DEVELOPMENT OF A BENTHIC ALGAE CULTIVATION SYSTEM FOR BIOENERGY APPLICATIONS, WASTEWATER TREATMENT AND CARBON CYCLING

by

NISHA VAIDYANATHAN

(Under the Direction of K.C. Das)

ABSTRACT

Benthic (attached) algae show significant potential to yield high biomass productivity and are effective in treating wastewater. The present study investigates a benthic algae cultivation system for biomass productivity for biofuels production and nutrient removal from a carpet industrial wastewater using two solid materials- Geotextile-fiberglass and Linen. Four horizontal biomat reactors were developed and a grid-method of biomass sampling was employed to establish daily productivity trends for the biomats. The maximum productivities achieved were in the range of 50 – 60 g m^{-2} d^{-1} for both materials and minimum productivities of 11.25 g m^{-2} d^{-1} for linen and 17.99 g m^{-2} d^{-1} for geotextile-fiberglass material. An average of 60-80% Total Nitrogen, >90% Nitrates, 80% Ammonia and 57.2% Total Phosphorus removals from wastewater by the benthic algae system was recorded. The biomass has been characterized with an energy value of 21 MJ/kg thereby making the entire system viable and feasible for bioenergy production.

INDEX WORDS: Benthic microalgae, industrial wastewater, biomass productivity, harvesting, nutrients removal, biofuels.

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DEDICATION

I wholeheartedly dedicate this thesis work to my dear Husband, Mom, Dad, Grandfather, Brother and Parents In-Law who have been supporting and encouraging me all through my hardships, gave me the mental strength to overcome few disappointments and nourished me with huge positive energy to complete my work successfully and reach greater heights in my career. I need their blessings and company throughout my life to keep me going successfully at all times.

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CHAPTER 1

INTRODUCTION

Biofuels have grabbed considerable attention of researchers in the past two decades in view of changing international perception about global warming and environmental pollution due to combustion of fossil based fuels. Biofuels contribute to clean energy, clean environment and provide energy security to the nation as they could be produced sustainably. Microalgae based biofuels have drawn lots of research interests in the recent times due to their great potential to meet the current nation's biofuel production target of 22 billion gallons by 2022 as mandated in the Energy Independence and Security Act of Fuels under Renewable Fuels Standard.

Microalgae are considered to be potent candidates for production of biofuels as they 1) grow fast and produce 5-10 times more biomass than terrestrial crops in an acre (Borowitzka and Borowitzka, 1990), 2) do not require agricultural lands for cultivation and hence do not compete with food prices 3) consume 1.83 kg of CO_2 for production of 1 kg of dry biomass and hence the process is carbon neutral thereby aiding to the reduction of global greenhouse gases in atmosphere (Demirbas, 2006) 4) can grow in poor and brackish waters such as municipal, industrial and manure effluents thereby contributing to effective wastewater treatment (Zhang et al., 2008; Oswald et al., 1957) 5) produce biomass rich in lipids, carbohydrates and proteins which can be converted to biofuels such as biodiesel, bioethanol and biomethane respectively. The most studied microalgal cultivation systems are the raceway ponds and photobioreactors operated for growing suspended algae. The raceways open systems are those in which algae are

grown in suspension to particular depths of growth medium (15 to 30 cm), and are designed based on the key concept that mixing exposes algae to sunlight and carbon dioxide which are the main inputs for growth. The major drawback with these culture systems is the ease of harvesting, due to the small size of algal cells (3-30 μm in diameter), low algal concentration in effluents and large volumes of water to be handled to recover the cells (Zhang, 2010). There are other open pond designs that include circular and unstirred ponds. The circular ponds are built with a diameter of 45 m and 30 to 70 cm in depth with a centrally pivoted agitator (Moheimani, 2005). The achievable biomass productivity with circular ponds is 15 g m^{-2} d^{-1} (Sheehan et al., 1998). The unstirred ponds are simply natural ponds with uncovered beds in which no mixing is provided and they are less than 50 cm deep (Borowitzka and Borowitzka, 1990). Nevertheless, these unmixed ponds are limited only to those microalgae that are capable of growing in poor conditions and also have a capability to outgrow possible contaminants (Chaumont, 1993). The advantages of these open pond systems are that they have relatively low construction and maintenance costs (Ben-Amotz, 2008), they are easier to scale up and they can be integrated with wastewater treatment. However, the major drawbacks of these systems include contamination with weed algae, low biomass productivities, high harvesting cost and huge water evaporation losses (Shen et al., 2009). All these drawbacks are overcome in photobioreactors (PBR) which are closed systems and hence have reduced contamination risks, better control of culture conditions and reduced losses of carbon dioxide. The photobioreactors are more superior to open ponds in that they can give twice higher biomass productivity and 30 times higher cell density (Chisti, 2007). However, the major limiting factor is the high construction cost involved with photobioreactors which is almost seven times than that required for open ponds per unit area (Shen et al., 2009). Therefore, increasing the productivity (net biomass weight per unit area per

day) and the cell density (hence reducing the harvesting cost) in a low-cost reactor system are the key challenges in mass cultivation systems. The estimated production cost of one kilogram of algae biomass with photobioreactors and raceways are $2.95 and $3.80 respectively, assuming that CO_2 can be provided from the flue gases and other industrial emissions at no cost (Chisti, 2007). As large quantities of biofuels will be required to replace the present fossil fuel needs, the cost of production of algae biomass has to be reduced to $0.20 -$0.25/kg to make algae biofuels economically viable (U.S. DOE, 2008).

Algae cultivation systems in which cells are grown in attached mode on solid substrates using wastewater have shown to yield high productivities and facilitate easy harvesting without spending more energy. A benthic algae cultivation system such as the Algal Turf Scrubber (ATS) has been thoroughly studied for wastewater treatment (including agricultural run-off and digested and undigested dairy manure effluents) and algal productivity purposes (Mulbry et al., 2008; Wilkie and Mulbry, 2002; Adey et al., 1993). A recent study was carried out by Johnson and Wen in 2010, with an attached microalgal culivation system using single-species inoculum in a closed set-up for evaluating biofuel production. However, in-depth studies of attached algae culture systems specifically targeted for biofuel production are required to understand the feasibility of large scale production while addressing the practical problems of mass cultivation in such systems. The present study investigates a benthic algae cultivation system in a greenhouse and carpet industrial wastewater as growth substrate for biomass productivity and nutrients removal. The objectives of the study were; 1. To develop a benthic algae cultivation system to do the experimental studies 2. Evaluate two judiciously chosen materials for biomass productivity and nutrients removal efficiency and 3. Analyze the biomass for bioenergy applications.

CHAPTER 2

LITERATURE REVIEW

1. Evolution of Algae-based Biofuels

The US has been facing a lot of energy and economical challenges for the past two decades as its fossil fuel reserves are depleting and its increased dependence on foreign oil. Approximately two thirds of its petroleum is imported from other countries and 60% of this petroleum is used as a transportation fuel. The use of petroleum-based fuels for transportation has harmful environmental impacts as its combustion results in huge emissions of green house gases (GHG) including carbon dioxide (CO_2), sulfur dioxide (SO_2) and nitrogen oxides (NO_x), which have detrimental global warming effects on the atmosphere. All of these threatening factors have turned nations' attention towards renewable sources of energy such as biofuels. Biofuels are a wide range of fuels including solid, liquid and gas fuel that are derived from biomass. The term biomass is defined as any material that has a biological origin such as wood, agricultural wastes, bio-solids, aquatic plants etc., and hence it is a renewable source of energy. Therefore biofuels are a clean source of energy providing environmental benefits and could be produced sustainably, thereby increasing energy security of the nation. They could also be made economically viable depending on the choice of feedstock used and the strategy and technology employed at every step of its production. Biodiesel is the most targeted form of biofuel, as it could directly be used in place of petrodiesel in internal combustion engines (ICE), is biodegradable, non-toxic and may significantly reduce the exhaust emissions including carbon

dioxides (CO_2), carbon monoxide (CO), hydrocarbon and particulate matter (PM) when burned as fuel (Qi et al., 2010; Demirbas, 2007). Currently the most important source of biodiesel in the US is soybeans. Other important sources of commercial diesel include rapeseed oil (Kusdiana and Saka, 2001), sunflower oil (Mohamed et al., 2003), palm oil, canola oil, corn oil, Jatropha (Barnwal and Sharma, 2005), animal fat, cotton seed and waste cooking oil (Kulkarni and Dalai, 2006). Another important form of biofuel is bioethanol produced from corn-based starch. All of these fuels are categorized as first generation biofuels derived from sugar, starch and vegetable oils, the sources of most of which include food crops. The increased demand for food crops for fuel results in high food prices, as the use of such crops as feedstock has to compromise the production of food. Therefore it is highly important to identify alternative sources of biofuels which could offset the challenges faced with the production of conventional biofuels described above. Microalgae has been recognized as a potential feedstock for producing biofuels offering a lot of benefits as compared to those produced from terrestrial feedstock. They are photosynthetic organisms which use carbon dioxide for their growth and convert it into usable form of biofuels, foods, feeds and high-value compounds (Chisti, 2007). Algae are capable of producing high productivities which are sufficient to meet the U.S. biofuel production target of 36 billion gallons by 2022 (U.S. DOE, 2008). The entire pathway of algae to energy could be exploited to produce a variety of biofuels. For example, the cultivated algal biomass is dried and the lipids are extracted and transesterified to produce crude oil. This crude oil is further processed and conditioned to produce biodiesel. The residual dried algae powder without lipids can be fermented to produce bioethanol or anaerobically digested to produce biomethane (Chinnasamy et al., 2010). Cultivation of algae does not interfere with the production of food as they can be grown in marginal lands. They possess the ability to grow in poor brackish waters, agricultural

run offs and municipal waste waters thereby offering an advantage of wastewater treatment and also eliminating the need to use fresh water which is scantily available in the US. 1 kg of dry algal production requires 1.83 kg of CO_2 (Chisti, 2007). Hence their cultivation can be done at the proximity of an industrial power plant where the emitted flue gases can be used as a carbon dioxide supply for algae growth, providing environmental benefits by reducing CO_2 emissions in atmosphere (Demirbas, 2006). Some microalgae possess oil content that can exceed 80% of the dry weight of dry biomass (Patil et al., 2008; Metting, 1996). These strains could be employed for mass cultivation for biodiesel production. Despite the many advantages that algae has to offer, their cultivation has major challenges that needs to be addressed and resolved before any technology is to be commercialized. The drawbacks are that algae yield high productivities only under optimum culture conditions such as ambient temperature, light intensity, pH and availability of nutrients such as nitrogen (N), phosphorus (P), CO_2, trace elements of minerals. All of these factors are difficult to maintain in algae reactor at all times and more importantly it is a great challenge to cultivate pure cultures of oil-rich species without contamination. Most of the currently employed cultivation technologies require large amount of inoculum preparation and are energy-intensive which again incurs a lot of cost associated with algal production. In sum, there is a need to analyze and evaluate all the above-mentioned factors in all the cultivation technologies currently available and opt out a single technology and optimize it to make the entire scenario of algae to biofuels environmentally and commercially viable.

2. Current Algae Cultivation Technologies

A variety of mass cultivation methods have been identified for maximizing algal productivity. However, the result depends on their performances in laboratory and field trials. The common objective for all the cultivation methods is to make optimal use of natural sunlight

and available nutrients. This can be achieved with proper engineering design of bioreactors. The most common designs used for algae mass cultivation are Open Ponds and Photobioreactors. Both designs are employed for cultivating suspended algae. Suspended algae also called phytoplanktons are algae which grow as free floating cells in suspension and they are microscopic in nature and hence called microalgae.

2.1. Open Ponds

Open ponds were designed based on the concept that algae are grown in conditions similar to those found in external environments. They are the oldest form of cultivation methods and are still commercially employed for algae cultivation for food and feed purposes. The most popular and used designs of open ponds are the raceways, circular ponds and unstirred ponds.

Raceways are usually constructed as singles or groups of raceways connected together. Depths of raceways vary between 15 and 30 cm and the culture is usually mixed using a paddle wheel. The purpose of mixing is to expose the cells to sunlight and CO_2 for their growth which is the concept of all open pond designs. A cell concentration of 0.5 g L^{-1} is maintained in raceways and an average productivity of 25 g m^{-2} d^{-1} could be achieved (Richmond et al., 1990). Velocities between 10 to 20 cm s^{-1} is maintained, however velocities greater than 30 cm s^{-1} would consume more energy which makes the technology non-viable (Sheehan et al., 1998). The water flow velocity is required to prevent the cells from settling and also to expose all the suspended cells to sunlight (Shen et al., 2009). Raceways are the most commonly used open pond systems for algae production because of their low construction and maintenance costs (Borowitzka, 2005). However, because of seasonal light and temperature changes, the raceways could not be completely relied on for consistent high biomass productivities all throughout the year.

Circular ponds are constructed similar to raceways, with a depth that varies from 30 to 70 cm and 45 m in diameter with a central rotating motor provided for mixing (Shen et al., 2009). These ponds have been used in the US for beta carotene production from algae and productivities up to 15 g m^{-2} d^{-1} have been reported with *Oscillatoria sp.* (Sheehan et al., 1998). The major drawback of circular ponds is when the rotating arm gets longer than 50 m diameter resulting in poor culture mixing efficiency (Lee, 2001).

Unstirred ponds are the simplest open pond design as they are not provided with any mixing equipment. These ponds are constructed with a depth less than 50 cm and are the most economical systems for suspended algal production (Borowitzka and Borowitzka, 1990). However, as algal cultures grown in these ponds are unmixed, only those species that are capable of outgrowing the contaminants and also can grow in poor conditions are benefitted from these designs (Chaumont, 1993).

2.2. Photobioreactors (PBR)

Photobioreactors are closed systems in which algal cells are not directly exposed to the atmosphere, instead they are covered with a transparent material to allow the cells to use maximum light intensity for their growth. Tredici in 2004 defined photobioreactors as enclosed systems for cultivating phototrophs in which a major proportion of light (>90%) does not impinge directly on the culture surface, but has to travel across a transparent material to reach the cells. Also the system does not allow or strongly limit direct exchange of gases and contaminants between cells in PBR and external atmosphere. These closed systems were designed based on the assumption that a high cell concentration is crucial for achieving high biomass productivity. The purpose of such a design is also to maintain monocultures of desired algae which can be grown

in mild and controlled culture conditions under artificial or solar light (Lee, 1986). The most commonly used designs are tubular and flat panel photobioreactors.

Tubular designs have come in various shapes and sizes. These include horizontal straight transparent tubes connected by U-bends to form flat loops (photostage) which are called serpentine photobioreactors. This design was first introduced by Tamiya et al., 1953. The reactors were made of glass or plastic as solar receptor and CO_2 and nutrients are provided in a separate vessel. Direct exchange occurs between the photostage and vessel with the help of a pump. One of the most famous designs was constructed at Batelle (Anderson and Eakin, 1985) for polysaccharides production using *Porphyridium cruentum*. Productivities ranging from 20 - 25 g m^{-2} d^{-1} were reported in these reactors. In later studies, the pump was replaced with an airlift design for culture circulation as they may increase the biomass productivity up to 75% (Gudin and Chaumont, 1991). Also airlift systems prevent damage to shear sensitive cells, at the same time supply CO_2 and degas O_2. Other important tubular designs include manifold photobioreactors in which a series of parallel tubes are connected by two manifolds at the ends for distribution and collection of culture suspensions respectively (Tredici, 2004). Manifold designs save 15% energy compared to serpentine reactors in which energy is spent in moving the culture suspension through the bends (Pirt et al., 1983). Manifold designs also include α-type PBRs containing cross transparent tubes arranged at an angle with the horizontal (Lee et al., 1995). The third group of tubular designs is helical PBRs also called biocoil PBR in which the reactors contain flexible tubing usually coiled around an upright structure like a cylindrical framework. The biocoil consists of a photostage made of PVC or polyethylene tubing wound helically around a cylindrical support. Sometimes the flexible tubes may be arranged parallel and

connected to the pumping system through manifolds, which allows more even flow and shorter tube length thus minimizing oxygen build up.

Flat panel PBRs on the other hand are those in which the reactors contain rectangular containers placed vertically or at an inclined position with a light path between 1 and 30 cm. Different designs of flat panel PBRs have been proposed. The first most designs are the flat alveolar panels in which the transparent material usually made of PVC, polyethylene or polymethyl methacrylate sheets are internally partitioned in a parallel manner. Inside the partitioned sections are contained the algae cell cultures which are connected to a pump for circulation. Double layer panels made of transparent PVC were experimented for culturing *Chlorella* (Ortega and Roux, 1986). The panels were placed horizontally in which the upper plates were used for algal growth and lower plates for thermoregulation. Productivities upto 24 $g\ m^{-2}\ d^{-1}$ was obtained in summer in 1.5 m^2 surface area of units. In later studies at the University of Florence, the flat alveolar panels were placed vertically or inclined at an angle perpendicular to the ground where mixing and oxygen degassing was done by bubbling air at the bottom of the reactors (Tredici et al., 1991). These are called vertical alveolar panels (VAP) and have been extensively used by the Florence group for mass cultivation of microalgae and cyanobacteria and also to study the effects of areal density and inclination on biomass productivities. The top and bottom sections of the inner partitioned alveoli were removed to allow communication of culture suspension among the entire panel volume. Other flat panel designs include glass plate PBRs in which the reactors containing flat glass chambers are connected in series fashion and inclined at an appropriate angle to maximize solar radiation reaching the algal cells (Hu et al., 1996). A similar parallel plate design was constructed which is the 110 L Green Wall Panel locate at Livorno, Italy. This system uses a flexible low density polyethylene to cut down the cost on

transparent tubes to culture *Nannochloropsis sp.* in two phases, in nutrient suffiecient and nitrogen starving (Shen et al., 2009). Productivities upto 30 g m^{-2} d^{-1} was reported in these reactors.

Although PBRs have advantages like higher biomass productivities, reduced contamination risks and better control of culture conditions compared to open ponds, their fabrication costs are very high to make it commercially viable for biofuel production purposes. An estimated field capital investment was $180 m. The other major drawback in closed systems is difficulty in scaling-up as compared to raceways, which again incurs huge material and operational costs (Tredici et al., 2004). Closed systems also face challenges like over-heating, oxygen accumulation, biofouling, cell damage etc.

2.3. Algal Turf Scrubber (ATS) Systems

While open ponds and photobioreactors are employed for culturing suspended algae, the algal turf scrubbers are used for cultivating benthic microalgae. Benthic algae also called attached algae are photosynthetic organisms which grow in attached mode on a solid support or substratum and utilize nutrients from external environments for their growth. Algal turfs are those small groups of benthic microalgae which grow on any solid support provided on a water surface thereby scrubbing CO_2 and other pollutants from water resulting in biomass production (Adey and McLean, 1980). The ATS systems were invented primarily for wastewater treatment purposes. The complex algal communities in the turf has the ability to effectively remove phosphorus, nitrogen, and other micronutrients from sewage or any source of poor quality waters (Adey et al., 1993). The concept of using algal turfs for purifying water was first identified while researching shallow tropical coral reefs which were used for microcosm studies as they support the planet's most productive photosynthetic systems (Adey and Loveland, 2007). Algae grow

abundantly on these coral reefs where they are subjected to strong water currents, intense wave action, abundant sunlight and nutrients and they result in large primary production of the plant material. This primary production in coral reefs occurs at a high rate and they are mostly accomplished by the algal turfs which include a major portion of filamentous algae (Adey and Hackney, 1989). Algae grown attached on coral reefs or on small square plastic mesh screens placed on warm, well-lighted and high water quality environments would typically contain 30 - 40 species of algae which include diatoms, blue-greens and red algae (Adey and Goertemiller, 1987). Usually the diatoms are the first colonizers and then the blue-green algae starts dominating the surface followed by red algae in high quality waters. In fresh waters, the diatoms and many species of green algae dominate and in poor waters, the number of species gets reduced to diatoms, blue-green and one or two species of green algae.

Similar to algae growing on coral reefs in natural aquatic environments, the ATS systems are simulated the same way in which the algal turf is grown on any solid surface (substratum) which is subjected to periodic wave surge action and light (artificial or natural) essential for algal growth. Therefore the ATS systems are engineered microcosm ecosystems dominated by algal turfs, used as water quality control devices; the high levels of photosynthesis and primary production produced by the algal turfs are used to control the quality of any wastewater provided in the model ecosystems (Adey and Loveland, 2007). The wave surge is an important physical demand for these algal turfs as it acts as a mixing system promoting the exchange of metabolites and nutrients needed for a high primary production. Harvesting of ATS is another significant factor in growing and maintaining a healthy primary production of algal turfs with limited grazers. As algal turf grows on the material, the depth of the turf increases and the bottom portions serve as a very good place for larvae of chironomid insects and other grazers to inhabit

and reproduce. This affects algal photosynthesis and decreases the efficiency of ATS systems. Therefore periodic harvesting of biomass is required to prevent build up of these grazers and maintaining a healthy community development which is essential for subsequent primary production of algal turfs. Algal filaments which dominate these turfs are simply long strands with no differentiation into complex structures, are photosynthetic and absorb all of the incoming light, available nutrients and carbon dioxide and convert them into oxygen and carbohydrates. The ATS model systems make use of these factors to produce algal turfs. Unlike the bacteriologiocal filters which remove ammonia and other pollutants and return to the aquarium water with depleted oxygen and plankton, high in dissolved nutrients and CO_2, the scrubber systems keep oxygen at saturated levels and remove all of the nutrients from water in a balanced process. Therefore this does not affect the community which is growing on the material. It is very evident that healthier that this community is maintained, higher is the efficiency of ATS systems to remove nutrients from wastewater effectively.

A lot of studies were performed with ATS to remove nutrients from dairy manure (Kebede-Westhead et al., 2003; Pizarro et al., 2002; Wilkie and Mulbry, 2002; Mulbry and Wilkie, 2001). One of the important studies performed was to remove phosphorus from natural waters using algal scrubbers (Adey et al., 1993). The element phosphorus has been a challenge to control, treat and reuse from human sewage, agricultural and urban runoffs and food processing wastes. Two types of ATS studies were then conducted to evaluate the removal of phosphorus from agricultural runoffs employing two biomass harvesting methods and biomass productivities were calculated for each of the methods. The two types of algal scrubbers were a floway type and a serial system consisting of four sets of four in-series scrubbers. The authors termed the ATS reactors as ‘raceways’. These raceways were provided with plastic screens for attached algae

production. The two harvesting methods used in this study were, manual harvesting where the plastic screens with fully grown algal turfs were harvested using a sharp piece of hard polystyrene plastic scraper and the other method was much faster which was a vacuum harvesting system. In both of these methods, after the biomass has been harvested, they were dewatered to between 90% and 97% water by squeezing the biomass in a paint strainer. This method was termed 'pressure sieve' and the water removed from the biomass using this method was called 'brown water' which was analyzed for total phosphorus and orthophosphates for the study. The average biomass productivity not including the brown water capture for floway was 21.16 g m^{-2} d^{-1} and for the serial plant scrubbers, it was 21.61 g m^{-2} d^{-1}. The mean productivity during the spring period was 47.77 g m^{-2} d^{-1} for the floway and 32.74 g m^{-2} d^{-1} for the serial system. The inlet total phosphorus concentrations ranged from 0.012 - 0.148 mg/L. The mean phosphorus content of the harvested biomass ranged from 0.34% to 0.43%. Total phosphorus removal rates during spring period with low nutrient supply ranged from 104 - 139 mg TP m^{-2} d^{-1}. Total phosphorus reduction in agricultural runoffs closely correlated with phosphorus yield from biomass removal in this study. Another study was performed by Craggs and his members to evaluate phosphorus removal from secondary effluent wastewater using ATS (Craggs et al., 1996). Manual harvesting method was performed and based on the mean phosphorus % (2.1%) in the harvested solids and mean productivity of 35 g m^{-2} d^{-1}, the yearly mean phosphorus removal was 0.73 ± 0.28 g m^{-2} d^{-1}. Much of the phosphorus removal from ATS and the high mean phosphorus content in solids were attributed to pH mediated precipitation in this study. Also the study revealed that phosphorus removal in ATS could be easily controlled by altering the hydraulic loading rate. Different studies were then conducted to evaluate the recovery of dairy manure nutrients in algae using ATS at different loading rates of anaerobically

digested manure (Mulbry et al., 2008; Pizarro et al., 2002; Wilkie and Mulbry, 2002). A further advancement in ATS studies were conducted recently by Jonson and Wen, in which they cultivated *Chlorella sp.* as biodiesel feedstock using dairy manure wastewater as growth medium (Johnson and Wen, 2010). Fatty acid analyses (FAME) were performed in the crude oil samples obtained from the ATS biomass and were evaluated for biofuel production. Polystyrene foam was used as a supporting material for algal attachment. A biomass yield of 25.65 g m^{-2} d^{-1} and a fatty acid yield of 2.31 g m^{-2} were achieved in the study. Also nutrients removal from manure wastewater was evaluated; 61% - 79% of total nitrogen and 62% - 93% of total phosphorus removals were achieved depending on the culture conditions.

In conclusion, the ATS systems effectively remove nutrients from any source of poor quality water and resulting in primary production of algal biomass. They rely on keeping the algae in place and bringing the nutrients to it, rather than suspending the algae in large volumes of culture media (Johnson and Wen, 2010). The growing turf biomass is harvested from the solid surface before it is dominated by larger macroalgae and other predators. Macroalgae deteriorates the quality of algal turf biomass and makes it ineffective for wastewater treatment and biofuel production and thus reduces the efficiency of the system (Adey and McLean, 1980; Mulbry et al., 2008). Hence the rate at which the algal turf biomass is harvested plays a crucial role in increasing productivity and nutrients removal.

3. Objectives

The ATS systems are superior to the suspended algae cultivation systems, in that they facilitate easy harvesting of algae with no significant loss of biomass thereby effectively cutting down the operational costs and result in high productivities. However, the ATS systems were not used for mass algae cultivation for biofuel production purposes in earlier studies, but the main

objective was to remove nutrients and pollutants from wastewaters. Hence further research is required to study fundamental engineering parameters for these cultivation systems to improve the system design which could increase biomass productivity though optimizing growth conditions and also selecting the right inoculum species and solid support materials for growing attached algae. The biomass obtained from such an improvised ATS system has to be further evaluated for its biofuel production potential.

The major objectives of the proposed study are:

1. To develop a benthic algae cultivation system and perform preliminary studies on the effect of using different inoculums and growth media on algae biomat productivity.
2. To evaluate the system performance with two solid materials to maximize biomass productivity.
3. To evaluate nutrient removal efficiency from industrial wastewater.
4. To characterize and evaluate the algal biomass obtained from the experimental studies for bioenergy applications.

CHAPTER 3

PRELIMINARY WORK ON STUDYING THE EFFECT OF WASTEWATER AND DIFFERENT TYPES OF INOCULUM ON BIOMASS PRODUCTIVITY IN A BETHIC ALGAE CULTIVATION SYSTEM

[1] Nisha Vaidyanathan, Senthil Chinnasamy, Ashish Bhatnagar, K.C. Das. To be submitted to Bioresource Technology.

Abstract: The algal turf scrubber (ATS) technology has been studied for the past two decades for purifying wastewater. However there have been very few studies on using this technology for algal biomass cultivation for biofuel production purposes. The present study focuses on developing a benthic algae cultivation system and evaluating the system by using two different growth media (fresh water enriched with nutrients similar to a BG 11 medium and carpet industrial wastewater) and three different inoculums (Mixed culture A, mixed culture B, *Ulothrix sp.* and control) on biomass productivity and they were performed as two separate experimental studies. In the growth media study, two solid materials were used which are Geotextile and Polymer fabric. The study resulted in wastewater performing better than enriched fresh water resulting in biomass productivity of 17.5 g m^{-2} d^{-1} on both materials (two replicates averaged). In the inoculum study, wastewater was used as the growth medium for biomat cultivation based on results from the previous study and geotextile material was used as the solid material. Three harvests were done, each after the end of a week, usually at the end of 7^{th} day when biomat reaches its peak productivity. Mixed culture B inoculum yielded a maximum productivity of 22.8 g m^{-2} d^{-1} in harvest 3 and performed consistently better than the other inoculums. An average of 80% of Total Nitrogen, 72% of phosphates and 93% of ammonia was removed from wastewater at the end of the experimental runs. Therefore the developed benthic algae cultivation system can yield high biomass productivities comparable to raceway ponds and photobioreactors, and also effectively treat industrial wastewater.

Keywords: Benthic algae, biomat reactor, biomass productivity, carpet industrial wastewater, biofuel production

1. Introduction

Open raceway ponds and tubular photobioreactors employed for the production of suspended algae (free algae cells grown in suspension in liquid medium) in large-scale have been observed with many demerits such as requirements of large volumes of inoculum, contamination, self shading, limited productivities and requirements of efficient harvesting technologies which significantly contributes to production and operational costs.

These problems call for unique algae mass cultivation system which can significantly improve biomass productivity and reduce the costs associated with it for value addition. A novel attached- algae cultivation method called the algal turf scrubber (ATS) technology where algae turfs are produced on horizontal surfaces (solid materials/substrates placed horizontally) using nutrients from natural water, was developed primarily to treat wastewater. Though ATS system has been widely studied for wastewater remediation, it has not been well researched and evaluated for bioenergy applications. In this project we propose to adapt the concept of ATS and develop an advanced algae cultivation system using industrial wastewater and evaluate the biomass production potential for bioenergy, bioremediation and economic feasibility of the proposed system. The objectives of the research to meet the goals are: (1) to develop an advanced algal bioreactor and evaluate its performance for biomass productivity, (2) to evaluate nutrient removal efficiency from wastewater and its kinetics for the proposed system, (3) to characterize and evaluate the obtained algal biomass for bioenergy applications, and (4) to compare the productivities achieved with the biomat reactors and open pond cultivation systems reported in literature.

Two preliminary studies were done to test the effect of using fresh water and wastewater and three different types of inoculum on biomass productivity. The maximum productivity

achieved was 22.8 g m^{-2} d^{-1} and the reactor systems could effectively remove 75-90% of nutrients from wastewater. The proposed benthic algae cultivation system would establish the fundamentals for engineering parameters which are viewed to be major constraints in a typical algae cultivation system. The research study would focus on high biomass productivity for bioenergy applications and efficient wastewater treatment, cost effectively, thereby benefiting the future researchers and industrialists to advance with a different perspective to solve the problems of energy and environment.

2. Methods

2.1. Reactor Development and Mechanics

A benthic or attached algae cultivation system was developed to perform preliminary bench scale experiments on achieving high biomass productivities and study fundamental engineering parameters which are significant in improving the design of the system. The system consists of four biomat reactors (Aquatic Eco-Systems, Florida) provided with a single reservoir of capacity of 553 L, on which the reactors are mounted horizontally with the help of a wooden plank at a slope of approximately 1%. A fabric or solid material is placed on the surface of each of the four reactors on which attached algae are grown. The reservoir usually contains water mixed with nutrients which form the medium for algal growth and also is provided with inoculum to initiate biofilm formation on the solid material. In the preliminary experimental studies, the materials used for growing attached algae were Geotextile and Polymer-fabric and the surface area of the materials were 0.297 m^2 which were cut exactly to fit into the biomat reactor. The materials were selected based on the criteria that they have to be reusable, robust enough to support the growth of different communities of attached algae, and easily procurable (Johnson and Wen, 2010). The reservoir is provided with four power head pumps purchased from a local store, to circulate

water to each of the biomat reactors. Each biomat reactor has two openings, one at the top which is used for water inlet and the other at the bottom which is the drain hole (Fig. 3.1). Water from the reservoir circulates to the biomat reactor placed on top with the help of water inlet, flows all through the material surface and drains back into the reservoir through the drain hole which is again re-circulated to the reactor. In this process of continuous water circulation through the reactor, the material forms a biofilm on its surface using the cells from the inoculums, which eventually grows into a thick algae biomat utilizing the nutrients provided in reservoir. The pump circulates water to the reactor at a flow rate of 6L min^{-1}.

2.2. Effect of using fresh water and industrial wastewater on algal biomass productivity

An experiment was conducted with the horizontal biomat reactors to study the effect of fresh water enriched with nutrients and wastewater on biomass productivity. As a single reservoir was provided for all the four reactors, two one-week runs were performed consecutively, one week with fresh water and the next consecutive week with wastewater. The results from both runs were comparable as there were no significant differences in culture conditions like ambient temperature, light intensity on the surface of the biomats, pH and nutrients such as nitrogen (N), phosphorus (P) and CO_2 were supplied to the fresh water according to the concentrations maintained in a BG 11 medium.

2.2.1. Wastewater collection

The wastewater used for the present study was procured from Dalton Utilities in Georgia, which are the worlds' most prominent carpet manufacturers. Nearly 40 - 55 million m^3 $year^{-1}$ of carpet industrial wastewater is generated everyday in Dalton and the wastewater which comes from different carpet industries are directed to a common wastewater treatment plant (Chinnasamy et al., 2010). Therefore, the wastewater that we procure each time for the

experimental studies are subjected to differences in nutrients concentrations. Periodical nutrients analyses were performed to record these differences during the startup of each new experimental run.

2.2.2. Inoculum preparation

Chlorelle minutissima and *Scenedesmus bijuga* strains were used as a mixed culture inoculum in the ratio of 1:1 at 10% (v/v) of medium used in the study. These phytoplankton species were used as inoculum in attached algae (biomat) cultivation, because the unicellular cells are reported to induce colonization of bacteria and diatoms aiding in biofilm formation (Hoagland et al., 1993; Cole, 1982). Also the cultures were readily available in our laboratory. 500 ml of each of the cultures were centrifuged at 5000 rpm for 10 mins and the concentrated cell pellet was resuspended in 2000 ml of BG 11 medium. In this way, stepwise scale up of the cultures was performed from 500 mL to 20 L. However for scaling up to 20 L, deionized water was used in place of BG 11 medium. The strains were maintained as pure cultures in the 20 L carboys. The cultures were bubbled with 5% CO_2 from a common air-CO_2 mixing system. Periodical biomass analyses were done to ensure the inoculums cell concentration is maintained at 0.1 g L^{-1}.

2.2.3. Biomass harvesting from the biomat reactors

After a mature biomat formation occurs in the reactors, which is usually 7 days, the entire material surface is harvested and collected in aluminum trays before biomat sloughing occurs. Sloughing of biomass occurs when well developed biomat loses strength to stay attached to the material surface due to hydrodynamic forces and is lost into the reservoir. The harvested biomass trays are stored in refrigerator at 4 ºC for future analyses. The biomass productivity values were

calculated in g m^{-2} d^{-1}, by actually dividing the dry weight of biomass (g) by the surface area of the material harvested (0.297 m^2) and 7 days.

2.3. Effect of using different inoculums on biomass productivity

Another experiment was conducted to study the effect of using three different inoculums on biomass productivity. While performing this experiment, the reactor system was slightly modified in which the single reservoir used in the previous study was replaced with four separate reservoirs (Rubbermaid 50 gal tote, The Home Depot, GA) for each of the four biomat reactors to facilitate to conduct this study. One reactor was used as control and the other three reactors were used for testing the three different inoculums respectively. The material used for the study was Geotextile fabric for biomat cultivation. The three inoculums include Mixed Culture A inoculum containing different species of diatoms, filamentous algae (species unknown) and bacteria which was collected from a swine pond at UGA swine center. The collected culture was mixed thoroughly and used as a single culture. The purpose of collecting culture from the swine pond was to introduce a natural, already established attached algae community as an inoculum. The second type of inoculum is called Mixed Culture B which consists of *Chlorella minutissima*, *Scenedesmus bijuga*, *Chlamydomonas globosa* and *Ulothrix sp.* which were used in the ratio of 1:1:1:1 at 10% (v/v) and the cultures were readily available in the laboratory. The scale-up and subculturing of the cultures were done as described in section 2.2.2. The idea of using planktonic and filamentous species in this inoculum was conceived from the fact that the single cells aid in quick colonization of bacteria and diatoms, while the filamentous algae help in developing biomat matrices by excess production of extra cellular polysaccharides (Hoagland et al., 1993). The third type of inoculum includes *Ulothrix sp.* only, to test if the use of filamentous algae

alone could increase biomass productivity. All the inoculums were used at 10% v/v of the medium in reservoir.

Three experimental runs were performed for three weeks consecutively. Each run lasted for 7 days and biomass was harvested using the method described in section 2.2.3. The inoculums were provided only for the first run, while for the other two consecutive runs the cells that already remained on the materials after harvesting served as inoculums. The three runs were done to test if there is an effect of harvesting on biomass productivities obtained in the next consecutive runs, to study the community patterns established by using the three different inoculums and also to test the robustness of the material if it supports biomat growth for long durations of experiments. The biomass productivity was calculated at the end of each run for all the four reactors and the results of the three runs were comparable.

2.4. Biomass compositional analyses

The obtained biomass samples were analyzed for its compositions to evaluate its potential for biofuel applications. The different analyses performed were; carbohydrates, lipids and protein estimation, moisture, elemental carbon, hydrogen, nitrogen and sulfur contents.

The dry biomass in the trays were powdered using a mortar and pestle, collected in transparent bags and stored in refrigerator at 4 ºC to prevent any degradation of elements and compounds. Carbohydrates in biomass samples were estimated using the phenol-sulfuric acid method as described by Dubois *et al.* in 1956. Percentage protein was calculated by using a conversion factor of 4.58 (Lourenço et al., 1998) multiplied with the % nitrogen analyzed from ultimate analysis. Neutral lipids in biomass samples were analyzed gravimetrically using an Ankom XT10 automated extraction system with hexane as the extraction solvent. A known quantity of 1 g of dry biomass sample was sealed in pre-weighed Ankom XT4 extraction bags

(W1) using an impulse sealer, dried in hot air oven at 60 ºC overnight, cooled in a desiccator for 2 h and weighed (W2) accurately to five decimal points in a weighing balance.. The bags were then placed in the Ankom system and the process was set to perform at 105 ºC for 2 h. After the extraction process was over, the bags were dried at 60 ºC overnight, cooled in a desiccator and weighed (W3) carefully. The % neutral lipid in the biomass sample was calculated using the formula;

% Neutral lipids = [(W2 – W3) / (W2 – W1)] x 100

Ultimate analysis for measuring elemental carbon, hydrogen, nitrogen and sulfur was done by using the LECO CHNS-932 (Leco Corp., MI, USA).

2.5. Nutrients analyses

The wastewater samples collected at the end of each run was analyzed for nutrients concentration. Aliquots of samples were filtered through 0.2 µm Whatman filter aids using a syringe prior to storage and analyses. The samples were analyzed for total nitrogen (TN) by persulfate digestion method and total phosphorus (TP) by molybdovanadate method with acid persulfate digestion. Nitrate, phosphate and ammonia were analyzed by cadmium reduction, ascorbic acid, and salicylate methods respectively. All analyses were done using a block digester (Hach DRB 200) and a spectrophotometer (Hach DR 2700, Loveland, CO).

2.6. Microscopy

Samples of wet biomass were collected periodically during the course of experimental runs for microscopic studies to study the pattern of microbial community development using different inoculums. This information may be helpful for carrying out future studies on benthic algae cultivation to develop biomats of desired algae.

3. Results and discussion

3.1. Reactor Operation

The biomat reactors were successfully developed and employed for the preliminary experimental studies. A submersible hose was provided in the reservoir for CO_2 bubbling. Approximately 5.5% of CO_2 (calibrated with GC) was bubbled into the reservoir water from a common air-CO_2 mixing system. The surface area of the materials placed on the biomat reactor is 0.297 m^2 and this number was used to calculate all the biomass productivity values in g m^{-2} d^{-1}. The flow rate of water in all the biomat reactors was evaluated to be 6 L min^{-1}. Therefore there were no significant differences in mechanics and operations among the biomat reactors.

3.2. Effect of using fresh water and waste water on algal productivity

3.2.1. Wastewater characterization and results

The results of wastewater analyses are given in Table 3.1. The values of TN, phosphates and ammonia-N were analyzed in three replicates to ensure there were no instrumental errors. The optimal mass ratio of C: N: P is 46.1:7.7:1 is suitable for benthic microalgae (Hillerbrand and Sommer, 1999). The Redfield ratio is 41.1:7.2:1 which is slightly lower than the ratio required for attached algae growth. The phosphorus values (P) for the samples in study were calculated from the PO_4 values by multiplying with 0.33 (which is the amount of phosphorus found in phosphate). The N: P value occurs to be 6.3:1 which indicates that N is not present in excess; however it appears to be sufficient to support algal growth.

3.2.2. Biomass productivity results

Two runs were conducted for two consecutive weeks, one week with fresh water and the next week with carpet industrial waste water, as only a single reservoir was provided for the biomat

reactors. The results from both runs were comparable as there were no significant differences in culture conditions (Fig. 3.2). Algae cultivated with wastewater on geotextile material yielded 57.1% higher productivity than that cultivated with fresh water. Similarly the treatment polymer fabric coupled with wastewater yielded 66.3% higher productivity compared to that with fresh water treatment. In conclusion, carpet industrial wastewater yielded higher productivities than fresh water and there were no significant differences in productivity among the two solid materials, geotextile and polymer fabric ($p<0.5$, 1-way ANOVA). The fresh water was enriched with nutrients similar to concentrations present in a BG 11 medium, and hence no control was used in this study.

3.3. Effect of using three different types of inoculum on biomass productivity

From the results of the previous study, wastewater yielded high biomass productivities and hence it was used as a growth medium for the present experimental study. Also the single reservoir used in earlier experiment was replaced with four reservoirs specific for each of the four biomat reactors to facilitate the current study. The biomass productivity results obtained by using three different types of inoculum in wastewater were analyzed (Fig. 3.3). The results were compared with the control which is mixed culture C consisting of *Chlorella minutissima* and *Scenedesmus bijuga* which was used in the previous experimental study with wastewater. Among the three different inoculums used against control, there was no significant difference in productivity obtained by *Ulothrix* and control for all the three harvests ($p<0.5$, 1-way ANOVA). Mixed culture B gave consistently higher results from harvest 1 through harvest 3, however a fourth consecutive run could not be continued due to overgrazing by predators such as flies and larvae of mosquitoes. Ulothrix and control (mixed culture C) also gave higher results from harvest 1 through harvest 3; however the productivity results were not as high as that compared

to mixed culture C. Mixed culture B yielded a productivity of 22.8 g m^{-2} d^{-1} during harvest 3, in wastewater which is observed to be approximately 45% higher compared to Ulothrix and control in harvest 3. Mixed culture A did not perform well as its productivity was greatly reduced with increasing number of harvests, the reason being an immediate invasion of larvae of mosquitoes during the second week of harvest. As this type of inoculum was collected from a swine pond, the species in the inoculums only supported the growth of larvae which fed on algal cells thereby decreasing the productivity to 3 g m^{-2} d^{-1} at the time of harvest 3. Overall mixed culture B performed well achieving a maximum productivity of 22.8 g m^{-2} d^{-1} which is comparable to that achieved in raceway ponds of about 25 g m^{-2} d^{-1} (Moheimani and Borowitzka, 2006) . An important point to be noted is that these inoculums were provided only during the first week of experimental run viz. harvest 1, while for the next two consecutive runs, no inoculum was provided. The cells that already remained on the materials after each harvest served as inoculum for the runs. However the productivity factors are attributed to the initial inoculums provided testing the robustness of the inoculums to prevent grazers and unwanted species to invade them during the first week run which served as the basis for the next two harvests. Also the initial microbial community established by using these inoculums played a significant role in subsequent productivities obtained in the other runs.

3.4. Biomass compositional analyses results

All the biomass samples obtained from both the wastewater and inoculum studies were analyzed for percentage carbohydrates, lipids, proteins and carbon, hydrogen, nitrogen and sulfur percentages using ultimate analysis (section 2.4.) and are presented in Table 3.2. The biomass samples obtained from the inoculum studies were pooled together to perform the compositional analyses. The samples obtained from both studies did not show significant differences in

compositions. The percentage netural lipids ranges from 6% - 8% which is low; however the energy in the biomass could be recovered through anaerobic digestion to produce biomethane (Chinnasamy et al., 2010).

3.5. Nutrients analyses

Nutrients analyses for final day water samples were performed only for the inoculum study. Samples of wastewater were collected from the reservoirs at the end of each experimental run to evaluate the percentage removal of nutrients by the algal biomass. An average of 80% of TN, 72% of phosphate and 93% of ammonia removal was achieved from the three experimental runs. This makes the benthic algae cultivation system also suitable for wastewater treatment.

3.6. Microscopy

Periodical microscopic studies were done to study the microbial community development achieved by using different inoculums for attached algae cultivation. It is interesting to note that the biomats showed majority of the species that were provided in the inoculum during the first two harvests. However during the third week runs, the biomats started developing more of filamentous algae such as *Oscillatoria sp.*, and lot of diatom species dominating the planktonic strains. The mixed culture B inoculum had both planktonic and filamentous algae and this type of inoculum developed more filamentous algae which were observed as thick biomats on the reactors. And it was mixed culture B which resulted in maximum biomass productivity compared to the other treatments.

4. Conclusion

In the first experimental study with encriched fresh water and carpet industrial wastewater, the latter performed well giving an average productivity of 17.5 g m^{-2} d^{-1} on both geotextile and polymer fabric materials. While in the inoculums studies, mixed culture B inoculum performed

better compared to other inoculums and control, yielding a maximum productivity of 22.8 g m^{-2} d^{-1} with wastewater and geotextile material. Significant nutrients removal from wastewater was also recorded; an average of 80% of TN, 72% of phosphate and 93% of ammonia was removed. The biomass compositional analyses also showed significant biofuel production potential of the algal biomats obtained in the experimental studies. In sum, the benthic algae cultivation system developed in the study could yield high biomass productivity with using the nutrients from industrial wastewater suitable for biofuel production and also effectively remove nutrients from wastewater.

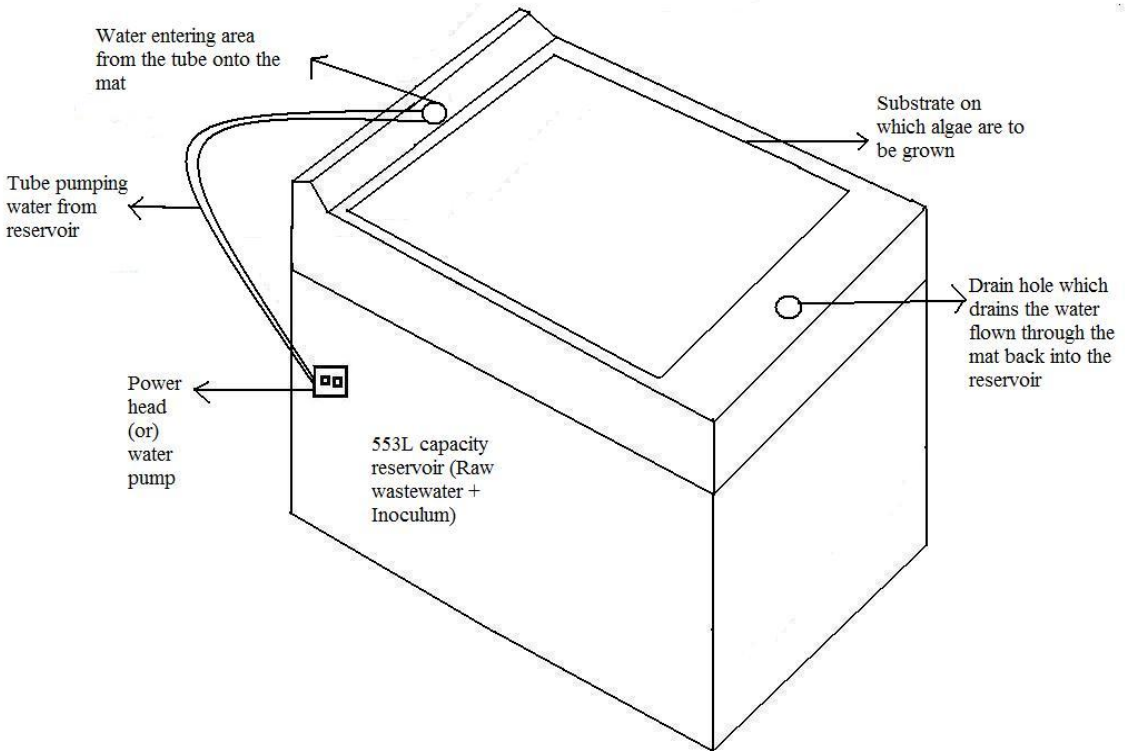

Fig. 3.1. Sketch of the benthic algae cultivation system showing the biomat reactor and reservoir

The cultivation system is provided with a biomat reactor on which the solid material is placed to support attached algae growth. A reservoir is placed below the biomat reactor and is provided with a submersible power head pump to circulate nutrients from water and inoculum over the biomat reactor surface with the help of the water inlet opening and drain hole.

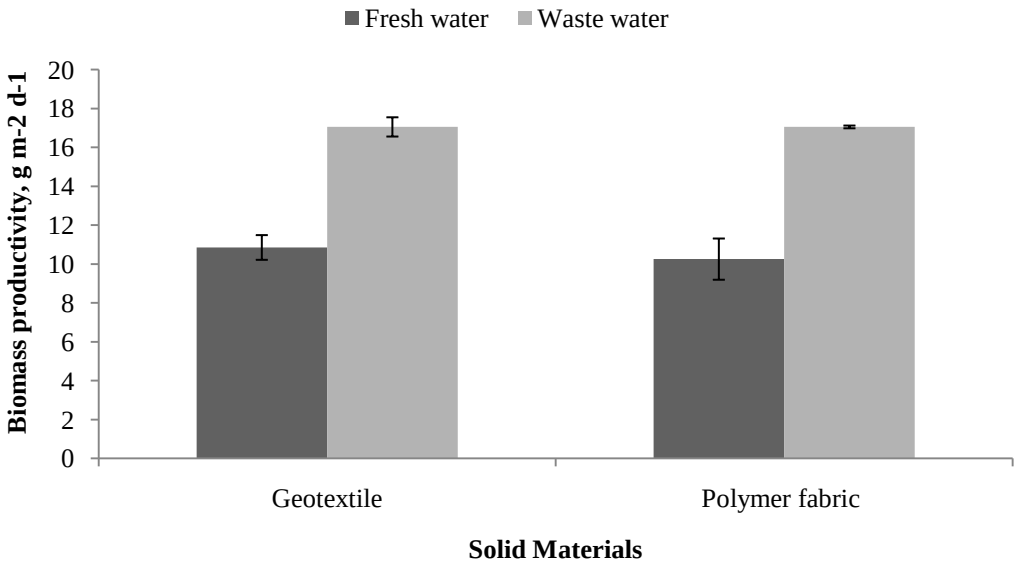

Fig. 3.2. Biomass productivity (g m^{-2} d^{-1}) results of fresh water and wastewater in Geotextile and Polymer fabric materials.

Productivity results obtained by using fresh water enriched with appropriate nutrients and wastewater in two solid materials used are compared. Wastewater performed better than fresh water yielding an average productivity of 17.5 g m^{-2} d^{-1} on both materials. The values obtained from doing two replications were averaged with the error bars shown in the graph.

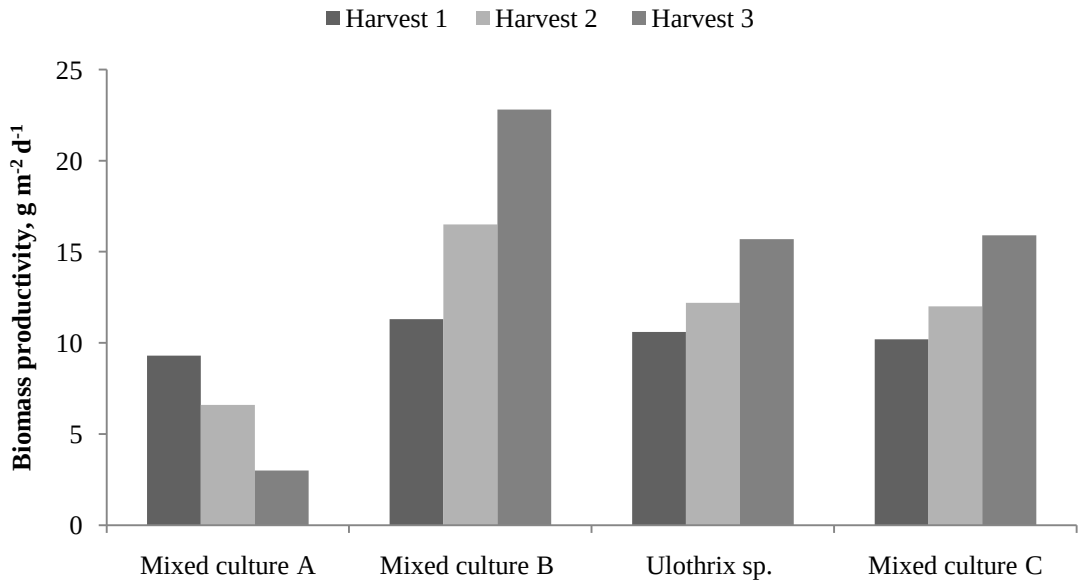

Fig. 3.3. Biomass productivity (g m^{-2} d^{-1}) results of all the three harvests obtained from three different inoculum compared against a control (mixed culture C)

Three harvests were done at the end of each experimental run (usually the 7^{th} day) and the productivity values were calculated for the different types of inoculums used for the study. Geotextile material was used as the solid support for algae biomat formation in the experiment. Mixed culture B inoculums resulted in consistently higher productivities compared to that obtained from other inoculums and control, giving a maximum yield of 22.8 g m^{-2} d^{-1} at the time of harvest 3.

Table 3.1

The table presents the Day 0 wastewater characterization results. As only a single reservoir was provided for this experimental study, a single sample represents the results for the entire system. However three replications were done for the nutrients analyses. The N:P ratio for the study was 6.3:1 which indicates that nitrogen (N) was not present in excess in wastewater, but sufficient enough to support attached algae growth. The Ph values ranged from 6.5-7.3, which could support green algae, diatoms and blue-greens colonization.

Sample	Parameter (ppm)			
Untreated carpet industrial waste water	**TN**	**PO_4**	**NH_4-N**	**pH**
	20.1 ± 0.81	10.6 ± 1.2	16.4 ± 0.77	6.5 - 7.3

Table 3.2

Biomass compositional analyses results of algal sample collected from the biomats for all experimental runs. The samples showed high percentage of carbon, and also protein of about 39% thereby making it suitable for biomethane production. The neutral lipids content was low (8.2%), however this has a major proporation of triacyglycerols (TAG) which is an important substrate for biodiesel production.

Paramters	Wastewater study	Inoculum study
% Carbohydrates	7.8 ± 0.8	8.5 ± 0.61
% Neutral lipids	8.2 ± 0.92	6.8 ± 1.1
% Protein	38.9 ± 0.66	42.3 ± 0.43
% Carbon (C)	46.45 ± 0.51	45.21 ± 0.72
% Hydrogen (H)	6.8 ± 0.44	6.3 ± 0.31
% Nitrogen (N)	8.5 ± 0.23	9.24 ± 0.15
% Sulfur (S)	0.54 ± 0.02	0.61 ± 0.04

CHAPTER 4

DEVEOPMENT OF A BENTHIC ALGAE CULTIVATION SYSTEM FOR BIOENERGY APPLICATIONS, WASTEWATER TREATMENT AND CARBON CYCLING

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Abstract: Benthic (attached) algae show significant potential to yield high biomass productivity and are effective in treating wastewater. The present study investigates a benthic algae cultivation system for biomass productivity for biofuels production and nutrient removal from a carpet industrial wastewater using two solid materials- Geotextile-fiberglass and Linen. Four horizontal biomat reactors were developed and a grid-method of biomass sampling was employed to establish daily productivity trends for the biomats. The maximum productivities achieved were in the range of 50 – 60 g m^{-2} d^{-1} for both materials and minimum productivities of 11.25 g m^{-2} d^{-1} for linen and 17.99 g m^{-2} d^{-1} for geotextile-fiberglass material. An average of 60-80% Total Nitrogen, >90% Nitrates, 80% Ammonia and 57.2% Total Phosphorus removals from wastewater by the benthic algae system was recorded. The biomass has been characterized with an energy value of 21 MJ/kg thereby making the entire system viable and feasible for bioenergy production.

Keywords: Microalgae, Attachment, Mass cultivation, Biomass productivity, Wastewater treatment.

1. Introduction

Depleting fossil fuel reserves and increased environmental hazards associated with its combustion have made renewable resources of energy an attractive alternative. Biomass which constitutes 47% of the total renewable energy consumption in the US is currently the largest renewable energy resource being used by the nation, recently surpassing hydropower energy (USDA-DOE, 2005). Biomass-derived fuels called biofuels are therefore sustainable providing energy security to the nation, environmentally safe to use, solve socioeconomic issues related to the rural sector and also contribute to nation's savings on foreign exchange by reducing dependence on foreign oil (Demirbas, 2006).The Energy Independence and Security Act of 2007 enacted a law under the Renewable Fuels Standard highlighting the U.S. biofuel production target of 22 billion gallons by 2022, out of which 21 billion gallons should come from cellulosic ethanol and other advanced biofuels. Though there are number of other potential biomass resources for biofuels, microalgae biomass feedstock show significant potential to meet this target due to multiple reasons; 1.Microalgae show rapid growth rates compared to terrestrial crops, completing their life cycle every few days 2. Their cultivation does not require agricultural lands and hence they do not interfere with food production (Chisti, 2007, 2008). 3. They have the ability to grow in all kinds of wastewaters, including industrial, sewage and brackish waters, thus offering a benefit of wastewater remediation (Bhatnagar et al., 2010; Zhang et al., 2008; Oswald et al., 1957) 4. They have great CO_2 biofixation potential thereby aiding to the reduction of greenhouse gas emissions into the atmosphere (Benemann, 2003) 5. They store energy in the form of lipids; average lipids content of many species vary from 1-70% and sometimes exceed 80% under nutrient-stressed conditions (Spolaore et al., 2006, Metting, 1996), and 6. Microalgae

biomass once cultivated could be routed to produce different biofuels such as biodiesel, biomethane, bioethanol and biohydrogen.

The most studied microalgal cultivation systems are the raceway ponds and photobioreactors operated for growing suspended algae. The major drawback with these culture systems is the ease of harvesting, due to the small size of algal cells (3-30 µm in diameter), low algal concentration in effluents and large volumes of water to be handled to recover the cells (Zhang, 2010). Though raceway ponds are technically simpler in operation, their drawbacks include highly inconsistent productivities due to environmental changes and predation, species contamination and high costs of inoculum and harvesting involved. All of these barriers are overcome in photobioreactors, however their fabrication is highly expensive and make it a tougher option for large scale cultivation. Benthic algae (attached algae) cultivation systems prove to be an effective option for algae cultivation because; 1. They are capable of achieving high productivities ranging from 25 – 40 g m^{-2} d^{-1} (Mulbry et al., 2008) 2. Harvesting of attached algae (mostly filamentous and blue-green algae) is easier and cost-effective; Occasionally there would also be no need of any device for harvesting, as these algae once they complete their life cycle get detached from the material (solid support on which algae attaches and grows) offering an advantage of 'self-harvesting' 3. Their cultivation is simple and inexpensive in that they cut down on the inoculum costs, as benthic algae have the ability to regrow from the residual cells that remained on the material after harvest. 4. They are effective in removing nutrients from wastewater (Kebede-Westhead et al., 2003; Mulbry and Wilkie, 2001). 5. Their fatty acid content is comparable to that of the suspended algal cultures making it more attractive for biofuel production (Johnson and Wen, 2010). A benthic algae cultivation system such as the Algal Turf Scrubber (ATS) has been thoroughly studied for wastewater treatment (including agricultural

run-off and digested and undigested dairy manure effluents) and algal productivity purposes (Mulbry et al., 2008; Wilkie and Mulbry, 2002; Adey et al., 1993). A recent study was carried out by Johnson and Wen in 2010, with an attached microalgal culivation system using single-species inoculum in a closed set-up for evaluating biofuel production. However, in-depth studies of attached algae culture systems specifically targeted for biofuel production are required to understand the feasibility of large scale production while addressing the practical problems of mass cultivation in such systems.

The present study investigates a benthic algae cultivation system in a greenhouse and carpet industrial wastewater as growth substrate for biomass productivity and nutrients removal. The objectives of the study were; 1. To develop a benthic algae cultivation system to do the experimental studies 2. Evaluate two judiciously chosen materials for biomass productivity and nutrients removal efficiency and 4. Analyze the biomass for bioenergy applications.

2. Methods

2.1. Reactor development, inoculum preparation and wastewater collection for algae biomat cultivation

Four sediment basins made of polyethylene were procured from Aquatic Eco-Systems, Inc., FL, and used as algae biomat reactors. The surface area of one reactor was 0.28 m^2. The reactors were mounted horizontally on a large piece of wooden plank made at a slope of approximately 1%. Below each reactor was placed a separate reservoir (Rubbermaid 50 gal tote, The Home Depot, GA) to hold wastewater and inoculum. The reservoirs were provided with submersible water pumps (Beckett pump, Model # G535AG20) purchased from a local store, for the purpose of water circulation over the biomat reactors. A material (solid support) was placed on the surface of each reactor on which the algae biomat grows in attached mode. The material was

pinned to a hard Styrofoam to ensure support and evenness of reactor surface. A floway system was designed to distribute water uniformly over the reactor surface. Each floway consisted of a hard PVC pipe (PVC Schedule 80 pipe) which had an inner diameter of 1.25 cm. The length of the pipe was cut to 45 cm to fit into the reactor's width and was threaded at both ends. Intermittent slits were made on one side and the non-threading section of the pipe at spacing of 2.5 cm to allow equal water discharge through the slits to the reactor surface. A pressure regulator was used to control the water flow rates through the floway system in the reactor. Proper tubing arrangements were made from the water pump in the reservoir to the floway system placed in the biomat reactor (Fig. 4.1).

2.1.1. Reactor operation and experimental strategy

On the start-up day of a fresh experiment, each reservoir was loaded with wastewater and inoculum (10 % v/v). The working capacity of a reservoir was 165 L (150 L of wastewater and 15 L of inoculum). Water (wastewater + inoculum) from the reservoir was circulated to the biomat reactor placed at the top. As the reactor was placed horizontally at 1 % slope, the water discharged from the floway system passes through the material surface and drains back into the reservoir through the drain hole, from where it is again circulated to the reactor by the submersible pump. In this continuous process of water circulation, the inoculum cells form a biofilm on the material and the cells further grow into an algae biomat by using the nutrients from wastewater. The process was continued until a matured algal biomat developed on the new material. This period of initial algae biomat formation takes about 10-12 days depending upon the environmental conditions and nature of the material. The biomat is then harvested from the material by scraping it off using a spatula, and the reservoir water is drained out into a large tote for safe disposal. The reservoir is again replenished with another set of wastewater (150 L) but

this time with no inoculum, and the next experimental run was started. The residual cells that already remained on the material after harvesting were used as inoculum for the new run.

2.1.2. Inoculum preparation

A mixed culture consisting of four algal strains was used as inoculum for biomat cultivation. The four strains, *Chlorella minutissima, Scenedesmus bijuga, Chlamydomonas globosa,* and *Ulothrix sp.,* were isolated from carpet industry wastewater following the procedure described by Chinnasamy et al. (2010). The algal strains were maintained as pure cultures in 500 mL of BG11 medium (Stanier et al., 1971). The four algal cultures were centrifuged at 8000 rpm for 10 minutes, the supernatant was discarded and the cell pellets were re-suspended in four Erlenmeyer flasks respectively, each containing 4000 mL of fresh BG11 medium. The flasks were incubated in an algae growth chamber at 25 ± 1 ºC and a light intensity of 75-80 µmol m^{-2} s^{-1}. After 7 days of incubation, the 4000 mL pure cultures were again scaled up to 20 L in carboys, in the same procedure described as above. The carboys were incubated in a growth chamber receiving a light intensity of 75-80 µmol m^{-2} s^{-1} and a temperature of 25 ± 1 ºC. The 20 L pure cultures were bubbled with 5 % CO_2 from a common air-CO_2 mixer at a flow rate of 2 L min^{-1}. The inoculum cultures were grown until a desired biomass density of approximately 0.1 g L^{-1} was achieved. 15 L of pure cultures from each of the four carboys were used for inoculation into the reservoirs whereas the remaining 5 L for maintenance and sub-culturing purposes for the subsequent runs. The four 15 L pure cultures were mixed in the proportion of 1:1:1:1 and used as a mixed culture inoculum at 10 % (v/v) for the initial algae biomat formation on the materials.

2.1.3. Wastewater collection

Untreated carpet industrial wastewater used in the present study was collected from Dalton, GA, once in 2 weeks. The wastewater was then stored in 1000 L capacity water totes placed in a

cool and shaded area and was used for the algal biomat experiments. Small samples were collected on day 0 of each run and subjected to chemical analyses to account for variation in chemical compositions of wastewater in each experimental run.

2.2. Evaluation of two harvesting methodologies for sampling of biomass from biomat reactors

Two harvesting methodologies were experimented for evaluating biomass productivity in the biomat reactors; the grid and non-grid method of harvesting. The grid harvesting method was chosen for the purpose of recording daily growth of biomass, so that a growth curve and peak productivity days could be established for such cultivation systems. The non-grid method is just the final day harvesting of biomass, and does not give information about the daily growth of algae biomat on the materials.

A preliminary experiment was conducted to evaluate and compare the peak day biomass productivities between the two harvesting methodologies, the result of which decided the biomass harvesting protocol for the forthcoming experiments with the biomat reactors. The two treatments in the experiment were the grid and non-grid harvesting methods and each treatment was replicated twice. The material used for biomat cultivation was Geotextile-fiberglass, also shortly referred as GF throughout the manuscript, which was actually two materials combined together (see section 2.3 for choice of material). After the initial algae biomat formation was achieved, the first experimental run was started (section 2.1.1). For the grid-method of harvesting, the total surface area of the material (0.28 m^2) was divided into 80 square grids of surface area 0.0036 m^2 each (6 cm x 6 cm) (Fig. 4.2). A paper template representing the material divided into grids was created for reference. The 80 square-grid template enables daily harvesting of biomass (4 replicate samples) from the material for 20 days. The 80 grids in the template were assigned random numbers from 1-20, 4 times each, representing the four biomass

replicate samples to be harvested on the respective days; for e.g. on day 4, 4 random grids (6 cm x 6 cm) were harvested from the biomat based on random grids on the template which were assigned the number '4', and similarly for day 5, 4 different grids were harvested based on the random grids assigned for day 5 in the template; Therefore the four replicates of biomass harvested on day 4 represents biomass growth from day 1 to day 4 and similarly for day 5, the samples represent growth from day 1 - day 5 and so forth.

After the startup of the first experimental run, the grid-method of biomass sampling was started on day 3, as bacterial biofilm conditioning on the material occurs in the first three days (day 0-day 2) on the material and would not be representative of algae biomass. The grid harvesting was done on two reactors and the non-grid harvesting on the other two biomat reactors. The non-grid harvesting just represents the harvesting done on the final day of the run (day 7 in the present study) when the biomass sloughing was about to occur, so that the peak productivities for both harvesting methods could be compared.

2.3. Evaluation of two fabric materials for biomass productivity and nutrients removal

Two fabric materials- Geotextile-fiberglass (GF) and linen were chosen for biomat cultivation on the reactors. GF was a combination of two materials used together as a single material for biomat production. The geotextile fabric was procured from Skaps Industries, Athens, GA, made of high quality polypropylene originally manufactured for the purpose of using it as a filter media for leachate solutions. The fiberglass portion and the linen material were purchased from local stores. The criteria for selection of the two materials were that they must be durable viz. they must be able to withstand multiple biomass harvests (be it manual scraping or any scraper device), easily procurable, and inexpensive (Johnson and Wen, 2010). An additional criterion taken into consideration for the present study was that the material surface must be

meshed or woven to provide some kind of resistance to the flow of water so that the cells get a grip on the material to attach and grow. Once the cells (mostly diatoms and filamentous algae) get hooked on the material surface, they produce extracellular polysaccharides (EPS) as mucilage which traps the other cells that come into contact, form a matrix and grow using the substrate nutrients (Hoagland et al., 1993).

2.3.1. Experimental strategy

After the initial biomat formation phase was completed, the first experimental run was started (section 2.1.1). The experimental run was continued until the peak biomat growth was recorded, which on an average occurred on the 7^{th} day. Harvesting was done after sloughing of biomass was observed, which usually occurred on the day after the peak growth of biomat. This harvesting day marks the final day of the first run and the entire run is referred to as E1H1, in which E1 denotes the first experimental run and H1 the first harvest, which spanned for 8 days. On the completion of E1H1, the reservoir was replenished again with 150 L wastewater and the second run was started which is referred to as E1H2, again spanning for 8 days. The experiment was continued until the completion of third run, E1H3. This entire set of three runs of harvests and re-growths (E1H1 to E1H3) each spanning for 8 days is referred as the first experimental set. The H2 and H3 refer to the 2^{nd} and 3^{rd} harvests respectively denoting the completion of the respective runs. Three-time harvesting and regrowth was done in order to establish biomass growth trends for the materials, to test if the materials gave consistent productivities, evaluate if there is an effect of subsequent biomat re-growths on productivity, test the durability of the materials, and assess the nutrients removal potential of the biomats. A second experimental set was done representing two runs E2H1 and E2H2, which is just the replicate of the first set. However the third run E2H3 could not be continued for more than 3 days, due to overgrazing of

the biomats by predators (fruit flies, mosquito larvae, rotifers and chironomids). In both sets of experiments, inoculation was done only once, during the startup of the first and second sets to initiate biomat formation on the fresh materials. For the subsequent runs within each set, the cells that remained on the material after each harvest served as inoculum.

2.3.2. Biomass and nutrients data collection

Daily samples of biomass were collected from the reactors using the grid-method of harvesting (section 2.2). The samples were collected in small pre-weighed aluminum trays (W1) and wet biomass-tray weights (W2) immediately after harvesting were determined using a weighing balance. The biomass trays were placed in hot air oven at 80 ºC for 24 h, kept in desiccator for 2 h, and their dry weights (W3) were recorded to 4 decimal points. The dry weights of biomass were calculated as follows;

Dry weight of biomass = (W3 – W1) g

The moisture content of the biomass samples were calculated using the formula;

Moisture content (% wet basis) = [(W2 – W3) / (W2 – W1)] x 100

To evaluate the removal of nutrients by the biomat reactors, 200 mL of wastewater was sampled from the reservoirs daily during the course of experimental runs, and stored in the refrigerator at 4 ºC for nutrients analyses. Aliquots of samples were filtered through 0.2 µm Whatman filter aids using a syringe prior to storage and analyses. The samples were analyzed for total nitrogen (TN) by persulfate digestion method and total phosphorus (TP) by molybdovanadate method with acid persulfate digestion. Nitrate, phosphate and ammonia were analyzed by cadmium reduction, ascorbic acid, and salicylate methods respectively. All analyses were done using a block digester (Hach DRB 200) and a spectrophotometer (Hach DR 2700, Loveland, CO).

2.4. Measurement of environmental parameters

The biomat cultivation studies were performed in a greenhouse to maintain optimum culture conditions. However, the major environmental parameters (temperature, pH, and light intensity) for all the experimental runs from E1H1 to E2H2 were measured on a daily basis, as significant variations in culture conditions between the runs would be expected. Temperature (ºC) and pH of water in the reservoirs were measured periodically using the pH-temperature meter (Fischer Scientific, Accumet portable AP62). The light intensity (μmol m^{-2} s^{-1}) on the biomats was measured using a photosynthetic active radiation (PAR) meter (LI-COR data logger and quantum sensor, LI-1400, Lincoln, Nebraska) during the afternoons when temperatures plateau. Data on maximum ambient temperature (deg F) for all the days of experimental runs were used from the website, www.georgiaweather.net for UGA-Watkinsville area. However, this was not representative of the ambient temperature in greenhouse. The microbial community succession on the biomats in order of dominance was studied for all the runs using light microscopy. In this way, an understanding of the community pattern development between each run as the biomat is regrown could be established and their relation to biomass productivities was evaluated.

2.5. Biomass compositional analyses

The dried biomass was powdered using a mortar and pestle and stored in refrigerator at 4 ºC prior to analyses.

Neutral lipids for all dry biomass samples were analyzed gravimetrically using an Ankom XT10 automated extraction system with hexane as the extraction solvent. A known quantity (1 g) of dry biomass sample was sealed in pre-weighed Ankom XT4 extraction bags (W1) using an impulse sealer, dried in hot air oven at 60 ºC overnight, cooled in a desiccator for 2 h and

weighed (W2) accurately to five decimal points in a weighing balance.. The bags were then placed in the Ankom system and the process was set to perform at 105 ºC for 2 h. After the extraction process was over, the bags were dried at 60 ºC overnight, cooled in a desiccator and weighed (W3) carefully. The % neutral lipid in the biomass sample was calculated using the formula;

% Neutral lipids = [(W2 – W3) / (W2 – W1)] x 100

Carbohydrates were estimates using the method described by Dubois et al. (1956). Percentage protein was calculated by using a conversion factor of 4.58 (Lourenço et al., 1998) multiplied with the % nitrogen analyzed from the ultimate analysis (elemental analysis of carbon, hydrogen, nitrogen and sulfur percentages). Proximate analysis (analysis of moisture content, fixed carbon content, volatile solids and ash content) was done using a LECO TGA701 proximate analyzer and elemental analysis was determined using a LECO CHNS932 analyzer. The calorific value of biomass samples was determined using a bomb calorimeter.

2.6. Data Analysis

Statistical analyses (1-way or 2-way ANOVA) using SAS software were done for determining significant differences in treatments used in the corresponding experimental studies. The percentage nutrient removal profiles were calculated by the formula;

% Nutrient removal = (Initial day - Final day) concentrations x 100

The biomass productivities were calculated by dividing the amount of biomass (g) by the area of biomass harvested (for grid type, it is 0.0036 m^2 and for non-grid type it is 0.28 m^2) by the number of days the biomass was grown (in grid type, it is the corresponding day on which it was harvested and for non-grid type, it is the final day of harvesting).

3. Results and discussion

3.1. Inoculum cell density and wastewater characterization

The biomass densities of the inoculum strains, *Chlorella minutissima, Scenedesmus bijuga, Chlamydomonas globosa,* and *Ulothrix sp.* were determined in interval of every 4 days until the desired concentration of approximately 0.1 g L^{-1} was achieved in each of the four cultures. After the desired cell concentration has been achieved, the cultures were mixed together and used as a mixed culture inoculum at the beginning of the biomat experiments. The initial cell densities of *Chlorella minutissima* in the inoculum were slightly higher than the other species as it was the fastest growing species among the other cultures.

The untreated carpet industrial wastewater has been characterized for nutrients concentration to support benthic algal growth. The day 0 analyses of wastewater for each run was recorded (Table 4.1). The influent total nitrogen (TN) concentrations for the first experimental set were considerably higher than that for the second replicate set. The ammonia and nitrates concentrations were below deductible limits in the test assays for the first and second sets respectively, indicating the nutrients were not present in the wastewater for those experimental sets.

3.2. Comparison of grid and non-grid biomass productivities

The biomass productivities were evaluated for the two harvesting methodologies. The final day results (day 7) of the non-grid harvesting on two reactors and that of the grid method harvesting (the four harvested grids from the reactors) on the other two reactors were averaged and compared (Table 4.2). The results showed the final day productivity determined by grid-harvesting method was recorded to be 29.3% higher than the productivity values obtained from non-grid harvesting method. However, the grid method was chosen as the harvesting protocol for

further experimental studies with algae biomat reactors. The purpose of choosing grid over non-grid harvesting is that the daily growth of biomat could be studied approximately and the day on which the peak productivity occurred could be recorded. This information helps in understanding the peak productivity days for the present benthic algae cultivation system developed for the study. This would help in preventing loss of biomass due to sloughing and harvestings could be done accordingly.

3.3. Performance of algae biomat reactors with two fabric materials

3.3.1. Biomass productivity potential of the two materials

The biomass productivity trends of GF and linen materials were evaluated for all the runs from E1H1 through E2H2 (Fig. 4.3). The average peak productivity achieved with GF material for E1 (H1-H3) was 55.8 g m^{-2} d^{-1} and that for E2 (H1-H2) was 23.33 g m^{-2} d^{-1}. Similarly, the average productivities achieved with linen material for E1 (H1-H3) and E2 (H1-H2) were 40.3 g m^{-2} d^{-1} and 11.84 g m^{-2} d^{-1} respectively. Overall, GF material gave significantly higher productivities compared to linen material ($p<0.05$). The moisture content of the biomass samples were in the range of 88% - 95% (wet basis), and the ash content ranged from 8% - 9% dry basis for all the runs. A huge decrease in peak productivities from E1 to E2 by 58% and 71% for GF and linen materials respectively was observed and recorded. This decrease may be due to the change in culture conditions each time when a fresh run was started. For example, in E2 runs, the light intensities recorded were much lower in the range of 960 - 975 µmol m^{-2} s^{-1} than that recorded for E1 experiments, which were in the range of 1060 - 1355 µmol m^{-2} s^{-1} (Table 4.3). Algal turf communities in exposed coral reefs reach peak productivities at midday light intensities of about 1400 µmol m^{-2} s^{-1} (Adey and Hackney, 1989). This could explain the higher peak productivities achieved in E1 runs, and relatively lower peak productivities in E2. Also the

productivity of benthic algae depends upon the local culture conditions that include water velocities, taxonomic composition of the biomats and composition of the coating and attachment strategies of algae (Ács, 1998). Productivities ranging from a maximum of 60.9 g m^{-2} d^{-1} during summers to 4.2 g m^{-2} d^{-1} in winters were reported for the ATS systems (Craggs et al., 1996). Earlier studies with series algal scrubber systems reported productivities ranging from 33 - 39 g m^{-2} d^{-1} (Adey et al., 1993). In earlier studies carried out with ATS systems, a wave surge was induced in the water flow over the biomat surface with the help of a surge pump, a concept which typically doubles algal productivity (Adey and Loveland, 1991). However in the present study, a wave surge was not used, instead meshed/woven surfaced materials were chosen for biomat production to give resistance to water flow and capture filamentous algae on the biomat. In sum, the benthic algae cultivation system developed for the present study was capable of producing high productivities on par with suspended algae culture systems.

3.3.2. Nutrients removal potential

The nutrient removals by the two materials were evaluated for all runs and plotted against their corresponding biomass productivities in those runs (Fig. 4.4 and Fig. 4.5). The first set E1H1-E1H3 shows nutrient profiles for total nitrogen (TN) and nitrates (NO_3), while the second set E2H1-E2H2 shows profiles for total phosphorus (TP), ammonia (NH_4) and TN. Total phosphorus was analyzed for the first set of runs; however the data are not reported in this paper as they were found to be erroneous due to measurement errors. It is observed that in all the runs of the first set, the nutrients get depleted as biomass productivity increased or peaked, except for E1H3 Linen treatment in which there was comparatively only 46% TN and 61% nitrate removals which also reflected in its relatively lower productivity of 21.7 g m^{-2} d^{-1}. Overall on an average there was >90% nitrate and >80% TN removals recorded in the first experimental set. From the

second set (E2H1, E2H2), the % TN removals on an average were not as significantly high as that achieved in the first set. However an average of 80% removal was recorded for ammonia (NH_4). Ammonia is one of the most commonly used forms of inorganic nitrogen by bacteria and phytoplanktons and also is invariably the preferred nitrogen source when it available (Reay et al., 1999; Wheeler and Kokkinakis, 1990; McCarthy and Carpenter, 1983). More importantly, there were no nitrates present in wastewater for this set. Hence the reason for high % NH_4 removal by the biomats recorded for this set. Interestingly there was no significant ammonia present in wastewater for the first set, where more than 90% nitrates were removed by the biomats. It is evident that the algae biomats grown in this system utilizes either of the inorganic nitrogen forms effectively when one of them was absent.

The % TP removals in second set were on an average 54.5% for GF and 61.5% for linen materials. Correspondingly in E2H1, GF and linen materials showed 81% and 61.2% P recovery in harvested solids. Mechanisms other than biomass uptake involved in phosphorus removal would be precipitation, adsorption/desorption and emigration through predation and larval emergence (Hydromentia, 2005). However P removal was not evaluated for the grazers present in the biomat in this study. Interestingly in E2H2, the GF material showed more than 100% P recovery in solids, suggesting that there had been additional phosphorus on the biomats not accounted from the influent wastewater source. It is likely due to measurement errors of the biomass which had large densities of chironomids (blood worms) that also accounted for a major % of phosphorus and were not separated from the algae biomass. Phosphorus exchange, consumption and excretion by chironomids have been well discussed (Gallepp, 1979). Hence, the very high % P in harvested solids not accounted from the influent does not represent the true % P of algae biomass. The % P of the biomass for this run was 1.36 ± 0.07. Literatures on ATS

systems have reported % P of algae biomass to be in the range of 0.3% to 2.1% depending on the type of wastewater or manure used in its production (Hydromentia, 2005; Craggs et al., 1996; Adey et al., 1993). In sum, from the first set, the subsequent re-growths of biomats did not affect the nutrients removal efficiency of the reactors significantly. E1H2 which had the highest influent TN concentration of 35 mg L^{-1} among the runs showed highest percentage TN removal of >90% for both materials. From the 2-way ANOVA analysis results, in the first set from E1H1-E1H3, GF material performed better than linen in nutrients removal ($p<0.05$), while in second set, there was no significant difference between GF and linen materials in nutrients removal efficiency ($p>0.05$). The first set showed an average of 80% TN and >90% NO_3 removal for all the three runs; while the second set showed a removal on an average of only 63% TN, 57.2% TP and 80% of ammonia.

3.3.3. Biomass productivity trends and their correlation with change in environmental conditions

In the first set of experiments (E1H1 to E1H3), both the materials almost showed a similar trend in which the productivity showed a sharp rise from day 5 - day 6, peaked on day 7, and dropped on day 8. For example, in E1H1, the GF material showed an increase in productivity by 255% from day 5- day 6, peaked on day 7 by an increase of 3% from day 6 – day 7 and dropped by 95% on day 8. And similarly for linen material in E1H1, an increase in biomass productivity by 245.6% from day 6 – day 7 and a decrease by 50.2% from day 7 – day 8 was recorded. This trend of a sharp rise in productivity from day 5 – day 6 has been occurring consistently with subsequent harvests and re-growths, E1H2 and E1H3 for both materials. However, the similar trend was not observed in the second replicate set (E2H1, E2H2) in which the peak productivities occurred well before day 6, and were also approximately two times lower than the first set for both materials. In E2H1 with GF material, there was 142% increase in productivity from

day 4 – day 5 despite reaching only a maximum of 17.99 g m^{-2} d^{-1} immediately followed by a 33.5% decrease on day 6; while for linen material there was only a 23.5% increase in productivity from day 3 – day 4 to its maximum and again a sudden drop in biomass yield by 8.3% on day 5 was observed. In E2H2, the GF material recorded a relatively high productivity of 27.79 g m^{-2} d^{-1} earlier on day 3. The rise in yield from day 2 – day 3 in this run was not recorded, as uniform algae biomat was not established on the materials by then. The peak productivity of 28.45 g m^{-2} d^{-1} on day 5 was only 2.3% higher from day 3, and again it dropped by 56% on day 5. The sudden drop in productivities immediately after reaching its peak is mainly attributed to the hydrodynamic forces imposed on the thick biomat and thereby leading to sloughing of biomass from the material. Biomats formed under conditions of low water velocities are thicker and may have higher biomass accumulation than those grown at high velocities, and also the former are less susceptible to shear forces while the latter are thinner and more stable (Vieira and Melo, 1999; Vieira et al., 1993). Due to the same reason, such biomats tend to grow faster and complete their life cycle earlier than thinner biofilms. The enormous rise in productivities in one day as observed from the trends in the first set of runs is attributed to a combination of environmental parameters associated with it that includes measured water temperature, pH of water, light intensities on the surface of biomats and maximum ambient temperature recorded for the peak day (Table 4.3). Also the taxonomic composition of the biomats played a major role in the rise in biomass growths which was again influenced by the environmental conditions at which the biomats were exposed. The microbial community of the biomats in order of dominance consisted of *Ulothrix sp.*, *Anabaena variabilis*, *Oscillatoria sp.*, *Phormidium sp.*, *Chlamydomonas globosa*, *Scenedesmus bijuga*, *Chlorella minutissima*, and a few unidentified species of filamentous algae. The generation times of the above identified filamentous and

cyanobacterial species were reported to vary from 7 to 25 hours (Iwai and Kitao, 1994). From table 3, it is evident that the first set of runs from E1H1-E1H3 were exposed to higher lighter intensities ranging from 1066 – 1363 µmol m^{-2} s^{-1} compared to the biomats cultivated in the second set which had measured light intensity of 970 µmol m^{-2} s^{-1} on an average. Though there was not a huge difference in light intensities between the two sets, it is likely that the composition of the biomats established during each run had an effect in their doubling times as a result of the slightest change in the culture conditions.

It was also observed that the peak productivity decreased with increased number of harvests from E1H1 to E1H3, but this decrease was not as significant in GF material as that observed in linen. However, the scenario occurred exactly the opposite for the second set (E2H1 and E2H2), where the peak productivity increased with increased number of harvests or re-growths, however a third run E2H3 could not be completed due to overgrazing by predators.

3.4. Biomass characterization

The compositional analyses for the biomass samples obtained from the first and second set of runs for both materials were determined (Table 4.4). All the biomass samples showed a higher protein content in the range of 40-50% and a comparatively lower lipids and carbohydrates. The neutral lipids for the samples were in the range of 3.3 - 8.6% and carbohydrates in the range of 9.1 - 21% as shown in the table. It is interesting to note that, despite the low influent TN concentrations in the wastewater, the biomass samples showed a comparatively high protein content which is again attributed to the composition of biomat that also included suspended solids and clumps of organic particulates. As the biomats had mixed microbial communities, also including bacteria and chironomids, the biomass samples would be expected to show slight variations in percentage elemental compositions. The energy value of the biomass samples were

determined to be 21 KJ g^{-1}, which are in the range of 20 - 25 KJ g^{-1} reported in literature (Ben-Amotz, 2007; Sheehan et al., 1998). The % neutral lipids obtained for present study could be converted to biodiesel and the energy present in the biomass could be routed to produce biomethane from anaerobic digestion process (Chinnasamy et al., 2010). Therefore, the benthic algal cultivation system developed for the present study yielded high biomass productivities and their biochemical compositions show significant potential for biofuel production.

4. Conclusion

Geotextile-fiberglass material performed better than linen, yielding a maximum biomass productivity of 58.66 g m^{-2} d^{-1} during early summer and a minimum of 17.99 g m^{-2} d^{-1} in the late summer period. An average of 60-80% TN, >90% nitrates, 80% ammonia and 57.2% TP removals from carpet industrial wastewater was recorded for the materials. The compositional analyses results of biomass samples showed significant potential for biofuel production. In sum, benthic algae cultivation system developed for the study was able to achieve high productivities and treat wastewater simultaneously thereby making the system suitable for large scale algae cultivation for bioenergy applications.

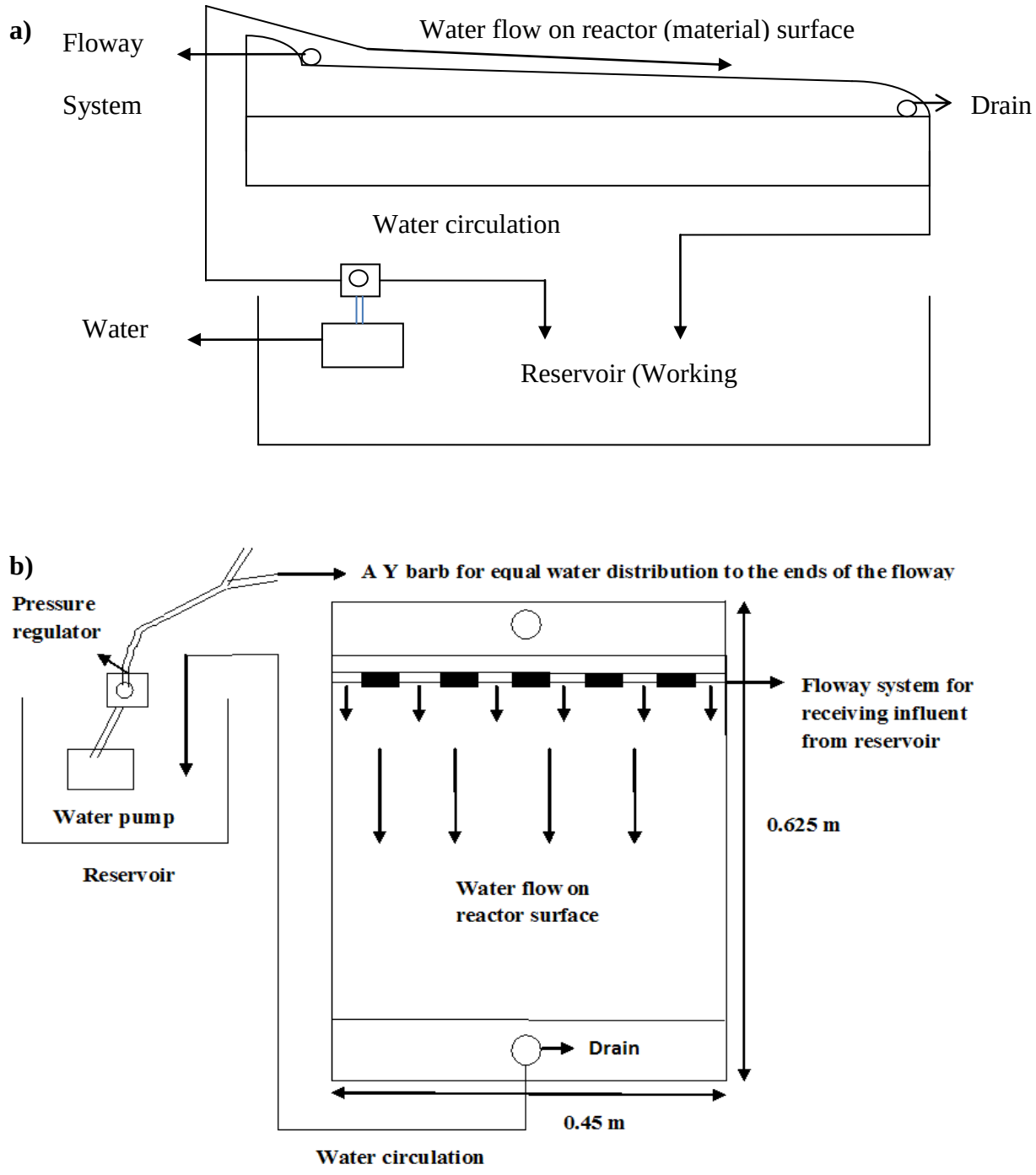

Fig. 4.1. Sketch of a biomat reactor with reservoir

a) Side-view of biomat reactor and a reservoir showing water circulation using a pump, over the material surface placed on the reactor. The water flow rates on the material were controlled using a pressure regulator b) Front-view of biomat reactor showing the floway system for uniform water distribution over the surface of reactor (material).

Fig. 4.2. Sample photograph of grid method of harvesting biomass

A photograph showing grid-method of biomass harvesting from a fully grown algae biomat on Geotextile-fiberglass material. The size of each grid was 0.06 m x 0.06 m. Four grids of biomass samples were harvested from the biomat reactors on all the days of experimental runs starting from day 3 till the final day of that run.

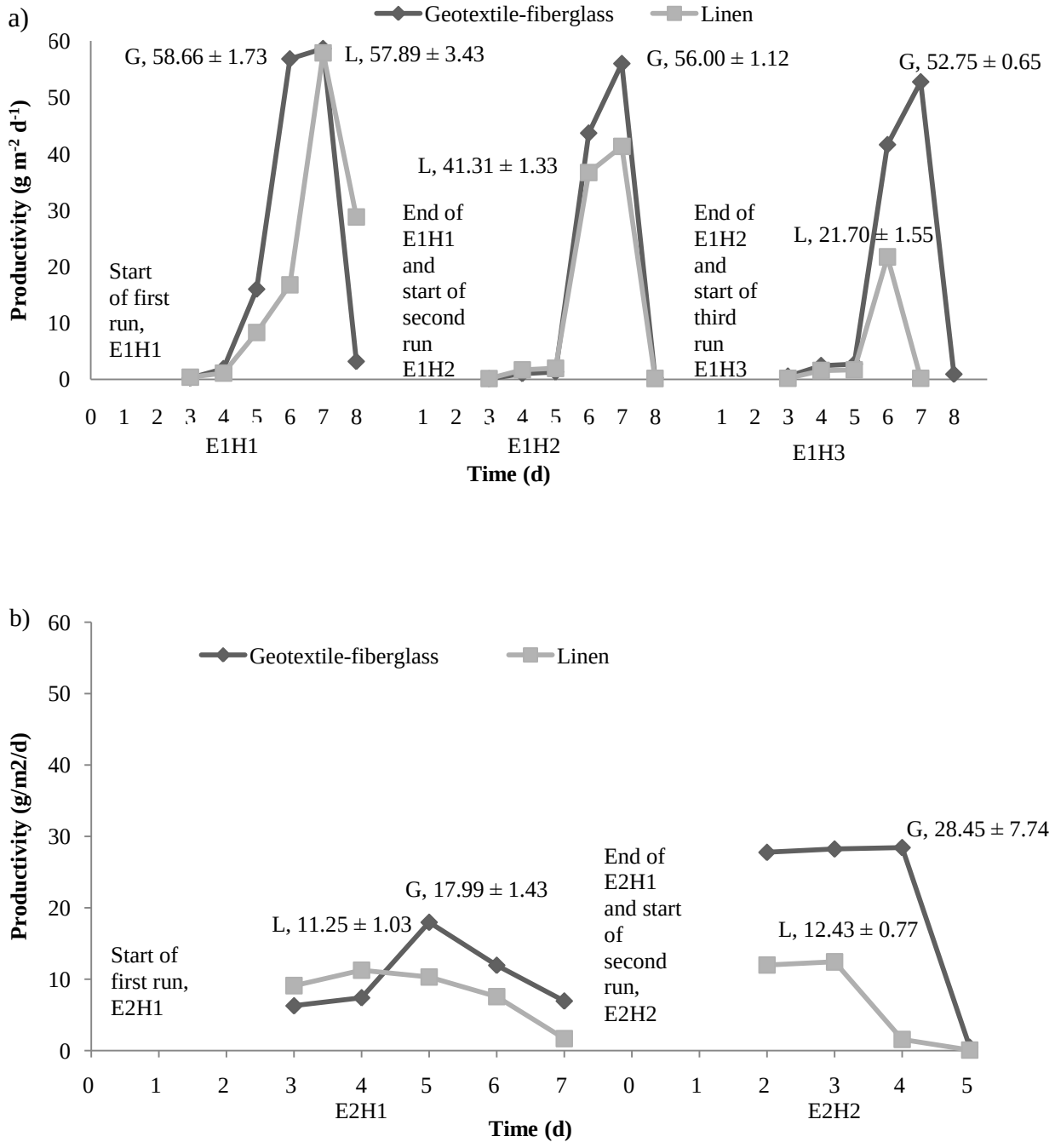

Fig. 4.3. Biomass productivity trends evaluated for first and second experimental sets.

Daily biomass sampling was done by grid method of harvesting and was started from day 3 for all the runs as bacterial conditioning on the materials was established on the first 2 days and were not representative of algae biomass. a) Productivity trends for runs, E1H1 to E1H3, each spanning for 8 days. b) Productivity trends for runs, E2H1 and E2H2. The peak productivities and their standard deviations are reported in the graph for each run and treatment.

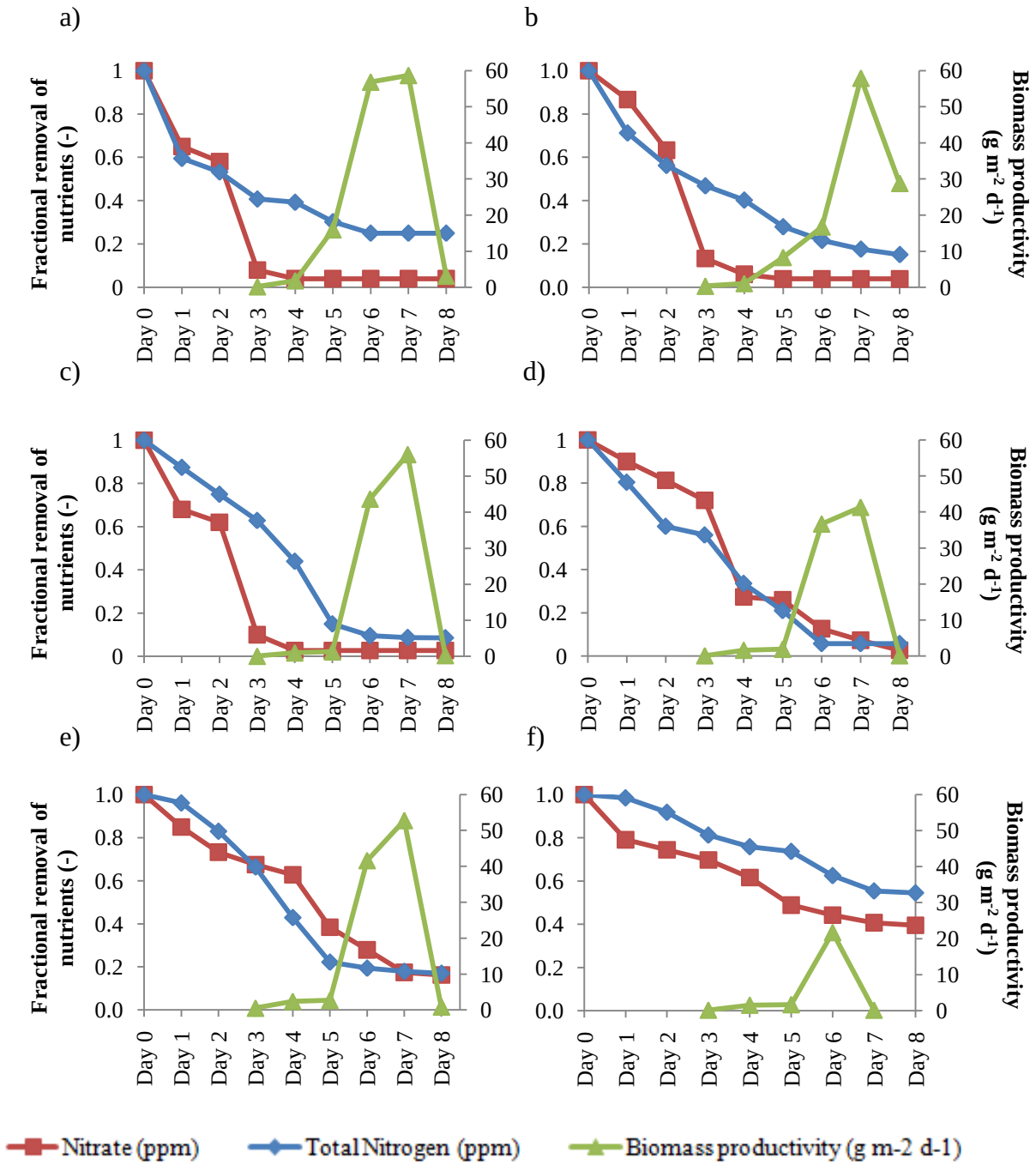

Fig. 4.4. Nitrate and Total Nitrogen removal profiles plotted against their respective biomass productivities of the first experimental set (E1H1 to E1H3)

The fractional removal of nutrients from reservoir (Concentration on that day/Initial concentration) and biomass productivities vs. Time (d) was plotted for the runs; a) E1H1 Geotextile-fiberglass b) EIHI Linen c) E1H2 Geotextile-fiberglass d) E1H2 Linen e) E1H3 Geotextile-fiberglass f) E1H3 Linen

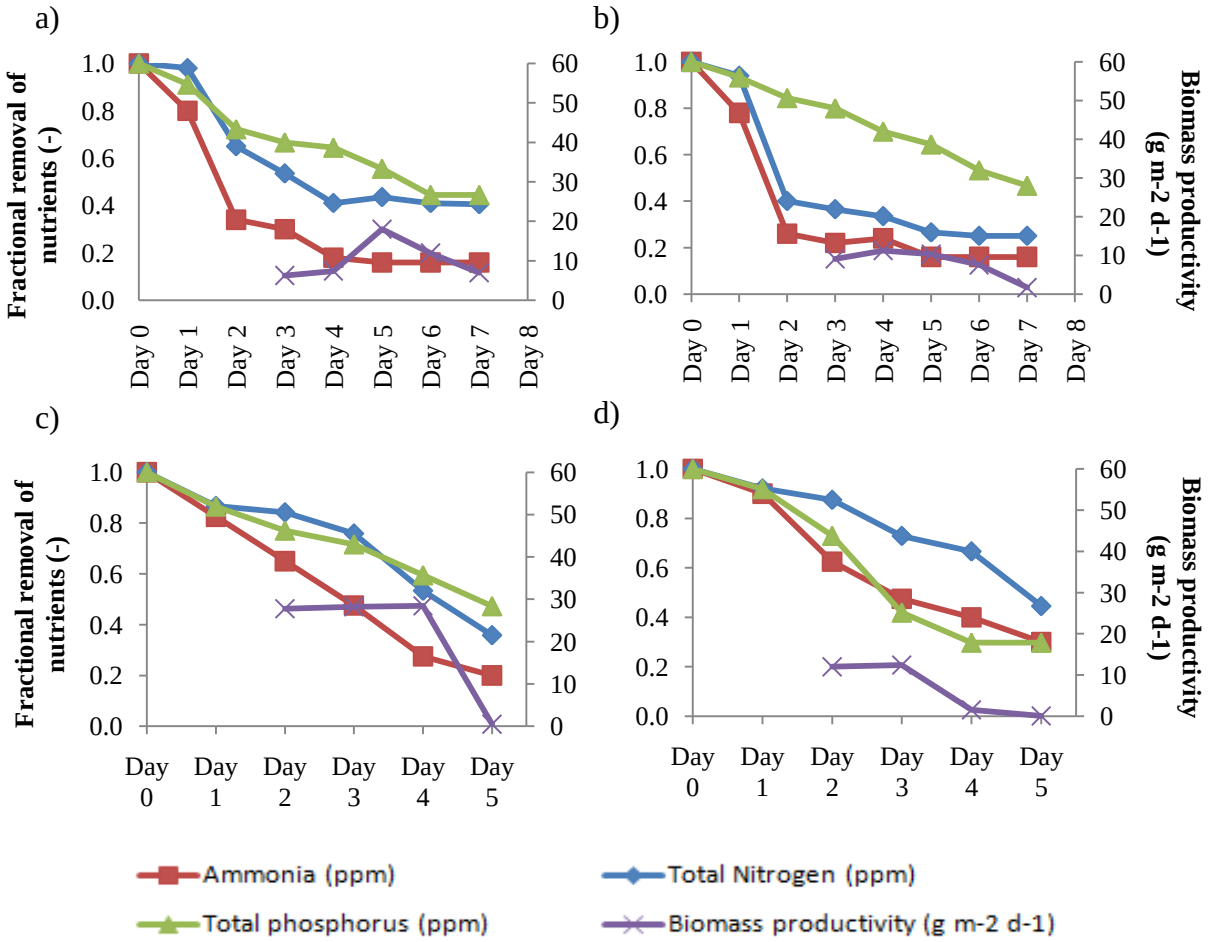

Fig. 4.5. Ammonia, Total Nitrogen and Total phosphorus removal profiles plotted against biomass productivities vs. Time (d) for the second replicate set

Ammonia, Total Nitrogen and Total phosphorus removal profiles from reservoirs is plotted against their corresponding biomass productivities for the second replicate set (E2H1 and E2H2) for the treatments Geotextile-fiberglass and Linen materials. a) E2H1 Geotextile-fiberglass b) E2H1 Linen c) E2H2 Geotextile-fiberglass d) E2H2 Linen. The third run E2H3 could not be completed due to overgrazing of the biomats by predators.

Table 4.1

Nutrients analysis of influent carpet industrial wastewater

Influent wastewater analyzed for Total Nitrogen, Total Phosphorus, Ammonia and Nitrate (mg L^{-1}) on day 0 of the runs E1H1, E1H2, E1H3 from first set and E2H1, E2H2 from second set. Total phosphorus for the first set of runs were analyzed, but were discovered to be erroneous due to a measurement error with the test assay.

Runs	Influent Total Nitrogen (mg L^{-1})	Influent Total Phosphorus (mg L^{-1})	Influent Ammonia (mg L^{-1})	Influent Nitrate (NO_3^-) (mg L^{-1})
E1H1	20	na	bdl	5
E1H2	35	na	bdl	7.5
E1H3	23.2	na	bdl	4.3
E2H1	10	4.5	2.5	bdl
E2H2	12	3.7	2	bdl

Influent Total Nitrogen = Total kjeldahl nitrogen (organic and reduced nitrogen) + ammonia + nitrate-nitrite
na - not available
bdl - below detectable limit

Table 4.2

Comparison of productivities between grid and non-grid harvesting methodologies of biomat reactors

The material used for biomat production was Geotextile-fiberglass. Non-grid harvesting represents the final day harvesting of biomass from the entire surface area of the reactor (0.28 m^2). The grid method of harvesting represents the harvesting made on four grids each of size 0.06 m x 0.06 m from the biomat reactors for the final day. The productivity values of both harvesting methodologies were compared.

Parameter	Non-grid harvesting (n=2)	Grid harvesting (n=2)
Biomass productivity data, (g m^{-2} d^{-1})	6.81±1.12	8.81±2.72

Table 4.3

Peak productivities and change in environmental conditions

Peak productivities and change in environmental condition that includes water temperature (ºC), Photosynthetic active radiation (PAR, µmol m^{-2} s^{-1}), maximum ambient temperature (deg F) and pH measured for all the runs on peak days.

Runs	Treatments	Peak productivity (g m^{-2} d^{-1})	Peak productivity day	Water temperature (ºC)	PAR (µmol m^{-2} s^{-1})	Maximum ambient temperature (deg F)	pH
E1H1	G-F	58.66 ± 1.73	7	35	1240	67.1	7.5
	Linen	57.89 ± 3.43	7	33.5	1169	67.1	7.2
E1H2	G-F	56.00 ± 1.12	7	33.5	1066	84.9	7.3
	Linen	41.31 ± 1.33	7	34	1077	84.9	8.0
E1H3	G-F	52.75 ± 0.65	7	31	1363	83.5	7.8
	Linen	21.70 ± 1.55	6	30	1352	83.5	8.1
E2H1	G-F	17.99 ± 1.43	5	26	974	88.5	8.4
	Linen	11.25 ± 1.03	4	27.5	973	89.6	8.5
E2H2	G-F	28.45 ± 7.74	4	26.3	970	97.7	8.0
	Linen	12.43 ± 0.77	3	26.5	965	96.1	7.8

Table 4.4

Compositional analyses of biomass obtained from each of the runs.

The daily biomass samples were pooled for each run and subject to analyses.

Runs	Treatments	%Proteins	%Lipids	%Carbohydrates	%C	%H	%N	%S	%Ash
E1H1	GF	44.4	8.22	19.5	48.8	6.6	9.7	0.7	8.44
	Linen	43.5	4.24	21	46.8	6.5	9.5	0.7	8.61
E1H2	GF	53.1	8.65	16.3	46.6	7.0	11.6	0.6	8.58
	Linen	54	7.47	17.5	47.3	6.9	11.8	0.7	8.7
E1H3	GF	48.5	3.34	10	45.8	6.1	10.6	0.7	8.46
	Linen	48.1	3.53	9.1	41.9	6.1	10.5	0.6	8.72
E2H1	GF	51.7	4.12	14.5	46.5	6.2	11.3	0.7	9.09
	Linen	51.2	4.20	15.1	42.1	6.6	11.2	0.7	8.59
E2H2	GF	43.5	5.65	16.7	45.5	6.5	9.5	0.6	9.15
	Linen	43	5.9	16.3	49.8	7.2	9.4	0.7	8.09

CHAPTER 5

CONCLUSIONS

Geotextile-fiberglass material performed better than linen, yielding a maximum biomass productivity of 58.66 g m^{-2} d^{-1} during early summer and a minimum of 17.99 g m^{-2} d^{-1} in the late summer period. An average of 60-80% TN, >90% nitrates, 80% ammonia and 57.2% TP removals from carpet industrial wastewater was recorded for the materials. The mixed culture inoculum used for the study initiated algal attachment and produced a matured biomat on the fresh materials. The subsequent harvests and re-growths did not affect the biomass productivity on the materials. However, the productivity of benthic algae depends upon a combination of factors that includes, water velocities, ph, water temperature, light intensity, ambient temperature and taxonomic composition of the biomat. The compositional analyses results of biomass samples showed significant potential for biofuel production. In sum, benthic algae cultivation system developed for the study was able to achieve high productivities and treat wastewater simultaneously thereby making the system suitable for large scale algae cultivation for bioenergy applications. However future research is needed in optimizing the best conditions for benthic algal productivity and assess cost economics of the cultivation system to make it a viable technology in the entire scenario of algae to biofuels.

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