR. Closed-loop system for growth of algae or cyanobacteria and gasification of the wet biomass. United States patent application US 20100003717. 2010.

- Oyler JR. Controlled growth environments for algae cultivation. United States patent application US 20080155890. 2008a.
- Oyler JR. Two-stage process for producing oil from microalgae. United States patent application US 20080160593. 2008b.
- Pal S, Mal D, Singh R. Cationic starch: an effective flocculating agent. *Carbohydr Polym* 2005; 59: 417-423.
- Passow U, Alldredge AL. Aggregation of a diatom bloom in a mesocosm: the role of transparent exopolymer particles (TEP). *Deep Sea Res Part II: Top Stud Oceanogr* 1995; 42: 99-109.
- Patwardhan AW. Rotating biological contactors: a review. *Ind Eng Chem Res* 2003; 42: 2035-2051.
- Pittman JK, Dean AP, Osundeko O. The potential of sustainable algal biofuel production using wastewater resources. *Bioresour Technol* 2011; 102: 17-25.
- Powles SB. Photoinhibition of photosynthesis induced by visible light. *Annu Rev Plant Physiol* 1984; 35: 15-44.
- Przytocka-Jusiak M, Baszczyk M, Kosinska E, Bisz-Konarzewska A. Removal of nitrogen from industrial wastewaters with the use of algal rotating disks and denitrification packed bed reactor. *Water Res* 1984; 18: 1077-1082.
- Putt R. Algae as a biodiesel feedstock: a feasibility assessment. Alabama: Center for Microfibrous Materials Manufacturing, Department of Chemical Engineering, Auburn University; 2007.
- Qureshi N, Annous B, Ezeji T, Karcher P, Maddox I. Biofilm reactors for industrial bioconversion processes: employing potential of enhanced reaction rates. *Microbiol Cell Factories* 2005; 4: 24.
- Rectenwald LL, Drenner RW. Nutrient removal from wastewater effluent using an ecological water treatment system. *Environ Sci Technol* 2000; 34: 522-526.
- Roeselers G, van Loosdrecht MCM, Muyzer G. Phototrophic biofilms and their potential applications. *J Appl Phycol* 2008; 20: 227-235.
- Roessler PG, Chen Y, Liu B, Dodge CN. Secretion of fatty acids by photosynthetic microorganisms. United States patent application US 20090298143. 2009.
- Roessler PG, Liu B, Kristof JR. Production of branched-chain alcohols by photosynthetic microorganisms. United States patent application. US 20100151545. 2010.

- Round FE. The ecology of algae. Cambridge: Cambridge University Press; 1984.
- Salerno M, Nurdogan Y, Lundquist TJ. Biogas production from algae biomass harvested at wastewater treatment ponds. 2009 Bioenergy Engineering Conference. ASABE Conference Presentation; 2009 Oct. Paper No.: Bio098023.
- Schwartz G, Massingill M, Van Olst J, Carlberg J. Method of developing a rapidly settling algal floc. United States patent application US 20100264094. 2010.
- Sears JT. Method, apparatus, and system for biodiesel production from algae. United States patent application US 20070048848. 2007.
- Sheehan J, Dunahay T, Benemann J, Roessler P. A look back at the US department of energy's aquatic species program—biodiesel from algae. Golden (CO): National Renewable Energy Laboratory (NREL); 1998 Jul. Report No.: NREL/TP-580-24190. Prepared for U.S. Department of Energy's Office of Fuels Development.
- Shelef G, Sukenik A, Green M. Microalgae harvesting and processing: a literature review. Haifa (Israel): Technion Research and Development Foundation Ltd; 1984.
- Shen Y, Yuan W, Pei ZJ, Wu Q, Mao E. Microalgae mass production methods. *Trans ASABE* 2009; 52: 1275-1287.
- Shepherd S. System for harvesting algae in continuous fermentation. United States patent application US 20100144017. 2010.
- Shigematsu S, Eckelberry N. Apparatus and method for optimizing photosynthetic growth in a photo bioreactor. United States patent application US 20090291485. 2009.
- Shipin OV, Rose PD, Meiring PGJ. Microbial processes underlying the PETRO concept (trickling filter variant). *Water Res* 1999; 33: 1645-1651.
- Sperling JA, Grunewald R. Batch culturing of thermophilic benthic algae and phosphorus uptake in a laboratory stream model. *Limnol Oceanogr* 1969; 14: 944-949.
- Staats N, De Winder B, Stal L, Mur L. Isolation and characterization of extracellular polysaccharides from the epipelagic diatoms *Cylindrotheca closterium* and *Navicula salinarum*. *Eur J Phycol* 1999; 34: 161-169.
- Stephen D, Morgenthaler GE. Methods for producing fish with high lipid content. Patent cooperation treaty application WO 2010059598. 2010

- Stephens J. Systems and methods for anaerobic digestion and collection of products. Patent cooperation treaty application WO 2010047815. 2010.
- Stephens J, Dickinson KW, Ogilvie K. Apparatus for containing, cultivating, and harvesting photosynthetic marine microorganisms within water. United States patent application US 20090137025. 2009a.
- Stephens J, Dickinson KW, Ogilvie K. Method for removing undesirable components from water while containing, cultivating, and harvesting photosynthetic marine microorganisms within water. United States patent application US 20090134091. 2009b.
- Stewart, III EA, Zivojnovich MJ. Apparatus and method for harvesting and collecting attached algal communities. United States patent US 6572770. 2003.
- Stumm W, Morgan JJ. Aquatic chemistry: an introduction emphasizing chemical equilibria in natural waters. Hoboken: John Wiley; 1981.
- Sukenik A, Shelef G. Algal autoflocculation-verification and proposed mechanism. Biotechnol. Bioeng 1984; 26: 142-147.
- Swanson AK, Postier BL, Sayre RT. Algal lipid production. Patent cooperation treaty application WO 2010132413. 2010.
- Tchobanoglous G, Burton FL. Wastewater engineering: treatment, disposal, and reuse. New York City: McGraw-Hill; 1991.
- Teixeira MR, Rosa MJ. Comparing dissolved air flotation and conventional sedimentation to remove cyanobacterial cells of *Microcystis aeruginosa*: Part I: The key operating conditions. Sep Purif Technol 2006; 52: 84-94.
- Torpey WN, Heukelekian H, Kaplovsky AJ, Epstein R. Rotating disks with biological growths prepare wastewater for disposal or reuse. J (Water Pollut Control Fed) 1971; 43: 2181-2188.
- Tredici MR, Zittelli GC. Efficiency of sunlight utilization: tubular versus flat photobioreactors. Biotechnol Bioeng 1998; 57: 187-197.
- Trent JD, Gormly SJ, Delzeit LD, Flynn MT, Embaye TN. Algae bioreactor using submerged enclosures with semi-permeable membranes. United States patent application US 20100216203. 2010.
- Uduman N, Qi Y, Danquah MK, Forde GM, Hoadley A. Dewatering of microalgal cultures: A major bottleneck to algae-based fuels. J Renew Sustain Energy 2010; 2: 012701.

- U.S. DOE. National algal biofuels technology roadmap. U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Biomass Program; 2010 May. Report No.: DOE/EE-0332.
- U.S. DOE. Review and evaluation of immobilized algae systems for the production of fuels from microalgae. Alexandria (VA): Solar Energy Research Institute; 1985 Nov. Report No.: SERI/STR-231-2798. Prepared for the U.S. Department of Energy.
- U.S. EPA. Wastewater technology fact sheet: Facultative lagoons. Washington DC: U.S. Environmental Protection Agency; 2002 Sep. Report No.: EPA 832-F02-014.
- Vandamme D, Foubert I, Meesschaert B, Muylaert K. Flocculation of microalgae using cationic starch. *J Appl Phycol* 2009; 22: 525-530.
- Vanhoutte J, Vanhoutte K. Modular continuous production of micro-organisms. United States patent application US 20090162920. 2009a.
- Vanhoutte K, Vanhoutte J. Method for harvesting algae or plants and device used thereby. Patent cooperation treaty application WO 2009037355. 2009b.
- Van Walsem J, Morgan F, Stuart J, Rainer P, Mcintire JR, Michonski SA, et al. Solar biofactory, photobioreactors, passive thermal regulation systems and methods for producing products. Patent cooperation treaty application WO 2010068288. 2010.
- Vasudevan PT, Briggs M. Biodiesel production—current state of the art and challenges. *J Ind Microbiol Biotechnol* 2008; 35: 421-430.
- Vick B. Glyphosate applications in aquaculture. United States patent application US 20100022393. 2010.
- Vick B, Fleischer D. Use of 2-hydroxy-5-oxoproline in conjunction with algae. United States patent application US 20090325270. 2009.
- Vonshak A, Richmond, A. Mass production of the blue-green alga *Spirulina*: An overview. *Biomass* 1988; 15(4): 233-247.
- Wei Q, Hu Z, Li G, Xiao B, Sun H, Tao M. Removing nitrogen and phosphorus from simulated wastewater using algal biofilm technique. *Frontiers Environ Sci Eng China* 2008; 2: 446–451.
- Weiss H. Method for growing photosynthetic organisms. United States patent application US 20080220486. 2008.

- Weissman J, Radaelli G. Systems and methods for maintaining the dominance of *Nannochloropsis* in an algae cultivation system. United States patent application US 20100183744. 2010.
- Weissman J, Radaelli G, Rice D. Systems and methods for maintaining the dominance and increasing the biomass production of *Nannochloropsis* in an algae cultivation system. United States patent application US 20100196995. 2010.
- Wells F, Snyder KM. Algaculture system for biofuel production & methods of production thereof. United States patent application US 20100068779. 2010.
- Whitton NM. Large scale photobioreactor using flexible materials, large bubble generator, and unfurling site set up method. United States patent application US 20080286851. 2008.
- Wilkerson BD, Chen JC, Pulse J, Guschin A, Weaver M. Systems, devices, and methods for biomass production. United States patent application US 20090047722. 2009.
- Wilkerson BD, Watters W. Illumination systems, devices, and methods for biomass production. United States patent application US 20090148931. 2009.
- Wilkie AC, Mulbry WW. Recovery of dairy manure nutrients by benthic freshwater algae. *Bioresour Technol* 2002; 84: 81-91.
- Willson B, Babbitt G, Turner C, Levitin P, Weyer-Geigel K, Ettinger A, et al. Diffuse light extended surface area water-supported photobioreactor. United States patent application US 20080160591. 2008.
- Willson BD, Turner CW, Babbitt GR, Letvin PA, Wickrmasinghe SR. Permeable membranes in film photobioreactors. United States patent application US 20090305389. 2009.
- Winsness DJ, Krablin R, Kreisler KE. Method of converting a fermentation byproduct into oxygen and biomass and related systems. United States patent application US 20070196892. 2007.
- Woertz I, Feffer A, Lundquist T, Nelson Y. Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. *J Environ Eng* 2009; 135: 1115–1122.
- Wolfstein K, Stal LJ. Production of extracellular polymeric substances (EPS) by benthic diatoms: effect of irradiance and temperature. *Marine Ecol Prog Ser* 2002; 236: 13–22.

- Wood A. A simple wastewater treatment system incorporating the selective cultivation of a filamentous algae. *Water Sci Technol* 1987; 19: 1251–1254.
- Woods RP, Legere E, Moll B, Unamunzaga C, Mantecon E. Closed photobioreactor system for continued daily in situ production, separation, collection, and removal of ethanol from genetically enhanced photosynthetic organisms. United States patent US 7682821. 2010.
- Wu BC, Stephen D, Morgenthaler GE, Jones DV. Systems and methods for producing biofuels from algae. United States patent application US 20100081835. 2010a.
- Wu BC, Stephen D, Morgenthaler GE, Jones DV. Systems and methods for producing biofuels from algae. United States patent application US 20100077654. 2010b.
- Wu Y, Wu X, Berzin I. Removal of ash and sulfur dioxide in flue gas with a combined multifunction impinging stream gas-liquid reactor. Patent cooperation treaty application WO 2007038605. 2007.
- Wuertz S, Bishop PL, Wilderer PA. *Biofilms in wastewater treatment: an interdisciplinary approach*. London: IWA Publishing; 2003.
- Yen HW, Brune DE. Anaerobic co-digestion of algal sludge and waste paper to produce methane. *Bioresour Technol* 2007; 98: 130-134.
- Youngs RO, Cook JR. Method and apparatus for separating particles from a liquid. United States patent application US 20100224574. 2010.
- Zeevalkink J, Kelderman P, Visser D, Boelhouwer C. Physical mass transfer in a rotating disc gas-liquid contactor. *Water Res* 1979; 13: 913-919.
- Zijffers JF, Janssen M, Tramper J, Wijffels RH. Design process of an area-efficient photobioreactor. *Mar Biotechnol* 2008; 10: 404-415.

CHAPTER 3
ROTATING ALGAL BIOFILM REACTOR AND SPOOL
HARVESTER FOR WASTEWATER TREATMENT
WITH BIOFUELS BY-PRODUCTS

1. Introduction

Algae are a promising source of feedstock for the production of biofuels and bioproducts, providing a source of triacylglycerides and free fatty acids to produce biodiesel (Sheehan et al., 1998). Harvested algae may also be fed to an anaerobic digester for methane production (Golueke et al., 1957; Gunaseelan, 1997), and/or used as a fertilizer, soil amendment, or livestock feed (Mulbry et al., 2008; Wilkie and Mulbry, 2002). Before these algae based biofuels and bioproducts can be utilized, suspended algae must be separated from the growth liquid. However, harvesting of suspended algae in a cost-effective way has proven to be difficult (Molina Grima et al., 2003; Uduman et al., 2010).

Using algae for tertiary treatment of wastewater also has several advantages, and many reports have discussed the need of integrating wastewater treatment and algae production (Lundquist et al., 2010; Pittman et al., 2011; U.S. DOE, 2010). In the United States, there are more than 7,000 facultative lagoon systems (U.S. EPA, 2002), representing a largely unused resource for algae production. Photoautotrophic biological assimilation of wastewater nutrients can be less expensive, more efficient, and ecologically safer than physical/chemical removal processes (Oswald, 2003). Compared to bacterial nitrification/denitrification operations, where the majority of the nitrogen is

removed as N₂ gas, algal treatment preserves nitrogen compounds in the produced biomass (Roeselers et al., 2007). However, acceptable nutrient levels in wastewater effluent cannot be achieved without sufficient harvest of the algal crop, and as with algae production for biofuels, this remains a challenge.

The majority of information on algae production for wastewater treatment or biofuels is related to suspended algae. Suspended algal cultures are most often grown using open raceway ponds or closed tubular reactors. Each approach has relative advantages and disadvantages, and several reviews have discussed these methods in detail (Carvalho et al., 2006; Chisti, 2007; Shen et al., 2009). Regardless of the relative benefits and limitations of the various approaches to suspended algae cultivation, all involve substantial challenges of biomass harvesting that can account for up to 30% of total costs (Molina Grima et al., 2003). Because of the challenges associated with harvesting suspended algae, there is interest in using surface-attached algae biofilm systems that are naturally concentrated and more readily harvestable (Hoffmann, 1998). Biofilm systems could reduce downstream processing costs related to algae harvesting; however, the approach is less studied than methods utilizing suspended algae.

In one attachment method, algae cells are immobilized using a matrix of carrageenan or alginate (Chevalier et al., 2000; Hameed and Ebrahim, 2007), but the high cost of the polymeric matrix prohibits use of this technique at large scales (Hoffmann, 1998). A few bench scale studies have used rotating disks of aluminum (Torpey et al., 1971) or polystyrene (Przytocka-Jusiak et al., 1984) to grow algae biofilms and reduce nitrogen and phosphorus levels in wastewater. The Algal Turf Scrubber grows filamentous algae on a plastic mesh by intermittently passing water over the surface

(Adey et al., 1993). It has been used at full scale for water treatment applications, but the filamentous algae product may not be as useful for biofuels as other species (Mulbry et al., 2008). More recently, Johnson and Wen (2010) designed a laboratory scale rocker system with a polystyrene bottom surface for attachment of a *Chlorella* culture grown in dairy wastewater. The attached culture gave higher yields than a suspended culture grown under similar conditions, and both cultures had similar lipid content. Beyond these reports, research relating to algae biofilm production is limited. Developing a scalable algae biofilm production and harvesting system suitable for wastewater treatment and biofuel production is needed.

The City of Logan, located in northern Utah, maintains a regional wastewater treatment plant (WWTP) consisting of 460 acres of open lagoons. The current load of total phosphorus (TP) entering downstream waterways is in excess of the determined loading capacity. As the largest point source contributor, the Logan Regional plant is required to reduce effluent TP levels by 48-62% (UDEQ, 2009), and levels may need to be brought from 4.1 mg l⁻¹ to 1.0 mg l⁻¹ and perhaps as low as 0.1 mg l⁻¹ (UDWQ, 2010). The City of Logan is exploring the possibility of using a full scale algae production and harvesting process to remove the phosphorus and nitrogen, with the goal of using the biomass as feedstock for the production of biodiesel, biomethane, and other bioproducts.

An algal biofilm system could be a part of achieving these goals. Bacterial contamination of unialgal cultures has been shown to enhance biofilm colonization by one to two orders of magnitude (Hodoki, 2005; Holmes, 1986). In addition, cells saturated with phosphate have a higher tendency to flocculate or adhere to a surface due to increased hydrophobicity (Qureshi et al., 2005). It is apparent that the algae and

bacteria mixed cultures and phosphate levels present in the Logan Regional WWTP as well as other WWTPs may be particularly useful for the development of an algal biofilm process.

The aim of this study was to develop an algae biofilm production and harvesting system for wastewater treatment with biofuels by-products using the Logan Regional WWTP as a testing and evaluation site. The objectives were:

1. Design a scalable algal biofilm production system and optimize key parameters.
2. Design a harvesting system to effectively harvest algae biomass.
3. Determine the wastewater treatment capability of the algal biofilm production and harvesting system.

To achieve the benefits of scalability, compactness, and good gas exchange, a Rotating Algal Biofilm Reactor (RABR) was designed. The RABR consists of a cylinder provided with a growth surface partially submerged in wastewater. The cylinder is rotated to alternately expose the growth surface to the wastewater and the air. Rotating biological contactors (RBCs) are used to grow bacterial biofilms for secondary wastewater treatment and are valued for their efficiency, compact design, good gas exchange, and high tolerance to shock loads (Patwardhan, 2003). The RABR design aims to maintain these benefits while optimizing for algae growth and tertiary wastewater treatment instead of bacterial growth and secondary treatment.

Reducing photoinhibition and photolimitation is another major design criterion for any photobioreactor, and care must be taken to ensure that cells do not reside too long in either the illuminated or dark zones (Chisti, 2007). Testing of light/dark cycles has

shown that maximum growth can continue even with a considerable dark period (Janssen et al., 2000). Light/dark cycling inherent in the rotation of the RABR can make use of the dark period, allowing the cylindrical construction of the RABR to support more growth area per illuminated/aperture surface area than flat growth surface designs.

2. Methods

2.1. Growth Substrata Test

Bench scale RABR units and water tanks were constructed using 3 inch (7.6 cm) diameter PVC pipe and acrylic plastic. The tanks were built to be 48 inches (121.9 cm) long, 6 inches (15.2 cm) wide, and 4 inches (10.2 cm) deep. Eight liters of wastewater effluent from the Logan Regional WWTP were added to each tank as seeding media. The RABRs were 40% submerged and operated at 4.8 rpm.

Eight substrata that qualitatively showed the ability to support algal attachment were chosen for further quantitative testing. Nylon, polypropylene, cotton, acrylic, and jute were tested in cord construction, and polyester, high thread cotton, and low thread cotton were in sheet construction. All cord materials were 1/4 inch (0.64 cm) in diameter except jute, which was 1/8 inch (0.32 cm) in diameter. Each type of material covered 36 square inches (232 cm²) of the reactor surface.

The concentration of total dissolved P (TDP) was measured and brought to 5 mg l⁻¹ using the Bristol's medium ratio of KH₂PO₄ to K₂HPO₄. Total dissolved N (TDN) was also measured and additional N was added in the form of NaNO₃ until the Redfield ratio N to P ratio of 16 to 1 (Stumm and Morgan, 1981) was reached. The reactors were

operated in fed batch mode with N and P added every 48 hours to bring concentrations back to these levels.

Plant growth fluorescent lights (plant & aquarium F40, General Electric) were placed over the reactors, and a light cycle of 14 hours on, 10 hours off was used throughout the experiment. Photosynthetically active radiation (PAR) was measured using an MQ-200 quantum meter (Apogee Instruments, Logan, UT), and had a value of $290 \mu\text{mol m}^{-2}\text{s}^{-1}$ at the uppermost surface of the RABR cylinders.

Harvested biofilms were dried at 105°C for 24 hours. All chemical analyses were performed using Hach analysis kits (Hach Company, Loveland, CO) in accordance with Standard Methods for the Examination of Water and Wastewater (Eaton et al., 2005).

2.2. Harvester Design

Biofilms grown on sheet substrata were harvested using a scraper blade. To harvest biofilms grown on cord substrata, a scalable spool harvester was designed and built. During harvesting, one end of the cord is threaded through an adjustable diameter scraper and then through a pulley system before it is reattached to the reactor in such a way that as the cylinder rotates, the entire length of cord is unwound, passed through the scraper, and rewound onto the reactor. The harvester is moved along the length of the reactor cylinder to prevent the rewinding cord from layering on top of itself.

2.3. Comparison to Suspended Cultures

Using the bench scale units described in section 2.1 with 1/4 inch (0.64 cm) diameter solid braid cotton cord as growth substratum, another experiment was designed to directly compare biofilm growth to suspended culture growth. Suspended cultures

were grown in reactor tanks of the same dimensions with the same light and nutrient conditions. The same wastewater sample was used to seed each type of reactor, and power input for mixing the suspended cultures was the same as power input for rotating the biofilm reactors at 4.8 rpm.

A 14 hour on, 10 hour off light cycle was used, and nutrients were added every 48 hours as in the substrata experiment. TDN and TDP averaged 26.2 mg l^{-1} and 3.7 mg l^{-1} , respectively, corresponding to levels at the Logan Regional WWTP. Biofilm mass was determined as described in section 2.1, and growth in the suspended culture reactors was determined by measuring the increase with time of the total suspended solids (TSS) according to Standard Method 2540 D (Eaton et al., 2005). After each biofilm harvest, the seeded cord substrata were reloaded onto the reactor to determine the secondary regrowth curve.

2.4. RABR-Enhanced Raceway Pond

Using white acrylic plastic, two outdoor tanks were constructed to be 96 inches (244 cm) long, 48 inches (122 cm) wide, and 16 inches (41 cm) deep. One tank was constructed to operate as a raceway pond, and the other constructed as a RABR modified raceway. The RABRs were constructed using five plastic 15 gallon (57 l) drums measuring 23 inches (58 cm) high and 16 inches (41 cm) in diameter. A length of 350 feet (107 m) of 1/4 inch (0.64 cm) diameter solid braid cotton cord was wound around each RABR along with randomly placed 10 feet (3.0 m) lengths for sampling biofilm growth progression.

The RABRs were 40% submerged and operated at 5.4 rpm, corresponding to a peripheral velocity of 0.38 feet per second (0.12 m s^{-1}). Each tank was filled with 535 l of wastewater. The paddlewheels for each tank consisted of four paddles and were operated at 5.4 rpm using 0.08 hp (0.06 kW) AC gearmotors (Leeson Motors, Grafton, WI).

To better represent conditions of continuous operation, the tanks were operated for 10 days to establish the biofilm base. The cords were then passed through the harvester and reloaded, and the tanks were drained and refilled before testing began. The experiment was conducted through the month of August.

For this experiment, TDP levels were brought to 3.5 mg l^{-1} using the Bristol's media ratio of KH_2PO_4 to K_2HPO_4 as before. If TDP levels of the wastewater used to inoculate the reactors were higher than 3.5 mg l^{-1} , no supplementation was made. Based on elemental analysis of the algae and on N and P uptake data from the previous experiments, an N to P ratio of 12 to 1 was chosen (19 mg l^{-1} TDN). Industrial grade urea was used as N source.

The reactors were operated until the criteria of $\text{TDP} < 0.5 \text{ mg l}^{-1}$ and $\text{TDN} < 3 \text{ mg l}^{-1}$ were met. Afterwards, for the RABR enhanced raceway, a new 535 l batch was started. For the regular raceway, leaving a 10-15% seed culture was found to eliminate the lag phase for the new batch.

Biofilm growth was estimated by harvesting the sacrificial 10 feet (3.0 m) portions of rope and extrapolating the results to the entire tank with the assumption that biofilm growth was uniform along the reactor. Harvested samples were lyophilized prior to weighing. Suspended algae in the raceway without RABRs were harvested using a Sharples T-1 continuous centrifuge.

The fatty acid methyl ester (FAME) potential of samples was determined using an acid-catalyzed in-situ transesterification process developed at Utah State University (Nelson, 2010). After transesterification, percent FAME was quantified by gas chromatography using relevant methyl ester standards.

2.5. Scale-Up Test

A scaled up RABR unit was designed to be suitable as a retrofit for facultative oxidation ponds or oxidation ditches. The unit was constructed using two aluminum Powerroll Wheels (Wade Rain, Tualatin, OR) measuring 76 inches (193 cm) in diameter. The wheels were placed 5 feet (1.5 m) apart on an aluminum shaft and ten sections of aluminum strip stock were attached to the outer circumference of each wheel, making the effective portion of the reactor a decagonal prism. Cotton cord measuring 1/4 inch (0.64 cm) in diameter with solid braid construction (WebRigging Supply, Lake Barrington, IL) was the most economical size available to cover the reactor surface area.

The scale-up experiment was conducted from mid-October to early November. The reactor was operated at 1.2 rpm to give a peripheral velocity of 0.38 feet per second (0.12 m s^{-1}) in a continuous flow channel measuring 6 feet (1.8 m) wide with water depth of 3 feet (0.9 m). The measurement area containing the reactor had a volume of approximately 8,000 l. With a flow rate of 3 gal min^{-1} (11.4 l min^{-1}), the hydraulic residence time between upstream and downstream sampling points was approximately 11.2 hours. Measurements and samples were taken upstream and downstream of the reactor to determine differences in TDP and TDN. Biofilm samples were harvested and

analyzed for FAME potential as described above. Figure 3 shows schematics of the experimental RABR units used in this study and the spool harvesting operation.

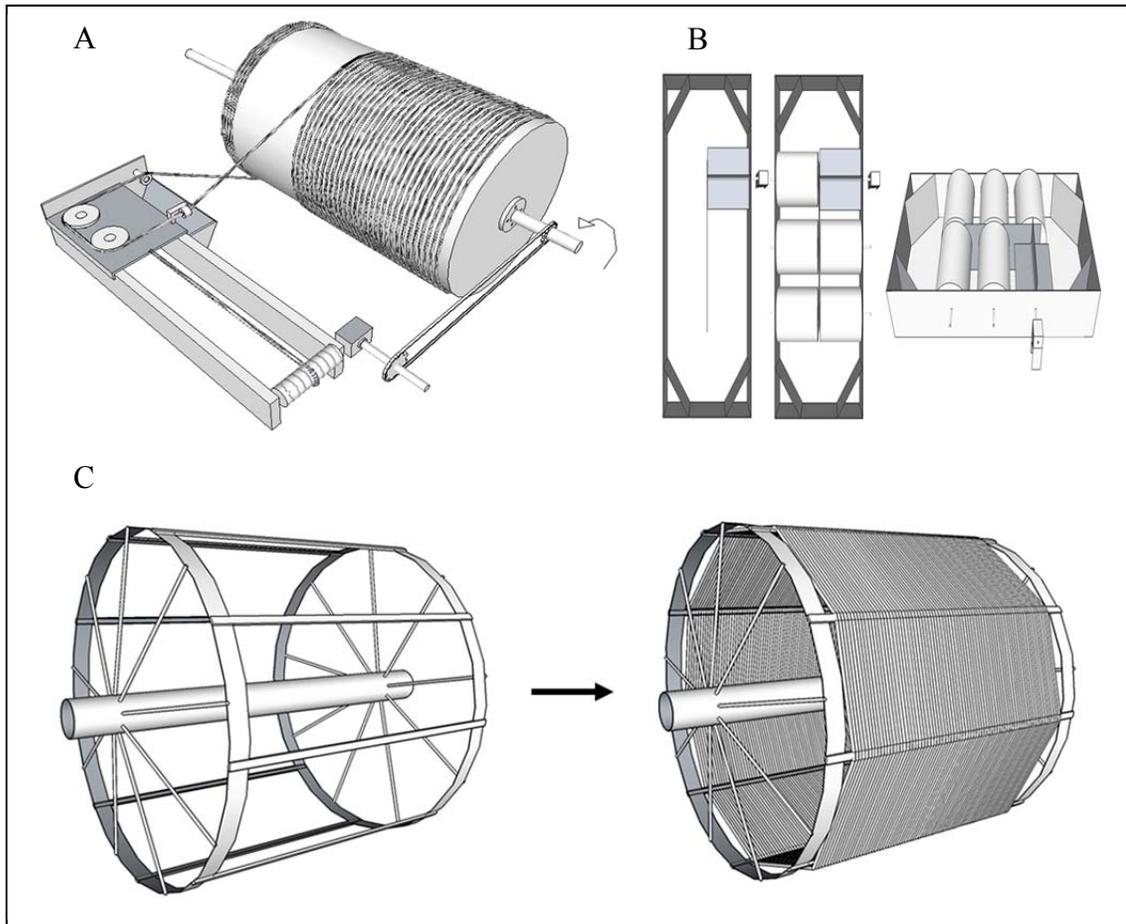


Figure 3: Schematics of experimental units used in this study. (A) Spool harvesting operation. (B) Plan view of raceway and RABR-enhanced raceway. (C) Pilot scale RABR framework and with substratum.

2.6. Statistical Analysis

All statistical analysis of collected data was performed using SAS software (Cary, NC). The substrata test was analyzed as a completely randomized design (CRD) with two crossed factors. The time factor had three levels and the substratum level had eight treatments. There were three replications for each factor combination for a total of 72

measurements. Model assumption of normally distributed residuals and constant variance were verified and no transformation of the data was necessary. Analysis of variance (ANOVA) gave F-values and p-values indicating a rejection of the null hypothesis that all treatments were similar. Post-hoc analysis using REGWQ grouping was performed to determine which substratum material produced significantly more biomass than all other materials tested.

The suspended culture comparison test was also analyzed as a CRD with two crossed factors. The time factor had six levels and the growth type factor had three levels consisting of: suspended growth, initial biofilm growth, and secondary biofilm growth. ANOVA was used to show that the growth type factor was significant, and REGWQ grouping at different time points was used to show when different growth types were significantly different.

The outdoor biofilm enhanced raceway test was analyzed as a repeated measures experiment with reactor type (regular raceway or RABR-enhanced raceway) as grouping factor. Three growth cycles were completed for each reactor type. The scale-up tests were performed to determine scalability of the RABR design and the aim was not to compare results to a relevant separate treatment.

3. Results & Discussion

3.1. Growth Substrata Test

Figure 4 summarizes the results of the substrata test on the basis of pond surface area or plan surface area of the tank. Polypropylene rope and nylon rope did not achieve any harvestable growth. The cellulose based natural materials performed better than any

of the synthetic polymers. A lag phase of ten days occurred before any harvestable biofilm growth was seen. Cotton cording reached a density of 56 g m^{-2} on a dry weight basis (DW), statistically more than any other material tested.

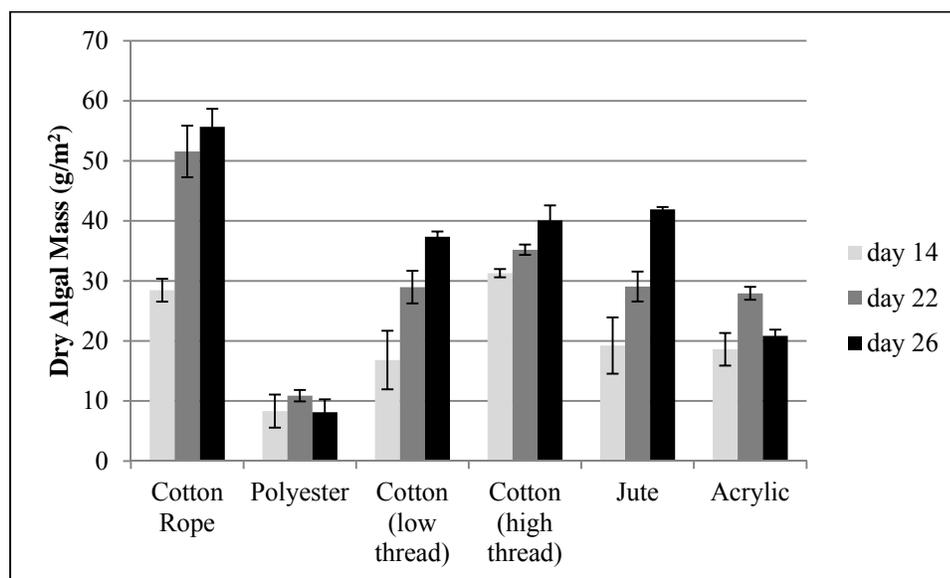


Figure 4: Algal biofilm formation on selected substrata for laboratory scale RABR.

As the results show, the attachment surface, or substratum, is a very important parameter in biofilm development. Today, most conventional RBC media disks for bacterial biofilms are made of polyethylene (Patwardhan, 2003). In this study, polyethylene and other synthetic polymers produced relatively low algae attachment and biofilm growth. Other research specific to surfaces that promote algae attachment are limited. Johnson and Wen (2010) found greater *Chlorella* attachment to polystyrene foam than to cardboard, polyethylene fabric, or loofah sponge, with polystyrene foam reaching a density of 26 g m^{-2} DW.

3.2. Comparison to Suspended Cultures

Although cotton cording gave statistically higher yields than any of the other materials tested, a direct comparison to suspended cultures grown under the same conditions was needed in order to better determine the potential of algae biofilm production using the RABR. A direct comparison of suspended growth production to biofilm production can often be difficult because of differences in the basis by which production values are reported. Attached growth is often reported on a surface area basis while suspended growth is reported on a volumetric basis. If full details concerning volume used and reactor dimensions are not described, conversion and direct comparison of the reported values is not possible. Because this experiment used tanks of the same volume and geometry, a direct comparison could be made by measuring the total biomass per reactor and dividing by the tank surface area or volume.

Figure 5 shows the growth curves of the initial biofilms, secondary biofilms, and suspended cultures on the basis of pond surface area and volume. It can be seen that the RABRs produced higher yields than the suspended reactors, and that the biofilm grows at a much faster rate after the initial harvest. This is most likely due to the residual biomass remaining on the substratum after harvesting that performed as a seed culture. The secondary growth curve more accurately represents the productivity of the reactor when operated continuously. The initial biofilm growth reached a density of $58 \text{ g m}^{-2} \text{ DW}$, similar to the results of the substrata test. The regrowth was able to reach a much higher density of $99 \text{ g m}^{-2} \text{ DW}$ after 18 days, corresponding to a bench scale productivity of $5.5 \text{ g m}^{-2} \text{ day}^{-1} \text{ DW}$.

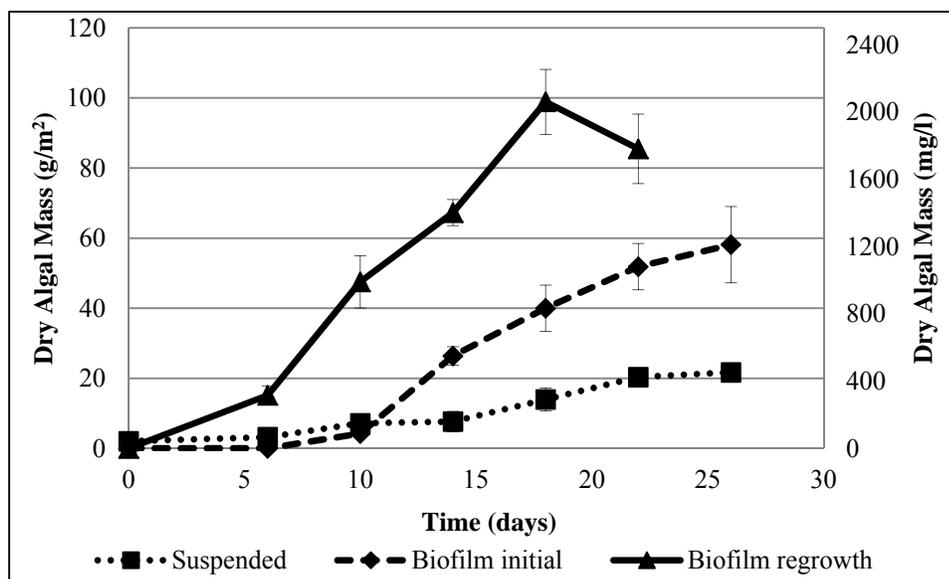


Figure 5: Growth curves of the initial biofilms, secondary biofilms, and suspended cultures at laboratory scale.

3.3. RABR-Enhanced Raceway Pond

Algae production by the raceway and RABR-enhanced raceway is shown in Figure 6. The maximum productivity of the RABR-enhanced raceway was $20 \text{ g m}^{-2} \text{ day}^{-1}$ DW occurring after 9 days of growth. The maximum productivity of the regular raceway was $7.4 \text{ g m}^{-2} \text{ day}^{-1}$ DW, occurring after 7 days of growth. All biomass harvested from the RABR-enhanced raceway was in the biofilm form, as the initial TSS was reduced by 90% after four days, leaving no harvestable algae in the suspended phase. Table 7 shows the reduction of TSS in the RABR-enhanced raceway. For wastewater treatment, the ability of the RABR-enhanced raceway to directly reduce TSS levels is an additional benefit that can aid in meeting TSS discharge limits. Mass balance calculations show that the attachment of suspended algae to the RABRs only accounts for 1.4-2.8% of the total biomass produced. The majority is produced from continued growth after reactor operation begins.

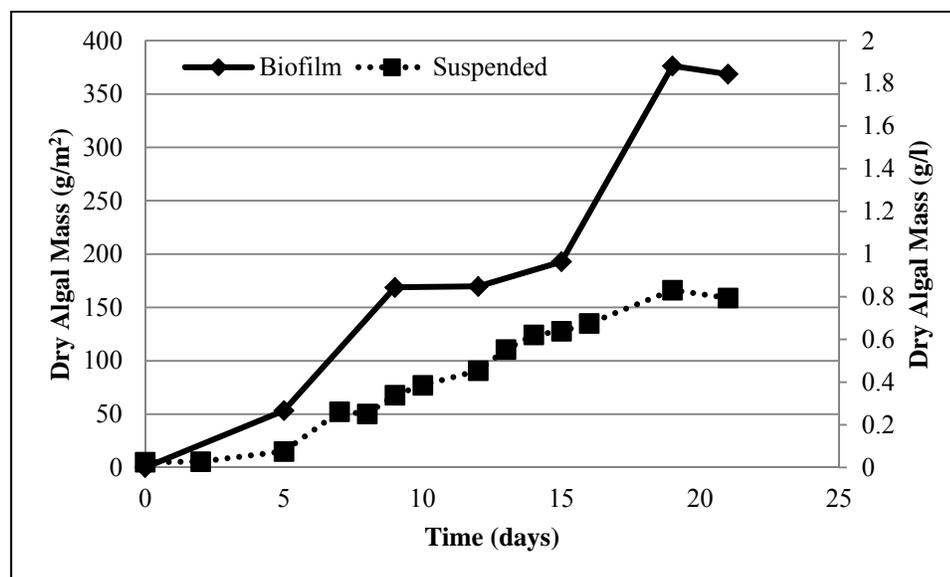


Figure 6: Algae biofilm production of RABR-enhanced raceway and suspended algae production of regular raceway in bench-scale (outdoor) units.

Table 7: Total suspended solids concentration and reduction in RABR-enhanced raceway.

Time (days)	TSS (mg l ⁻¹)	TSS Reduction (%)
0	27.3 ± 4.6	0
1	21.2 ± 5.3	22.3 ± 9.4
2	15.5 ± 6.4	45.6 ± 12.6
3	6.5 ± 2.1	75.3 ± 2.8
4	4.0 ± 0.8	86.7 ± 1.9

The concentration of harvested biofilms ranged from 12-16% solids, which is comparable to performance using centrifugation (Uduman et al., 2010). After the *in-situ* transesterification procedure, the FAME content of the biofilms and suspended algae on a dry weight basis measured 11.2-12.4% and 11.4-13.8%, respectively. Combining these figures with the biomass productivity figures gives maximum FAME productivities of 2.1-2.3 g m⁻² day⁻¹ for the RABR-enhanced raceway and 0.9-1.0 g m⁻² day⁻¹ for the

regular suspended culture raceway. Figure 7 shows the algae production of the RABR-enhanced raceway and the corresponding FAME content over time.

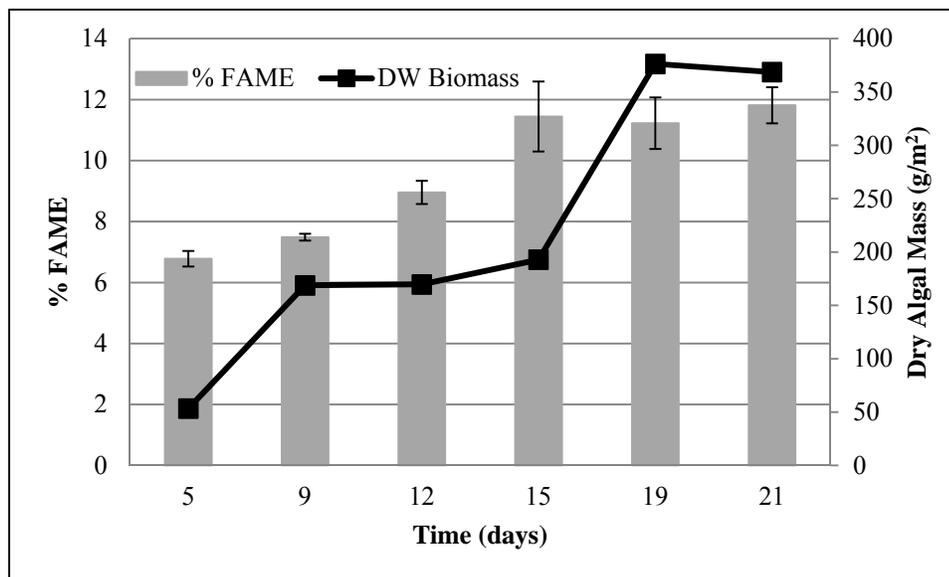


Figure 7: Algae biofilm production of a RABR-enhanced raceway and corresponding FAME content after in-situ transesterification in bench-scale (outdoor) unit.

Visual characterization using microscopy revealed that the suspended cultures of the regular raceway were dominated by species of *Chlorella* and *Scenedesmus*, although some *Pediastrum* were present as well. The biofilm cultures of the RABR-enhanced raceway contained a variety of algae, including *Pediastrum*, *Chlorella*, *Nitzschia*, *Navicula*, *Crucigenia*, *Synedra*, and various *Diatoma*.

3.4. Scale-Up Test

Figure 8 shows the TDN and TDP levels upstream and downstream of the scaled-up RABR. Using the differences to determine uptake by the biofilm, the maximum uptake rates were $4.1 \text{ g m}^{-2} \text{ day}^{-1}$ and $22.1 \text{ g m}^{-2} \text{ day}^{-1}$ for TDP and TDN, respectively. This gives an N to P ratio of 12 to 1. Maximum productivity occurred after 12 days of

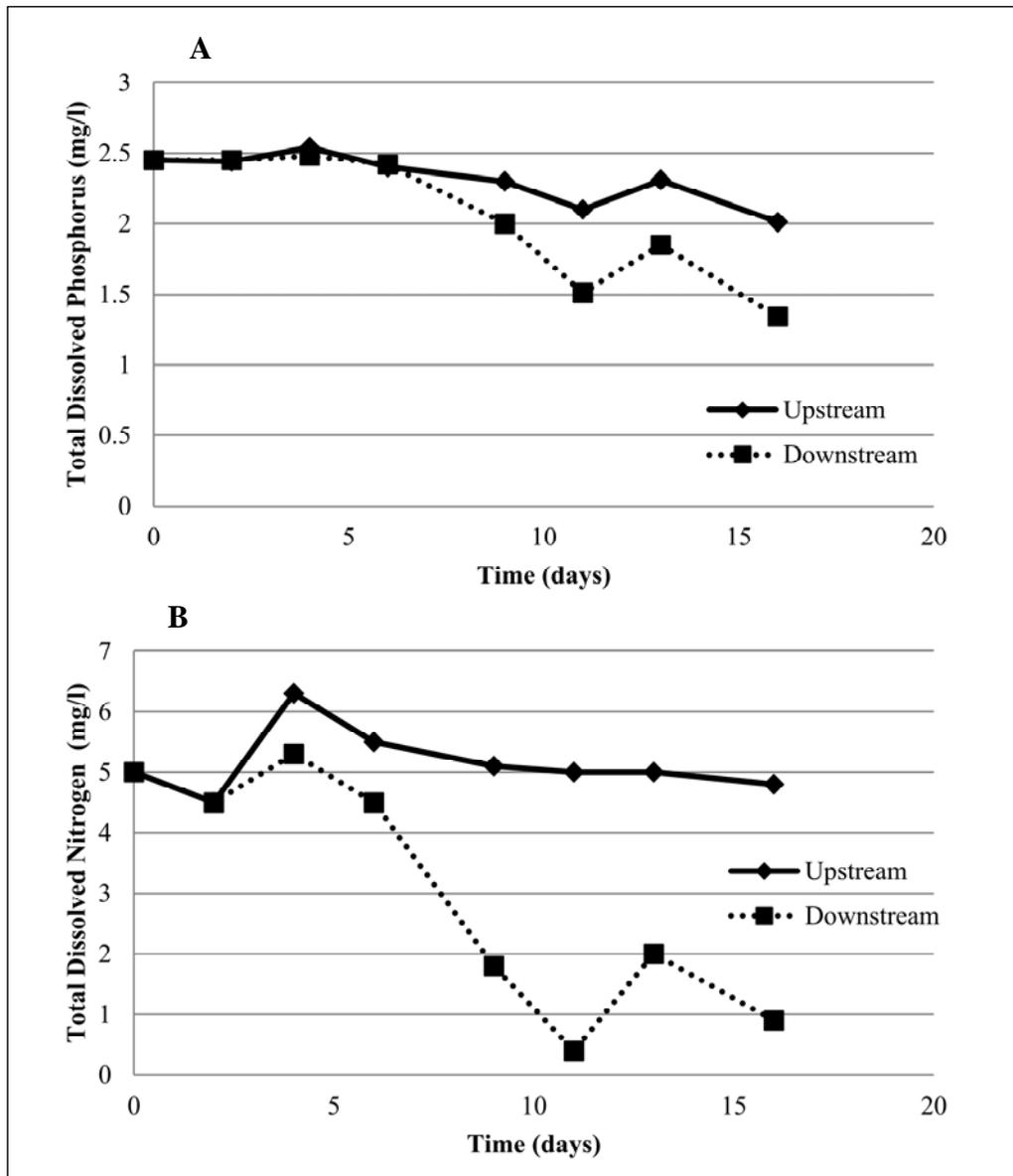


Figure 8: Nutrient concentrations of wastewater upstream and downstream of pilot scale RABR. (A) Total Dissolved Phosphorus. (B) Total Dissolved Nitrogen.

growth, when the total biomass of the RABR measured $377 \text{ g m}^{-2} \text{ DW}$, corresponding to a productivity of $31 \text{ g m}^{-2} \text{ day}^{-1} \text{ DW}$. Because nutrient loading was approximately the same, the high productivity of the scaled-up RABR relative to the initial tests is likely due to other factors such as seasonal variations and changes in the mixed culture of algae in the wastewater. Regardless, the performance of the RABR at a field relevant scale in the late fall season shows potential for beneficial use in the fields of wastewater treatment and biofuels production.

3.5. Energy Balance

For energy input, the scaled-up RABR unit required approximately 100-150 inch pounds (11-17 $\text{N}\cdot\text{m}$) of torque to rotate at 1.2 rpm (0.13 rad s^{-1} used for calculation). Using 200 inch pounds (23 $\text{N}\cdot\text{m}$) as an example, and assuming an electric motor efficiency of 0.7, the power requirement for rotation can be calculated as 4.1 W for the entire unit or 1.4 W m^{-2} . This value ignores the water pumping requirements that would already be necessary to a wastewater treatment plant and represents only the additional power required for RABR implementation. Because biofilm harvesting adds a negligible amount of friction for a short time, it does not add to the power demand. The heating value of algae grown at the wastewater plant has been measured as 21.4 kJ g^{-1} , and this value was multiplied by the algae productivity of $31 \text{ g m}^{-2} \text{ day}^{-1}$ to give an energy output of 7.7 W m^{-2} . Subtracting from the input requirement gives a net positive output of 6.3 W m^{-2} .

The paddlewheel of a $1,000 \text{ m}^2$ raceway pond producing $25 \text{ g m}^{-2} \text{ day}^{-1}$ requires 0.2 kWh kg^{-1} produced algae (Collet et al., 2011), leading to an area based power

requirement of 0.2 W m^{-2} . This is lower than the area based power requirement to rotate the pilot scale RABR (1.4 W m^{-2}), but the benefit is lost when harvesting of the suspended algae is considered. As an example, the raceway model was given a depth of 30 cm (Collet et al., 2011), giving a total volume of 300 m^3 . A power requirement of 1 kWh m^{-3} for centrifugation (Molina Grima et al., 2003) and an assumed residence time of 5 days (Hoffmann, 1998) will contribute an additional power requirement of 2.5 W m^{-2} . When harvesting is considered, the total power requirement for the raceway of 2.7 W m^{-2} is approximately double the requirement for the RABR.

3.5. Performance Comparison

Table 8 compares the biomass productivities and FAME/lipid productivities of the reactors of this study to other values reported in the literature. Table 9 compares the spool harvesting method for the algae biofilms of this study to other common suspended algae harvesting methods. Care should be taken when comparing geographically disperse studies with results that may not have had similar nutrient loads, weather, or algae culture composition. Nevertheless, the production and harvesting results of this study compare well to other reported values, suggesting that the RABR with spool harvester is a feasible approach to the production and harvesting of algae in wastewater.

4. Conclusions

Results of the study fulfilled the original objectives. The RABR design is capable of effective algal biofilm growth, and has potential for implementation at full scale. The pilot scale RABR achieved a productivity of $31 \text{ g m}^{-2} \text{ day}^{-1} \text{ DW}$. The algal biofilms grown on the RABR were able to reduce nutrient concentrations in the wastewater, with

P and N removal rates of $4.1 \text{ g m}^{-2} \text{ day}^{-1}$ and $22.1 \text{ g m}^{-2} \text{ day}^{-1}$, respectively. The spool harvesting method effectively removed the biofilms from the cotton cord substratum, yielding a concentrated product of 12-16% solids. The FAME content of biofilms after an in-situ transesterification procedure was 11.2-12.4%, giving a FAME productivity of 2.1-2.3 $\text{g m}^{-2} \text{ day}^{-1}$. Considering that lipid accumulation was not optimized in this study, these values represent a promising baseline for future improvements. Results of this study indicate that the RABR with spool harvester represents a promising approach to the production and harvesting of algae in wastewater. No apparent constraints are currently identified for scale up to full scales comparable to RBCs, including materials and power. Construction and operation of a full-scale RABR with spool harvester represents the next phase of this research.

Table 8: Comparison of algae biomass productivities and FAME/lipid productivities.

Reactor Type	Biomass Productivity (g m ⁻² day ⁻¹)	FAME/Lipid Productivity (g m ⁻² day ⁻¹)	Wastewater Type	Reference
Biofilm Based				
RABR	20-30	2.2 (FAME)	Municipal	This study
Polystyrene disks	2.2	–	Industrial	Przytocka-Jusiak et al. (1984)
Polycarbonate flow lanes	2.9	–	Municipal	Guzzon et al. (2008)
Polystyrene rocker system	2.59	0.2 (lipids)	Dairy	Johnson and Wen (2010)
Algal turf scrubber	5-20	–	Dairy	Wilkie and Mulbry (2002)
Suspended Based				
Raceway	7.4	1.0 (FAME)	Municipal	This study
Open tank with air & CO ₂ sparging	13	2.8 (lipids)	Dairy	Woertz et al. (2009)
Raceway	10-25	–	Not wastewater	Shen et al. (2009)
Tubular reactor	35-48	–	Not wastewater	Chisti (2007)

Table 9: Comparison of harvesting methods.

Harvesting Method	Solids Concentration (after harvest)	Recovery	Reference
Spool harvester	12-16%	70-85% ^a	This study
Centrifugation	12-22%	>90%	Shen et al. (2009), Uduman et al. (2010)
Tangential filtration	5-27%	70-90%	Uduman et al. (2010)
Sedimentation	0.5-3%	10-90%	Shen et al. (2009)
Dissolved air flotation	3-6%	50-90%	Shen et al. (2009), Uduman et al. (2010)

^a According to stoichiometric calculations based on P uptake using a formula of C₁₀₆H₁₈₁O₄₅N₁₆P (Stumm and Morgan, 1981)

References

- Adey W, Luckett C, Jensen K. Phosphorus removal from natural waters using controlled algal production. *Restor Ecology* 1993; 1: 29-39.
- Carvalho AP, Meireles LA, Malcata FX. Microalgal reactors: a review of enclosed system designs and performances. *Biotechnol Prog* 2006; 22: 1490-1506.
- Chevalier P, Proulx D, Lessard P, Vincent WF, De La Noiüe J. Nitrogen and phosphorus removal by high latitude mat-forming cyanobacteria for potential use in tertiary wastewater treatment. *J Appl Phycol* 2000; 12: 105–112.
- Chisti Y. Biodiesel from microalgae. *Biotechnol Adv* 2007; 25: 294-306.
- Collet P, Hélias A, Lardon L, Ras M, Goy RA, Steyer J-P. Life-cycle assessment of microalgae culture coupled to biogas production. *Bioresour Technol* 2011; 102: 207-214.
- Eaton AD, Franson MAH, Association AWW, Federation WE. Standard methods for the examination of water & wastewater. American Public Health Association; 2005.
- Golueke CG, Oswald WJ, Gotaas HB. Anaerobic digestion of algae. *Appl Microbiol* 1957; 5: 47-55.
- Gunaseelan VN. Anaerobic digestion of biomass for methane production: A review. *Biomass Bioenergy* 1997; 13: 83-114.
- Guzzon A, Bohn A, Diociaiuti M, Albertano P. Cultured phototrophic biofilms for phosphorus removal in wastewater treatment. *Water Res* 2008; 42: 4357–4367.
- Hameed MSA, Ebrahim OH. Biotechnological potential uses of immobilized algae. *J Agric Biol* 2007; 9: 183–192.
- Hodoki Y. Bacteria biofilm encourages algal immigration onto substrata in lotic systems. *Hydrobiologia* 2005; 539: 27-34.
- Hoffmann JP. Wastewater treatment with suspended and nonsuspended algae. *J Phycol* 1998; 34: 757-763.
- Holmes PE. bacterial enhancement of vinyl fouling by algae. *Appl Environ Microbiol* 1986; 52: 1391-1393.
- Janssen M, de Bresser L, Baijens T, Tramper J, Mur LR, Snel JFH, et al. Scale-up aspects of photobioreactors: effects of mixing-induced light/dark cycles. *J Appl Phycol* 2000; 12: 225–237.

- Johnson MB, Wen Z. Development of an attached microalgal growth system for biofuel production. *Appl Microbiol Biotechnol* 2010; 85: 525-534.
- Lundquist TJ, Woertz IC, Quinn NWT, Benemann JR. A realistic technology and engineering assessment of algae biofuel production. Berkeley, CA: Energy Biosciences Institute; 2010.
- Molina Grima E, Belarbi E-H, Ación Fernández FG, Robles Medina A, Chisti Y. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnol Adv* 2003; 20: 491-515.
- Mulbry W, Kondrad S, Buyer J. Treatment of dairy and swine manure effluents using freshwater algae: fatty acid content and composition of algal biomass at different manure loading rates. *J Appl Phycol* 2008; 20: 1079-1085.
- Nelson D. Transesterification and recovery of intracellular lipids using a single step reactive extraction. All Graduate Theses and Dissertations 2010. Available from: <http://digitalcommons.usu.edu/etd/642>
- Oswald WJ. My sixty years in applied algology. *J Appl Phycol* 2003; 15: 99–106.
- Patwardhan AW. Rotating biological contactors: a review. *Ind Eng Chem Res* 2003; 42: 2035-2051.
- Pittman JK, Dean AP, Osundeko O. The potential of sustainable algal biofuel production using wastewater resources. *Bioresour Technol* 2011; 102: 17-25.
- Przytocka-Jusiak M, Baszczyk M, Kosinska E, Bisz-Konarzewska A. Removal of nitrogen from industrial wastewaters with the use of algal rotating disks and denitrification packed bed reactor. *Water Res* 1984; 18: 1077-1082.
- Qureshi N, Annous B, Ezeji T, Karcher P, Maddox I. Biofilm reactors for industrial bioconversion processes: employing potential of enhanced reaction rates. *Microbial Cell Factories* 2005; 4: 24.
- Roeselers G, Loosdrecht MCM van, Muyzer G. Phototrophic biofilms and their potential applications. *J Appl Phycol* 2007; 20: 227-235.
- Sheehan J, Dunahay T, Benemann J, Roessler P. A look back at the US department of energy's aquatic species program—biodiesel from algae. National renewable energy laboratory (NREL) report: NREL. TP-580-24190. Golden, CO; 1998.
- Shen Y, Yuan W, Pei ZJ, Wu Q, Mao E. Microalgae mass production methods. *Trans ASABE* 2009; 52: 1275-1287.

- Stumm W, Morgan JJ. Aquatic chemistry: an introduction emphasizing chemical equilibria in natural waters. Hoboken: John Wiley; 1981.
- Torpey WN, Heukelekian H, Kaplovsky AJ, Epstein R. Rotating disks with biological growths prepare wastewater for disposal or reuse. *J (Water Pollut Control Fed)* 1971; 43: 2181-2188.
- U.S. DOE. National algal biofuels technology roadmap. U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Biomass Program; 2010.
- U.S. EPA. Wastewater technology fact sheet: facultative lagoons. U.S. Environmental Protection Agency; 2002.
- UDEQ. Middle bear river and Cutler Reservoir TMDLs - public draft. Salt Lake City, UT: Utah Department of Environmental Quality, Division of Water Quality TMDL Section; 2009.
- UDWQ. UDWQ POTW nutrient removal cost impact study: analysis of city of Logan's wastewater lagoon facility. CH2M Hill. Prepared for Utah Division of Water Quality; 2010.
- Uduman N, Qi Y, Danquah MK, Forde GM, Hoadley A. Dewatering of microalgal cultures: A major bottleneck to algae-based fuels. *J Renew Sustain Energy* 2010; 2: 012701.
- Wilkie AC, Mulbry WW. Recovery of dairy manure nutrients by benthic freshwater algae. *Bioresour Technol* 2002; 84: 81-91.
- Woertz I, Feffer A, Lundquist T, Nelson Y. Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. *J Environ Eng* 2009; 135: 1115–1122.

CHAPTER 4

DESIGN OF A FULL-SCALE WASTEWATER TREATMENT
AND BIOFUEL PRODUCTION SYSTEM

1. Introduction

Data collected from the pilot scale RABR unit can be used in the design of a full scale RABR-based wastewater treatment system. The system can be designed to reduce phosphorus levels in the Logan Regional WWTP to either 1.0 mg l^{-1} or 0.5 mg l^{-1} . The estimated production of algae biomass can then be used to calculate the theoretical production of biodiesel and/or biomethane in such a full scale system.

The Logan Regional WWTP treats an average of 15 MGD of wastewater on a 460 acre site consisting of seven facultative ponds. Figure 9 shows an overview of the plant. The majority of secondary treatment occurs in the first two sets of parallel ponds A and B. The later ponds are intended for further polishing. For this analysis, pond D will be considered as the RABR treatment zone for P removal and algal biofilm production.

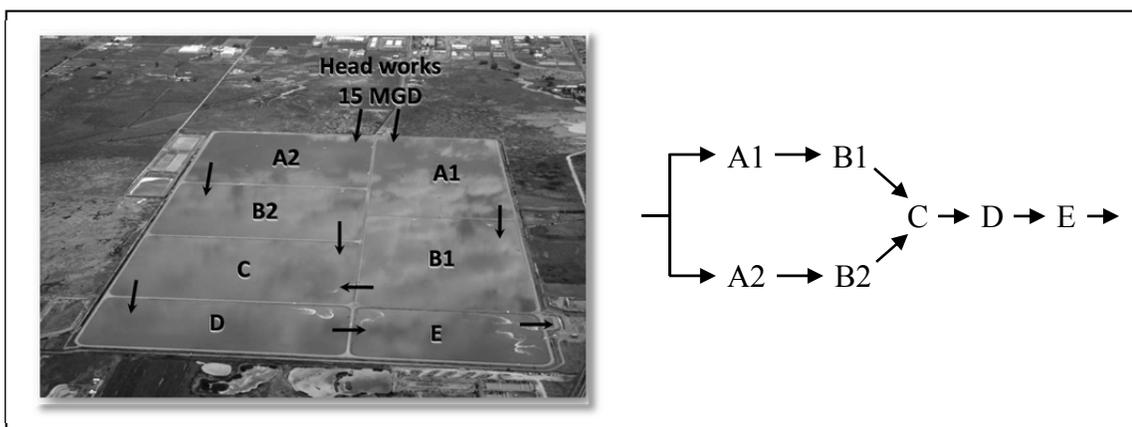


Figure 9: Logan wastewater plant pond distribution and direction of wastewater flow.

2. Specifications and Parameters

The size of the pilot scale RABR is 76 inches in diameter and 60 inches in length. Adding 24 inches to the diameter measurement and 6 inches to the length measurement for clearance gives a modular plan area of 4.26 m² per unit. The total phosphorus levels in pond D measured from July 2010 through April 2011 averaged 2.51 mg l⁻¹. Pond D measures 276 m by 612 m with an area of 168912 m². Table 10 shows the biomass yield and productivity of the pilot scale RABR over the time period of operation. Maximum productivity is achieved at day 12 and a growth and harvesting cycle of this length is used in this full scale design analysis. Table 11 shows the P uptake of the pilot scale RABR over the 12 day cycle. The P uptake in g day⁻¹ is calculated from the measured difference between the influent and effluent P levels using the values for active reactor volume (7650 l) and hydraulic retention time (0.47 days). The average uptake value through the 12 day cycle is 3.92 g day⁻¹.

Table 10: Algae biomass yield and productivity of pilot scale RABR unit.

Time (days)	Total Biomass		Productivity	
	(g)	(g m ⁻²)	(g day ⁻¹)	(g m ⁻² day ⁻¹)
0	0	0	0	0
8	580	235	73	29
12	928	377	77	31
16	832	337	52	21
22	940	381	43	17
27	1426	579	53	21
34	1374	557	40	16

Table 11: Phosphorus levels and phosphorus uptake of pilot scale RABR unit.

Time (days)	Influent P (mg l⁻¹)	Effluent P (mg l⁻¹)	P difference (mg l⁻¹)	P uptake (g day⁻¹)
0	2.4	2.4	0	0
1	2.3	2	0.3	4.88
3	2.1	1.51	0.59	9.60
5	2.31	1.85	0.46	7.48
8	2.01	1.34	0.67	10.90
12	2.01	1.66	0.35	8.46
Average				3.92

3. Full Scale Design Data and Summary

The specifications and parameters described above are used to calculate the full scale design data shown in Table 12. A large number of RABR units would be needed to reduce P levels to the required concentrations, but the area for these reactors is available in pond D, and a P removal and biofuel production process based on a RABR system in pond D appears to be feasible. A design and performance summary for the pilot scale RABR is given in Table 13.

Table 12: Phosphorus removal and biomass and biofuel yields using RABR units in pond D of the Logan City wastewater plant.

Specifications		
Average P concentration	2.51 mg l ⁻¹	
Wastewater flow	56.7 X 10 ⁶ l day ⁻¹	
Algae production per RABR unit	77 g day ⁻¹	
P removal per RABR unit	3.92 g day ⁻¹	
Area of pond D	168912 m ²	
Plan area of RABR with clearance	4.26 m ²	
Phosphorus removal & biofuel yields		
Required effluent P concentration	1.0 mg l⁻¹	0.5 mg l⁻¹
Required P removal	1.51 mg l ⁻¹	2.01 mg l ⁻¹
Required P removal	85.6 kg day ⁻¹	114.0 kg day ⁻¹
No. of RABR units	21850	29085
Area required for RABRs	93081 m ²	123902 m ²
Percentage of pond D area used	55%	73%
Algae production	1682 kg day ⁻¹	2240 kg day ⁻¹
FAME production ^{a,b}	67 gal day ⁻¹	89 gal day ⁻¹
Biomethane production ^c	412 m ³ day ⁻¹	549 m ³ day ⁻¹
Biomethane energy potential ^d	4169 kWh day ⁻¹	5549 kWh day ⁻¹
Electricity generation ^e	1251 kWh day ⁻¹	1665 kWh day ⁻¹

^a Assuming a FAME content of 12% (w/w) after *in situ* transesterification (see p. 77)

^b Density of biodiesel = 0.801 kg l⁻¹ (Vijayaraghavan and Hemanathan, 2009)

^c 245 l CH₄ per kg algae (Sialve et al., 2009)

^d CH₄ heating value = 55,500 kJ kg⁻¹ (NIST, 2011)

^e Assuming 30% electricity generation efficiency

Table 13: RABR design and performance summary.

Production		
Biomass	FAME	Power requirement
31.4 g m ⁻² day ⁻¹	2.2 g m ⁻² day ⁻¹	1.4 W m ⁻²
Harvesting		
Solids concentration	Recovery	Power requirement
12-16%	70-85%	negligible
Wastewater Treatment		
N removal rate	P removal rate	
22.1 g m ⁻² day ⁻¹	4.1 g m ⁻² day ⁻¹	
RABR Design & Operation		
Diameter	76 inches	
Length	60 inches	
Rotation	1.2 rpm	
Peripheral velocity	0.38 ft s ⁻¹	
Submersion level	40%	

References

- NIST Chemistry WebBook [Internet]. National Institute of Standards and Technology. Reference Database No. 69. Available from: <http://webbook.nist.gov/chemistry/>.
- Sialve B, Bernet N, Bernard O. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnol Adv* 2009; 27: 409-416.
- Vijayaraghavan K, Hemanathan K. Biodiesel production from freshwater algae. *Energy and Fuels* 2009; 23: 5448-5453.

CHAPTER 5

RECOMMENDATIONS FOR FUTURE RESEARCH

1. RABR Operation*1.1. Biofilm Harvesting*

Further research to address harvesting of the biofilm would also be beneficial.

The spool harvesting procedure used in this study was designed to allow for an automated and scalable harvesting process, but there are still several engineering details that need to be worked out before this is possible at full scale. At large scales, a more efficient may be to shear off the biofilms by spraying with water. The biofilm flocs could then be collected in a sedimentation basin. From observations during this study, biofilm flocs are expected to sediment well. Quantitatively studying the integrity of biofilm flocs after removal from the RABR would provide information to determine whether sedimentation of algae biofilm flocs is a possible method for harvesting algae.

1.2. Operation Parameters

Several important RABR operation parameters have yet to be optimized. For this study, the rotation, peripheral velocity, and submersion level were selected according to the operation of RBCs. Optimization of these parameters for algal biofilm growth instead of bacterial growth is recommended for future research. Related to rotation speed is the flow of wastewater around and through the RABR. There are several potential avenues of research relating to the fluid dynamics of a RABR system.

1.3. Repetition at Pilot Scale

The pilot scale data gathered in this study were from one unit that was in operation for six weeks. While the results from this testing were promising, more repetitions would be beneficial for a greater understanding of RABR mechanics. The pilot scale RABR was also operated from mid-October to late November 2010, giving a representation of RABR performance in the late fall season. Seasonal variations in phosphorus removal, biomass growth rate, biomass production, biofilm composition, and possibly FAME potential are all possible. A better understanding of these seasonal variations is needed. In addition to seasonal variations, day to day variations in weather likely effect RABR performance as well. Temperature and PAR levels will need to be monitored and a better understanding of their effects on RABR performance determined.

1.4. Substratum Durability

The solid braid cotton cord used as substratum in these experiments showed the greatest attachment and biofilm formation, but compared to other available substratum materials, particularly synthetic polymers such as nylon, polypropylene, and polyethylene, the durability is low. It is suspected that the much higher yields of cotton cord outweigh the limitations of lower durability. Indeed, most synthetic polymers tested were unable to show any biofilm formation. However, the true durability of cotton cord in continuous RABR operation is not known. Determining this durability is needed to calculate operation and maintenance costs of the RABR.

2. System Design

2.1. Anaerobic Digestion & Nutrient Recycle

Biofilms formed on the RABR have yet to be analyzed for biomethane potential after anaerobic digestion. A related research objective could involve recycling of nutrients from the digester effluent and an optimization of a full system design that includes RABR-based wastewater treatment and biomethane production.

2.2. Lipid Accumulation

No optimization of lipid production was attempted in this study. Nutrient management and/or stress induction could possibly increase the biofuel value of algal biofilms produced by the RABR. If increases are possible, they will need to be balanced against overall biomass productivity and nutrient uptake of the biofilm.

3. Water Quality

Depending on pH, calcium levels can affect the bioavailability of phosphates due to calcium phosphate precipitation and phosphate adsorption to calcium carbonate. Calcium concentrations and perhaps magnesium concentrations are water quality variables that need further analysis in all wastewater-based algae production studies. Because the RABR can offer good gas exchange and increased CO₂ transfer to the wastewater, it can keep pH levels lower, thereby leaving more phosphorous and calcium in the dissolved phase. This potential benefit of the RABR has yet to be measured.

CHAPTER 6

SUMMARY

Algae are capable of reducing nitrogen and phosphorus concentrations in wastewater through biomass assimilation, and if harvested, offer the added benefit as a source of feedstock for the production of biofuels and bioproducts. The integration of microalgae-based biofuel and bioproducts production with wastewater treatment has major advantages for both industries. However, major challenges to the implementation of an integrated system include the large-scale production of algae and the harvesting of microalgae in a way that allows for downstream processing to produce biofuels and other bioproducts of value. Difficulties in harvesting, concentrating, and dewatering the algae have limited the development of an economically feasible treatment and production process. When algae are grown as surface attached biofilms, the biomass is naturally concentrated and more easily harvested, leading to less expensive removal from wastewater, and less expensive downstream processing in the production of biofuels and bioproducts.

In this study, a novel rotating algal biofilm reactor (RABR) was designed, built, and tested. The RABR achieved effective nutrient uptake from wastewater and algae biomass production ($31 \text{ g m}^{-2} \text{ day}^{-1}$) at pilot scale. An efficient spool harvesting technique was also developed in order to obtain a concentrated biosolids product (12-16% solids) suitable for further processing in the production of biofuels and bioproducts.

The algal biofilms grown on the RABR were able to reduce phosphorus and nitrogen concentrations in the wastewater at pilot scale, with P and N removal rates of 4.1

$\text{g m}^{-2} \text{ day}^{-1}$ and $22.1 \text{ g m}^{-2} \text{ day}^{-1}$, respectively. The FAME content of biofilms after an acid-catalyzed in-situ transesterification procedure was 11.2-12.4%, giving a FAME productivity of $2.1\text{-}2.3 \text{ g m}^{-2} \text{ day}^{-1}$. Considering that lipid accumulation was not optimized in this study, these values represent a promising baseline for future improvements.

Results of this study indicate that the RABR with spool harvester represents a promising approach to the production and harvesting of algae in wastewater. No apparent constraints are currently identified for scale up to full scales comparable to RBCs, including materials and power. Construction and operation of a full-scale RABR with spool harvester represents the next phase of this research.