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Microalgal bioremediation of nutrients in wastewater and carbon dioxide in flue gas

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MICROALGAL BIOREMEDIATION OF NUTRIENTS IN WASTEWATER AND CARBON DIOXIDE IN FLUE GAS

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ABSTRACT

Microalgae are potential biofuel feedstocks that can provide solutions to the twin challenges of energy security and environmental pollution. They have great potential for the removal of excess nitrogen and phosphorus from wastewater including the farm runoff. They can capture carbon dioxide in the flue gas from coal fired power plants thereby reducing greenhouse gas and also producing algal biomass, which can be converted into biofuel. In this study, the nutrient consumption of *Scenedesmus* algae was investigated in a batch culture and the biomass yield was determined. Algae were able to utilize carbon in the form of soluble carbonates which can be derived from carbon dioxide. Dewatering of microalgal biomass is a major obstacle in large scale cultivation. Harvesting algal culture in different proportions, 10-90% volume, revealed that the less-frequent removal of larger volume produced similar amount of biomass but required lower labor cost. Chitosan was found to be an effective bioflocculant for the separation of algae via the flocculation-aided settlement method. Approximately 30% reduction in the rate of biomass yield was observed when the water from the flocculation-aided harvesting process was recycled for the subsequent cultivation. The prevalence of herbicides such as atrazine in aquatic systems can present a problem if wastewater is utilized in the cultivation of microalgae due to its potential toxicity. The maximum tolerance level of atrazine for the *Scenedesmus* algae was found. This study showed the potential of using microalgae to reduce nitrogen and phosphorus in wastewater. The ability of algae to use soluble carbonate salts for their growth could be implemented to sequester carbon dioxide from power plants when the algae ponds are not located near them.

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1. INTRODUCTION

1.1. MICROALGAE FOR SUSTAINABLE ENERGY AND ENVIRONMENT

The search for alternate forms of energy is growing more intense with the depletion of fossil fuels and the accumulation of greenhouse gases that make current practices and conditions unsustainable. Biofuels are considered as best alternate fuels because they are made from nontoxic and biodegradable resources. They can utilize carbon dioxide from various sources, and they produce less SO_x, NO_x, and particulate emissions when burned. They contribute to sustainable development by reducing the greenhouse gas emissions and thus mitigate the effects of climate change and global warming.

Biodiesel is prepared mainly from soybeans in the US and canola in Europe; sunflower, corn, and rapeseed are also significant sources. Biodiesel is also obtained from a number of sources like coconut, palm oil, jatropha, animal fat, and algae. Fuel produced from algae does not compete with food supplies as that produced from other oil crops does. Microalgae can produce biodiesel with a yield 10 to 20 times higher than the other oil crops (1).

Currently, production of biodiesel from oil crops contributes to only 0.3% of the global demand for transport fuels (2). Biofuel production from row crops cannot be increased from arable land without acutely reducing the food supply. Microalgae, however, can be grown on saline and wastewaters without interfering with agricultural production. Biofuels from algae have a significant potential to meet the fuel requirements of the world (2).

Microalgae are simple photosynthetic microorganisms that can efficiently use the sun's energy to convert water and carbon dioxide from the air into biomass. They are composed of 6% to 52% proteins, 7% to 23% lipids, and 5% to 23% carbohydrates, and they are species dependent. The protein content in several species of algae is significantly higher with a C/N ratio of 10.2 (3).

Microalgae have several advantages over other biodiesel feedstocks. They have greater light conversion efficiency. The annual yield of biomass per hectare is higher for algae compared to other biofuel sources. They can be harvested in batches throughout the year. They can be grown from human sewage and agricultural wastewater run-off, thus fulfilling the "waste to energy" ideal and minimizing fresh water use. They can fix CO_2 efficiently from various sources, including the atmosphere, industrial exhaust gases, and soluble carbonate salts. The CO_2 in flue gases from a coal-fired power plant can be fed directly to algae cultivated for biofuel production. Microalgae can use growth nutrients such as nitrogen and phosphorus from a variety of wastewater sources, like: agricultural run-off, concentrated animal feed operations, and industrial and municipal wastewater, thus contributing to wastewater treatment. They can also grow rapidly; certain species can double their biomass within 24 hours. Finally, microalgae are not used for food like other potential biodiesel feedstocks such as corn, palm, canola or soybeans (2, 4).

Algae can be isolated from unique aquatic environments to identify the algal strains with the highest growth rates and lipid content. The microalgae *Chlorella vulgaris*, *Spirulina maxima*, *Nannochloropsis sp.*, *Neochloris oleabundans*, *Scenedesmus obliquus,* and *Dunaliella tertiolecta* have been identified with higher oil content and are hence suitable for biofuel production (7). The microalga *Neochloris oleabundans* and

Nannochloropsis sp. was found to have an oil content of 29.0% and 28.7%, respectively (5, 6). These algae were shown to accumulate 50% oil when grown with reduced nitrogen supply. The algae *Scenedesmus obliquus* was identified with "best fatty acid profile in terms of linolenic and other polyunsaturated fatty acids," and thus very suitable for biodiesel production (7).

Various biotechnology approaches to microalgae cultivation have the potential to improve algal strains rapidly. The production of algal oils can be increased by "metabolic engineering through genetic manipulation". Lipid production in selected algal strains can be increased by cloning and modifying genes. These transformations improve the growth rate of the strains and also enhance the production of tri acyl glycerol (TAG) and other lipids (8).

1.2. MICROALGAE CULTIVATION

Algae are most commonly cultivated in batch cultures and harvesting is done before the next batch is started. It is easier to control the environmental conditions when the algae are grown in batches. The batch culture is started with the algal inoculum and the growth media consisting of the nutrients in a reactor. The algal culture is mixed by shaking or impeller mixing to promote the nutrient and gaseous exchange. Carbon dioxide gas is supplied to the culture based on the pH. Light is provided to the algae cultures by either natural or artificial light sources (9).

An initial lag phase during which the specific growth rate is submaximum level is to be expected in the batch growth of algae. This lag represents a period of physiological adjustment due to changes in nutrient or culture conditions. The lag phase may be

eliminated when cells at a later exponential growth phase are used as inoculum. The lag phase is followed by the exponential phase, at which point the cells have adjusted to the new environment and begin to grow and multiply. During this phase, cells grow and divide as an exponential function of time as long as mineral substrates and light energy are saturated. The exponential phase is followed by the stationary phase during which metabolism slows and cells cease rapid division. The growth rate slows as a result of nutrient depletion and the accumulation of toxic products. It is equal to the death rate of cells in the stationary phase. The stationary phase is followed by death phase. The cells start to die as nutrients are depleted. Cell division stops, and algae no longer reduce. Algae in this phase may be invaded by bacteria or other species. Green algae also turn brown during the death phase (9).

Ideal growth conditions for microalgal cultures are strain specific, and the biomass productivity depends upon many factors. These include abiotic factors like temperature, pH, water quality, minerals, carbon dioxide, light cycle and intensity, and biotic factors like cell fragility and cell density. Mechanical factors include mixing, gas bubble size and distribution, and mass transfer; these are of particular concern in photobioreactors (2).

Algae are grown heterotrophically using organic compounds and autotrophically with CO_2 supply. Culture productivity increases with optimal mineral nutrition. Nitrogen and phosphorus are essential nutrients in all algae growth medias (2). Mineral ions are essential for supporting cell structure and metabolism and also to facilitate osmoregulation (10).

The two most important factors that affect algae biomass productivity are light and temperature. The energy for growing algae is provided by light through photosynthesis. Light energy must be effectively utilized to achieve higher biomass productivity. Temperature influences the rates of all chemical reactions related to algal growth and metabolism (11). The temperature change on algae cultures affects the biochemical composition of the cells specifically lipids and proteins. Light and temperature have a significant effect in the metabolism, enzyme activities and cell composition of algae (12).

Algae cultivation depends on pH levels, and optimum pH influences the carbon availability. pH affects the metabolism and biochemical composition of cells. In both photoautotrophic and heterotrophic cultures, pH is controlled by the addition of strong acids, alkalies, and CO_2 (2). Specific pH levels are controlled and maintained by the CO_2-H_2CO_3-HCO^-_3-CO^{2-}_3 carbonate system in mass cultures. These are the most important buffer present in fresh waters. The level of pH rises as a result of CO_2 fixation during photosynthesis as OH^- accumulates in the growth solution. Sparging of carbon dioxide into the culture media is the most convenient method of pH control and can also increase yield in mass algal cultures (9).

Microalgae are most commonly mass cultivated in open ponds, which are very economical for commercial algae production as long as the species can be maintained. Raceway ponds are the most common form and are constructed in different shapes and sizes. These ponds consist of a rectangular grid, with each grid containing an oval-shaped channel. The water is moved in the pond continuously by a paddle wheel. A dry weight of 1 g per liter and productivity of 60 to 100 mg $L^{-1}day^{-1}$ biomass is obtained when the paddle wheel is operated at the depths of 15 to 20 cm (13). Algal wastewater treatment

ponds have retaining walls or dug trenches. Dense cultures are required to maintain stability in the ponds, and paddle wheels make the raceway ponds expensive. However, algae biofilm that attaches on the surfaces is easy to remove since the system is open and easily accessible. Open ponds do have drawbacks, such as they lose water by evaporation and their productivity is limited by contamination with unwanted species (2).

Microalgae are also cultivated in photobioreactors. They save water and chemicals compared to open ponds. Productivity is increased five times with respect to reactor volume (14). Higher biomass productivity is achieved in these reactors although they are expensive to be constructed. Closed photobioreactors are usually designed as flat panel reactors, tubular reactors, plate reactors, or bubble column reactors (15). Light must be uniformly distributed throughout the entire volume of the photobioreactor so that the algae cells are exposed to moderate light intensity. The tubular reactors are constructed like a fence to distribute light efficiently. Sunlight is "diluted" horizontally and vertically when the fences are aligned in a north/south direction. Such systems permit algal productivity of 47 g dry weight $m^{-2}day^{-1}$ (16).

Mixing is an important aspect in photobioreactors. They require mixing to prevent settling of cells and to ensure the circulation of nutrients, CO_2 and O_2 to the algae cultures (17). The light is also distributed by mixing as each algal cell moves through dark and light zones of the reactor. Microalgae waste excess energy quickly as fluorescence and heat at high light intensities that can also cause photo inhibition. Mixing exposes the algae to the low and high light cycle and save energy because the "low light phase channels the energy in photosystems into downstream metabolic processes" (18).

Microalgae grown in photobioreactors are often damaged because of high liquid velocities and turbulence. Air bubbles are known to cause shear stress to the algae cells and are a major problem in these reactors (19). The algae cultures are shielded from shear damage by using carboxymethyl cellulose. It prevents the cell attachment to gas bubbles (20).

1.3. MICROALGAE HARVESTING METHODS

Harvesting is the most important step in the production of algal biomass as it accounts for 20–30% of the production costs (21). The very small size of microalgae (3-30 μm) and its low concentration in the culture medium (below 500 mg/L) makes the cell recovery, a very challenging process (9). The harvesting cannot be done by a single process because of the several species of algae with varying characteristics like shape, size and motility that influence their settling (22).

Centrifugation is one of the most commonly used techniques to harvest microalgae. It is a separation process in which algae settles by sedimentation. Algae for aquaculture applications are harvested by centrifuging to produce concentrates with longer shelf-life. The settling of algae in a centrifuge depends on the residence time of the algae culture and the settling depth (23). These are the centrifuges that are commonly used for centrifuging microalgae: tubular bowl, disc-stack bowl, and a scroll discharge decanter (9).

Filter presses are used to recover fairly large microalgae, but are not suitable for smaller microalgae like *Scenedesmus, Dunaliella, and Chlorella*. The chamber filter press and the belt press are the most common devices; both operate at pressure or under

vacuum conditions. A rotary drum precoat filter uses diatomaceous earth or cellulose as a filter aid to form a cake or precoat. The algae suspension is then filtered through the precoat layer. The filtered algae biomass is collected with a thin layer of the filter aid (23).

Algae harvested using membranes are found to have higher recovery (70%-89%) than other conventional processes (24). Membranes are available in a wide range of pore sizes and offer better filtration rates. Microfiltration separates particles using a membrane of pore diameters between 100 and 10000 nm. It is more applicable to fragile cells. Ultrafiltration uses a membrane that has a pore diameter size between 1 and 100 nm. It is often used in the biomedical industry to separate pigments, proteins and enzymes (25). Membrane filtration is not generally used to produce algal biomass in commercial processes. It is employed in small aquaculture farms to harvest algae for feeding shellfishes (23).

The major costs involved in centrifugation are depreciation and maintenance of equipment; those involved in cross-flow filtration are membrane replacement and pumping. Centrifugation may be more attractive for large scale production (> 20 000 L), whereas cross-flow filtration is suitable for small-scale (< 2000 L) operations (9).

The harvested algae biomass is dried so that the product can be stored without spoilage when algae are used as aquaculture feed. The drying process accounts for 30% of total algal production costs. Spray drying, freeze-drying, drum drying, and sun drying are used to dry microalgae. Spray drying is used for high-value products, but it may lead to degradation of pigments or vitamins in algae (although these may be protected by the addition of antioxidants before drying). Freeze-drying or lyophilization is mostly used to

dry microalgae in research laboratories. Oil can be easily extracted from freeze dried algae for biodiesel production. Drum drying involves the treatment of a wet slurry or paste over a hot rotating drum. The process consumes more energy, but it is suitable for the food industry because the product is safe from bacteria (4, 23).

Flotation is a process in which a gas or air is bubbled through the liquid to be clarified. The particles are adsorbed by the rising bubbles and are removed when they reach the surface of the liquid. The bubbles can attract smaller particles easily. This process can be used for the separation of algae with particle diameter of less than 500 μm (22). Air flotation techniques may be either dissolved or induced. This process is among several new harvesting methods proven to be efficient (26).

The efficiency of dewatering microalgae by sedimentation depends on the time of removal of algae. It is influenced by the intercellular interactions of algae in the different growth phases during cultivation. The optimum harvesting time is determined by the zeta potential of the microalgal culture. It was found that algae cultures harvested during the stationary phase had a higher rate of settling than those harvested during the exponential phase. Algae cells in the exponential phase are highly stable and electrostatically repel each other. The reduced rate of metabolism of the algae during the stationary phase contributed to the aggregation of algae cells. It was found that algal cultures stored in darkness settled faster compared to daylight conditions (27).

1.4. MICROALGAE FLOCCULATION AS A HARVESTING AID

Algae have high negative surface charges during the logarithmic phase of their growth. They remain widely dispersed in water because of these repulsive charges. Algae

settle and forms clusters after longer residence times when they approach decay phase. This process is called autoflocculation. It can also occur by organic polymers excreted by algae. The other factors responsible for autoflocculation include nutrient (nitrogen and phosphorus) limitation, restricted CO_2 supply, and coprecipitation of magnesium, calcium and carbonate salts (4).

Autoflocculation is also found to take place by changing the conditions of algae cultivation. Algal flocs are formed when pond agitation stops and the CO_2 supply is cut off, causing an increase in pH. On flocculation with various pH values, *Scenedesmus* sp. showed no flocculation for pH values between 5.0 and 7.5, while at pH values above 8.5, almost 95% of the algal biomass was found to be removed (4).

The algae cells form precipitates with the addition of chemicals. Multivalent metal salts like ferric chloride [$FeCl_3$], aluminum sulfate [$Al_2 (SO_4)_3$, alum] and ferric sulfate [$Fe_2(SO_4)_3$] are used to flocculate algae (23). Alum is an efficient flocculant for *Scenedesmus* and *Chlorella* to produce fuel. The metal salts are not intended for flocculation when considering the algae for use in some aquaculture applications (28).

Algae can also be flocculated by using cationic polymers or polyelectrolytes (29). The negative charge on algae is attracted to the positive charge of cationic flocculants. Algae particles are bound together by polymer flocculants through a process called bridging. This process fails to occur at high ionic strengths. Dosage between 1 and 10 mg/L of the polymer is required to flocculate fresh water algae (30). Flocculation by polymeric flocculants was mainly found to depend on the molecular mass, ionic strength and dose of the polymer and also the pH and concentration of the algae culture (31).

Chitosan is a cationic polymer (deacetylated polymer of β-N-acetyl-D-glucosamine) prepared from the exoskeleton of marine crustaceans. Chitinous shellfish wastes are subjected to alkaline hydrolysis. Chitin is converted to chitosan by deacetylation with 50% NaOH at 130-150˚C. The structure of chitosan is shown in the Figure 1.1. Chitosan concentration of 50 mg/L was found to completely settle (96%) *Scenedesmus obliquus* at pH between 7.5 and 8.5 (4). Chitosan has several advantages over other conventional flocculants: It does not produce any toxic effects, it is required in very low concentrations (4). Algae can also be immobilized in a chitosan matrix and used in tertiary treatment of wastewater (32).

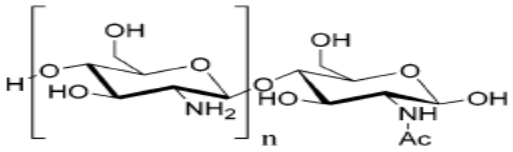

Figure 1.1. The structure of chitosan.

1.5. APPLICATIONS OF MICROALGAE

Microalgae are mainly considered as a potential biofuel source due to their lipid content. They are a main source of animal feed especially in aquaculture. They also serve as medicines to mankind. They are also a source of valuable byproducts and find their use in cosmetics. They are of immense benefit to the environment as they address pollution problem. They can capture the carbon dioxide from flue gas generated from coal fired power plants thereby reducing the green house effect. They can also utilize the nutrients from wastewater for their growth contributing to wastewater treatment and bioremediation of metals.

1.5.1. Lipid Production by Microalgae. Microalgae produce oil due to their high lipid content. Biodiesel can be produced from non polar tri acyl glycerols (TAG). Microalgae may vary from about 1% to 85% of their dry weight in lipid content (1). Nutrient stress was found to increase the lipid content to higher than 40% (33). The growth conditions that can enhance the lipid content in microalgae are high light intensity (34), low temperature (35), and high iron concentration (36). TAGs and polar lipids (phospholipids and glycolipids), are produced under higher and lower irradiances, respectively (8, 37). Nitrogen limitation in some algae was found to increase the lipid content up to 70% of the dry biomass in batch cultures. TAGs were found to be accumulated up to 80% of the lipids in the nitrogen starved algae cells (37, 38). The diacylglycerol acyl transferase is influenced under nitrogen stress and the fatty acid acyl-CoA is converted to triglyceride (39).

Photosynthesis in algae cultures takes place at a reduced rate under nitrogen limitation. The biochemical composition of the algae cell is changed from proteins to either lipids or carbohydrates. Carbohydrates are formed with no loss in productivity, whereas lipids are produced with a decrease in algae productivity (38). It has been reported that algae cells accumulate oil under nitrogen depletion even when the growth or cell division is inhibited. Sheehan et al. suggested that the oil productivity could be enhanced by “controlling the timing of nutrient depletion and cell harvesting” (6).

Chlorophyll also serves as a nitrogen reserve under nitrogen depletion conditions. It is an “intracellular nitrogen pool” in algae that can support cell division when the external nitrogen supply to the algae cultures is depleted. It can help both in cell growth and lipid enhancement thereby producing oil (40).

Growth rates and biochemical composition of microalgae vary according to the conditions of cultivation. The lipid production was found to be enhanced by fed-batch cultivation wherein the nitrogen source is intermittently supplied to the algae cultures (41). Wu et al. found that the lipid productivity increased by 26.4% in the fed-batch cultivation of *Chlorella* using 0.025 g/L urea over that of the batch mode (39). It is an efficient method for producing microalgal lipids.

1.5.2. Biofuels from Microalgae. Algal biomass can be converted to biofuels by various biological and thermal processes. Algae biomass is partially oxidized at high temperatures around 800 to 900ºC during gasification. Syn gas is produced by gasification of algal biomass. It is a mixture of hydrogen, methane, and carbon dioxide. It can be used as an engine fuel and also in the production of ammonia fertilizers and methanol (42, 43). Liquefaction is a process in which the algae biomass produces heavy oil at low temperature and high pressure in the presence of hydrogen. Wet algae can be directly subjected to liquefaction (44). Algae biomass is heated in the absence of oxygen at 500ºC during pyrolysis. It produces char, oil and gas when run in a fluidized bed reactor (45). Algae produce ethanol on fermentation. Yeast is used to ferment the sugars sugars to ethanol. Algae oil on transesterification with an alcohol produces biodiesel (42).

Microalgae also produce methane by anaerobic digestion (46). This process can recycle the nitrogen and phosphorus present in algal waste after lipid extraction through mineralization and use it for algae growth. This greatly minimizes the fertilizer use (6, 47). Methane can be used as fuel and helps to offset the cost and energy in the microalgae-to-biofuel process. Oligo nutrients such as iron, cobalt, and zinc present in algae have also been found to activate methanogenesis, along with carbon, nitrogen, and

phosphorus (48). Methane can also be produced by fermentation because of the presence of lipid, starch, and protein and absence of lignin in algae (2).

1.5.3. Microalgae as a Food, Medicine and Cosmetic Source. The presence of nearly all vital vitamins (e.g., A, B_1, B_2, B_6, B_{12}, C, E, nicotinate, biotin, folic acid, and pantothenic acid) in microalgae make them suitable for use in both human and animal nutrition (49, 50). Microalgae are used in human nutrition in various forms, such as tablets, nutritional supplements, and food colorants (33). *Chlorella* consists of β-1,3-glucan, which is a vaccine (51). The β carotene content in *Dunaliella Salina* makes it suitable for use in food supplements (52). The high protein content in *Anthrospira* is utilized as a nutritional supplement. This alga is widely used in medicine to cure hyperlipidemia, arrest hyper tension and protect against kidney failure (53, 54).

Microalgae are used as animal feed mainly in aquaculture and also to pets and farm animals. Mollusks and shrimp feed on microalgae for larval nutrition directly or indirectly through live prey that have already fed on algae (3, 56). The microalgae *Chlorella, Tetraselmis, Isochrysis, Pavlova, Phaeodactylum, Chaetoceros, Nannochloropsis, Skeletonema*, and *Thalassiosira* have been frequently used in aquaculture owing to their higher protein content (55, 56). The presence of eicosa pentaenoic acid makes the marine algae *Nannochloropsis* to be used as a rotifer feed (57).

Microalgae are also a source of number of other valuable byproducts. Microalgae extracts are extensively used in cosmetics. They are used in face, skin, hair care and also in sun protection products (58). The other products obtained from carotenoids of algae are natural food colorants (orange juice), animal feed supplements especially for salmon and therapeutics (59).

1.5.4. Nutrient Removal from Wastewater using Algae. Microalgae are increasingly used in wastewater treatment for their potential to remove nitrogen and phosphorus from wastewaters generated by different sources. The nutrients are removed from wastewater through direct uptake into the algae cells and stripping ammonia at high pH (60). Wastewater treatment using algae has many advantages. It offers the feasibility to recycle these nutrients into algae biomass as a fertilizer and thus can offset treatment cost. Oxygen rich effluent is released into water bodies after wastewater treatment using algae (4). The addition of carbon is not required to remove nitrogen and phosphorus from wastewater. *Chlorella* (61), *Scenedesmus* (62), and *Spirulina* (63) are the most widely used algae for nutrient removal.

Municipal wastewaters typically have organic and ammonia nitrogen concentrations ranging from 25 to 45 mg/L and phosphorus concentrations of 4 to 16 mg/L (64). The nitrogen and phosphorus present in wastewater are removed by algae in tertiary treatment. Some algae can also be used to remove organic matter. Some constituents of wastewater are in high concentration depending on the type of wastewater that can possibly inhibit algae growth. These constituents are mainly urea, ammonium, organic acids, phenolic compounds, and pesticides (farm run-off) that can limit the use of wastewater to grow algae (67). Kim et al. reported 95.3% and 96% removal of nitrogen and phosphorus, respectively, by *Chlorella vulgaris* in 25% secondarily treated swine wastewater after four days of incubation (65). Travieso et al. treated distillery wastewater from an anaerobic fixed-bed reactor in a microalgae pond and obtained 90.2%, 84.1%, and 85.5% organic nitrogen, ammonia, and total phosphorus removal, respectively (67).

Hodaifa et al. used industrial wastewater from olive oil extraction to remove potassium salts and other minerals with *Scenedesmus obliquus* (67).

Nitrogen constitutes about 7% to 10% of microalgae cell dry weight (12). It is the most important nutrient for algae growth that constitutes the proteins. Ammonia, nitrite, nitrate, and urea are used as nitrogen sources in microalgae cultivation. Urea is the organic nitrogen source used in large-scale algal cultivation, especially for *Chlorella* and *Scenedesmus*, because of its lower cost (4). Nitrate is used as a nitrogen source in many green algae. Nitrate taken up by the cells is reduced to nitrite by a "NADH-dependent" nitrate reductase. The nitrite is then reduced to ammonium by a located "NADPH-linked" nitrite reductase. The resulting ammonium is assimilated to form amino acids by glutamine synthetase and glutamate synthetase (68).

Phosphorus is a macronutrient that plays an important role in growth and metabolism of algae. It is required for most cellular processes, that involving energy transfer and nucleic acid synthesis (69). The two most important phosphorus forms used by algae are HPO_4^- and HPO_4^{2-}. Organic phosphates are found in larger concentrations in water than inorganic phosphates. They must be hydrolyzed by extracellular enzymes such as phosphoesterases or phosphatases. Algae stores phosphorus mainly in the form of polyphosphates and metaphosphates. These compounds are present in granular form in algae under excess phosphorus conditions and disappear in limiting conditions. Polyphosphates are present as "acid-soluble or acid-insoluble" forms. Algae utilize the soluble form for metabolism. Algae stores the insoluble form when phosphate amounts present in the culture is limited (70). The phosphorus consumption rate in algae depends

on phosphorus concentration in both the environment and the cells, and on pH, temperature (71).

Iron is the next vital nutrient after nitrogen and phosphorus required by algae. It greatly affects the growth and biochemical composition of algae because of its redox properties. It is the most important nutrient involved in various processes such as photosynthesis, respiration, nitrogen fixation, and DNA synthesis (12). Chlorophyll may be degraded when iron becomes limiting. Iron is also responsible for enhancing phytoplankton biomass in oceanic waters (36).

High-rate algal ponds (HRAP) offer a great potential for performing wastewater treatment using algae. Wastewater after primary or secondary treatment is fed to a race track reactor 0.3 to 0.4 m in depth. Algae and bacteria are cultured in these reactors. Algae are continuously mixed to keep the cells in suspension and expose them periodically to light. Algae and bacteria remove organic matter by a “mutual relationship”. Algae provide the dissolved oxygen required for bacterial decomposition of organic matter and bacteria provide carbon, nitrogen, and phosphorus essential for algal growth by degrading wastewater components (72).

Algae remove the nutrients in HRAPs directly through uptake and harvesting of the biomass. Nitrogen and phosphorus are removed indirectly by ammonia-nitrogen volatilization and orthophosphate precipitation, respectively. Directly and indirectly, the growth rate of algae controls the efficiency of nitrogen and phosphorus removal. The efficiency of nutrient removal in a HRAP is determined by cellular retention time, solar radiation, and temperature (72). These ponds have been successfully used for the treatment of anaerobic effluents from pig waste (73).

The other mechanism of wastewater treatment is the immobilization of algal cells. It eliminates the harvesting step which is most difficult in the treatment process. A gel matrix prevents cells from freely moving in its environment (74). Immobilized cells have increased reaction rates because of higher cell density. Further, they show no cell wash out. As a result, they are preferable to their free living counter parts (75).

Microalgae are entrapped in gel beads to remove nitrogen and phosphorus from wastewater. Travieso et al. reported higher nutrient removal from raw sewage treatment through internal immobilization of *Chlorella vulgaris* in sodium alginate beads (76). Tam and Wong have studied the entrapment of *Chlorella vulgaris* in calcium alginate beads, and they observed complete ammonium and 94% phosphate removal by adsorption on the alginate beads (77). Bashan et al. developed a novel approach to immobilize *Chlorella vulgaris* with the "microalgae-growth-promoting-bacterium" *Azospirillum brasilense* strain Cd in polysaccharide beads. Co-immobilization of the two microorganisms increased the nutrient removal than that by microalgae; 100% of ammonium, 15% of nitrate, and 36% of phosphorus were removed within six days, compared to 75% of ammonium, 6% of nitrate, and 19% of phosphorus by the microalgae alone (78).

Pesticides and herbicides are prevalent in aquatic systems due to runoff from agricultural fields. They are toxic to many aquatic organisms, particularly phytoplankton. Herbicides like atrazine in the aquatic systems could cause a problem if that wastewater is used in the cultivation of algae. Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is one of the most commonly used herbicides in the United States to protect the

crops from weeds. It is moderately water soluble (33 mg/L at 25ºC). It can enter the surface waters through precipitation by movement from soils (79).

Atrazine is toxic to algae and it decreases the rate of photosynthesis. It reduces the growth rate and changes the biochemical composition. It also reduces cell size. The "nutritionally altered" algae may have an adverse effect on higher trophic levels because of the less energy obtained by consumers (79).

The variation in algal response towards atrazine was found to be mainly due to the differences in its uptake and "binding of atrazine within the cell". Weiner et al. explained the atrazine uptake must have been through intracellular mechanism. They also claimed that the difference in algal sensitivity to atrazine may be because of the "amount of atrazine each species bioconcentrates from the surrounding medium" (80).

Behra et al. tested different concentrations of atrazine to determine its short term tolerance to algae. The green alga *Scenedesmus subspicatus* was unaffected of its growth rate or net photosynthetic activity at the atrazine concentrations of 1, 5, and 20 μg/L (81). Atrazine concentrations that affect the growth rate of phytoplankton have been reported from as low as 1 μg/L for communities to 1000 μg/L for single species (82, 83, 84). Atrazine concentrations up to 20 μg/L are tolerable in aquatic ecosystems on the basis of toxicity data, obtained from laboratory bioassays and field studies (85).

1.5.5. Remediation of Organics and Heavy Metals. Microalgae require different metals for biological functions. Selected microalgae are cultivated to enhance the specific bioremoval of certain metals. They have a potential to reduce the metal contamination in aquatic systems (86).

Metals are taken up by algae through adsorption. At first, the metal ions are adsorbed over the cell surface very quickly just in a few seconds or minutes; this process is called physical adsorption. Then, these ions are transported slowly into the cytoplasm in a process called chemisorption (86).

Polyphosphate bodies in algae enable fresh water unicellular algae to store other nutrients. Several researchers have established that metals such as Ti, Pb, Mg, Zn, Cd, Sr, Co, Hg, Ni, Cu are sequestered in polyphosphate bodies in green algae. These bodies perform two different functions in algae; provide a "storage pool" for metals and act as a "detoxification mechanism". The alga *Scenedesmus obliquus* was also found to accumulate some metals on increasing the amount of phosphorus in the media. It was able to accumulate increased Cd and Zn with higher phosphorus concentrations, whereas Se accumulation was found to be inhibited (87).

Shehata et al. cultured *Scenedesmus* in different concentrations of copper, cadmium, nickel, zinc, and lead to evaluate their effects on the growth of algae. The concentration of metal that reduced *Scenedesmus* growth was 0.5 mg/L for Cu, 0.5 mg/L for Cd, 2 mg/L for Ni and 2 mg/L for Zn (88). The nickel solution was less toxic than copper for *Scenedesmus* growth. The alga tolerated high lead concentrations up to 30 mg/L.

Microalgae are capable of growing on a number of carbon compounds. Industrial wastes rich in these organic compounds are used to grow algae thereby contributing to bioremediation. Microalgae grown heterotrophically also offer several advantages over autotrophic mode. It requires minimal light and reduces the cost of harvesting the biomass. The rate of biomass production is also higher compared to autotrophic mode (89). Wu et al. reported higher biomass and lipid content in *Chlorella protothecoides*

when grown on acetate, glucose, or other organic compounds (90). Ethanol, glycerol, and fructose may also serve as carbon sources depending on the alga species.

The cost of the microalgae-to-biodiesel production could also be reduced by using cheaper carbon sources (91). Glucose can be replaced by corn powder hydrolysate (CPH) or molasses as an organic carbon source. CPH contains some components beneficial to *Chlorella protothecoides* and produced 55.2% lipids in the cells with a dry cell weight concentration of 15.5 g/L (92). It also reduced the cost of biodiesel production compared to glucose. Microalgae were also grown on industrial waste water containing nitrogen. Mono sodium glutamate waste after dilution has also been used as an inexpensive fermentation medium for *Rhodotorula glutinis* to produce lipids (93).

1.5.6. Algal Flue Gas Sequestration from a Power Plant. Carbon dioxide capture by algae is a feasible technology for mitigating the emissions of fossil fuels. CO_2 fixation by algal cultures is not only a method for greenhouse gas mitigation but can also be used for producing algae biomass, which can then be converted into a biofuel. According to Bilanovic et al., "Microalgae can use up to 9% of incoming solar energy to produce 280 tons of dry biomass per ha^{-1} y^{-1} while sequestering roughly 513 tons of CO_2" (94).

The flue gases emitted by coal fired power plants constitute 7% of the world CO_2 emissions. The concentration of CO_2 present in the flue gases is up to 15% as estimated by IPCC criteria (95). Most of the microalgae species were found to tolerate SO_x and NO_x in the flue gas up to 150 ppm (96). SO_x may form sulphurous acids, causing a drop in pH levels that can become a problem for some species when present above 400 ppm. Metals such as nickel, vanadium, and mercury are also present in flue gas in

combinations that depend on the fuel used. The presence of the metals nickel and vanadium, higher than 1.0 and 0.1 ppm, respectively, have shown to decrease algal productivity. Mercury was not found to have any adverse effects on algae growth. Some algae may also bioconvert mercury from one form to another; offer the potential for remediation of toxic metals (97).

The carbon source for algae in the outdoor ponds are CO_2 enriched air or bicarbonate salts. Many microalgal species use sodium carbonate and sodium bicarbonate for their growth. The carboanhydrase enzymes present in these algae convert carbonate to free CO_2 (98). Inorganic carbon was also transported in some algae through a bicarbonate carrier. The bicarbonate resulted in a decrease of pH levels of algal culture. Low pH levels must be maintained in the culture so that free CO_2 are available to the algae cultures (99).

The supply of carbon dioxide enriched air is regulated in various times of the day to increase algal productivity. It is not required by algal cultures throughout the daylight period. The concentration of CO_2-air mixture was suggested to be made lower during the dawn and the dusk and higher at midday to reduce the carbon losses. The molar fraction of the injected gas had an effect in both the biomass yield and carbon losses. Biomass increased by 20% and the carbon losses were reduced by 60% on decreasing the molar fraction of the supplied CO_2 gas from 1.00 to 0.40 (100).

The algae biomass production also depends upon the interaction between CO_2 concentration and temperature. *Chlorella vulgaris* showed significant growth at 30ºC, and the growth improved significantly when the CO_2 level was raised from an ambient 0.036% to 6% (101). FitzGerald and Rohlich developed a process to remove nutrients

from municipal and industrial wastewaters using algae as it grew well at high CO_2 levels (102). CO_2 was bubbled through sewage to enhance the removal of nitrogen. The efficiency of high-rate oxidation ponds was found to be increased when CO_2 was supplied.

1.6. RESEARCH OBJECTIVES

Microalgae are valuable resources that have an immense potential to solve the nation's energy and environmental challenges. The overall objective of this research was to investigate the use of microalgae in the bioremediation of nutrients in wastewater and sequestration of carbon dioxide in flue gas.

Microalgae mainly require the nutrients nitrogen and phosphorus for growth and reproduction. The effluent from the secondary treatment in wastewater treatment is rich in nitrogen and phosphorus. This water cannot be directly discharged into the water bodies as it can cause eutrophication and other harmful ecological impacts. Algae can be cultivated in wastewater during tertiary treatment thereby producing biomass which can be used as feed for animals and for producing biofuels like biodiesel and bioethanol. Algae not only recycle the nutrients into biomass but also purify the wastewater by producing oxygen which can aid in the bioremediation of heavy metals and xenobiotic compounds. Harvesting of biomass is a major challenge in algal treatment systems and industrial-scale processing of microalgae for biodiesel production. A sustainable dewatering method should be developed to make microalgae, a commercial feedstock for biodiesel production.

The specific objectives of this study were:

- To determine the rate of phosphorus consumption by algae
- To evaluate the growth of algae using water recycled from chitosan flocculation process
- To find the tolerance and inhibition levels of the atrazine in *Scenedesmus* algae

- To find the optimum dewatering or harvesting rates considering the frequency of harvesting and biomass yield

Microalgae also need carbon dioxide for photosynthesis. Algae can be mass cultivated in open ponds or photobioreactors to maximize carbon dioxide conversion to biomass. Algae can mitigate greenhouse gases by consuming the carbon dioxide from anthropogenic sources like flue gas emitted by coal fired power plants. The carbon dioxide can be converted to soluble carbonates and then used to grow microalgae. This can be done when the power plants are not located near the algae ponds. The specific objective of this study was to evaluate the growth of algae with sodium carbonate and sodium bicarbonate.

2. EXPERIMENTAL PROCEDURE

2.1. MATERIALS

Algae strains isolated from natural habitats in local areas are most likely to establish and thrive at those local conditions and were found to be suitable for large scale cultivation. *Scenedesmus* was isolated from a pond in Jefferson City, Missouri. It is a ubiquitous organism belonging to the chloroccalean genera and dominant microalgae in fresh water lakes and rivers (105). It was found to have high growth rate, 20-40% lipid content and resisted contamination in mass cultures (38). The microscopic image of the algae *Scenedesmus* is shown in the Figure 2.1. This alga was used throughout this research.

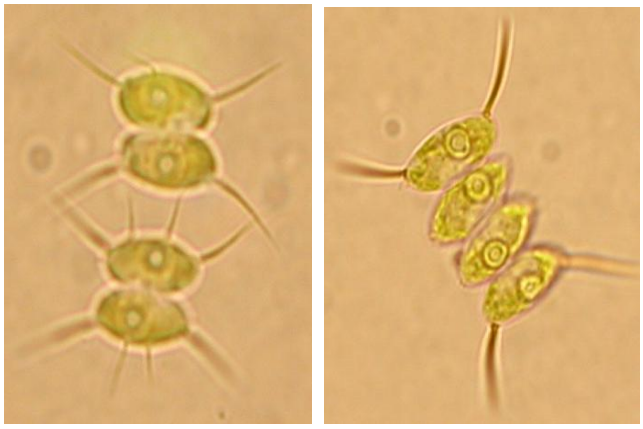

Figure. 2.1. *Scenedesmus* algae.

The nutrient media used to grow the algae were F/2 or Miracle-Gro. The F/2 medium has two forms. F/2 A consists of ferric chloride, cobalt chloride, and EDTA; F/2

B contains sodium nitrate and mono sodium phosphate. Miracle-Gro is a nutrient media derived from ammonium sulfate, potassium phosphate, potassium chloride, urea, urea phosphate, boric acid, copper sulfate, iron EDTA, manganese EDTA, sodium molybdate, and zinc sulphate. Proline F/2 algae food was purchased from Aquatic Ecosystems (Apopka, Florida). Miracle-Gro All-Purpose Plant Food (The Scotts Miracle-Gro Company, Marysville, Ohio) was purchased from a local store. The nutrient content of the F/2 and Miracle-Gro media are shown in the Table 2.1. Chitosan powder was purchased from Federal Laboratory Corporation (Alden, New York).

Table 2.1. Nutrient content of F/2 and Miracle-Gro media.

Serial #	Constituents	F/2	Miracle-Gro
1.	Nitrogen	9.33%	24%
2.	Phosphate	2%	8%
3.	Iron	0.82%	0.15%
4.	Manganese	0.034%	0.05%
5.	Cobalt	0.002%	-
6.	Zinc	0.0037%	0.06%
7.	Copper	0.0017%	0.07%
8.	Molybdate	0.0009%	0.0005%
9.	Vitamins	B1 (0.07%) & B12 (0.0002%)	-
10.	Biotin	0.0002%	-
11.	Soluble potash	-	16%
12.	Boron	-	0.02%

Fisher Scientific (St. Louis, Missouri) supplied sodium nitroprusside, sodium tetraborate, sodium thiosulphate, sodium hydroxide, ammonium molybdate, ammonium metavanadate, potassium metaphosphate, and ammonium chloride. Sodium hypochlorite, with ≥ 4% available chlorine, was obtained from Sigma Aldrich Chemicals (Milwaukee, Wisconsin). Atrazine was purchased from Chem Service (West Chester, Pennsylvania).

These standards and solutions were prepared for the nitrogen and phosphorus determination in water**.** A stock solution of 0.1427 M ammonium chloride was prepared for ammonium determination in water. For use in the spectrophotometer, 1 mL of this solution is diluted into 100 mL. One mL of the standard solution contained 0.02 mg N or 0.0244 mg NH_3.

A standard solution of 0.0032 M phosphate (KH_2PO_4) was prepared for phosphorus determination in water. One mL of the standard solution contained 100 µg PO_4^{3-}-P.

A phenol solution was prepared by mixing 11.1 mL liquefied phenol with 95% ethanol. A 0.0167 M sodium nitroprusside solution was then prepared. Alkaline citrate was prepared by dissolving 200 g of trisodium citrate and 10 g NaOH in deionized water. A fresh solution of 100 mL alkaline citrate solution was mixed with 25 mL of sodium hypochlorite to create an oxidizing solution. These reagents were prepared for nitrogen determination in water.

Vanadate molybdate reagent was prepared for phosphorus determination in water by heating 0.0674 M of ammonium molybdate and 0.0356 M of ammonium metavandate, cooling it with 300 mL concentrated HCl, then diluted it to 1 liter.

Chitosan solution was prepared for flocculating algae by mixing 100 mg of chitosan powder with 0.1 M HCl solution. It was heated at 30ºC for about an hour to dissolve it completely, then diluted with 100 mL of tap water to obtain a solution containing 1.0 mg chitosan per mL of solution.

2.2. ALGAE REMEDIATION FOR THE REMOVAL OF NITROGEN AND PHOSPHORUS

This study used microalgae to remove phosphorus from water. Phosphorus is one of the most important limiting nutrients for algae growth. The phosphorus content of the water filtered from the algae culture was measured to account for its removal.

Scenedesmus algae were grown in an 8-gallon tank of water to which 40 mL of F/2 media A and B had been added (Figure 2.2). The nitrogen and phosphorus concentration in the F/2 media were 123 mg/L and 8.5 mg/L, respectively. Algae were grown in natural sunlight. The cultures were not mixed or provided air supply initially for 6 days. After thoroughly mixing the contents of the tank, the optical density (OD) of the algae was determined daily by sampling 1 mL of algae daily and measuring it in a spectrophotometer (Thermo Electron Corporation, Genesys, Madison, Wisconsin) at a wavelength of 600 nm. The pH of the algae culture was also measured using a pH meter (MiniLab IQ 120, Carlsbad, California). The algae growth was compared to the concentration of phosphorus in water filtered from the culture. On day 7, after the pH level had reached 9, it was allowed to drop to 5.4, by supplying carbon dioxide to the algae culture through air stones. The same experiment was repeated by using Miracle-Gro as the nutrient media. Miracle-Gro media (15.5 g) was added to the algae in 8 gallon of water. The Miracle-Gro contained 122 mg/L nitrogen and 13.4 mg/L phosphorus.

Carbon dioxide was supplied on day 5 and the pH changed from 8.9 to 7.4. Phosphorus concentration in water filtered from the algae culture was also measured when Miracle-Gro was added.

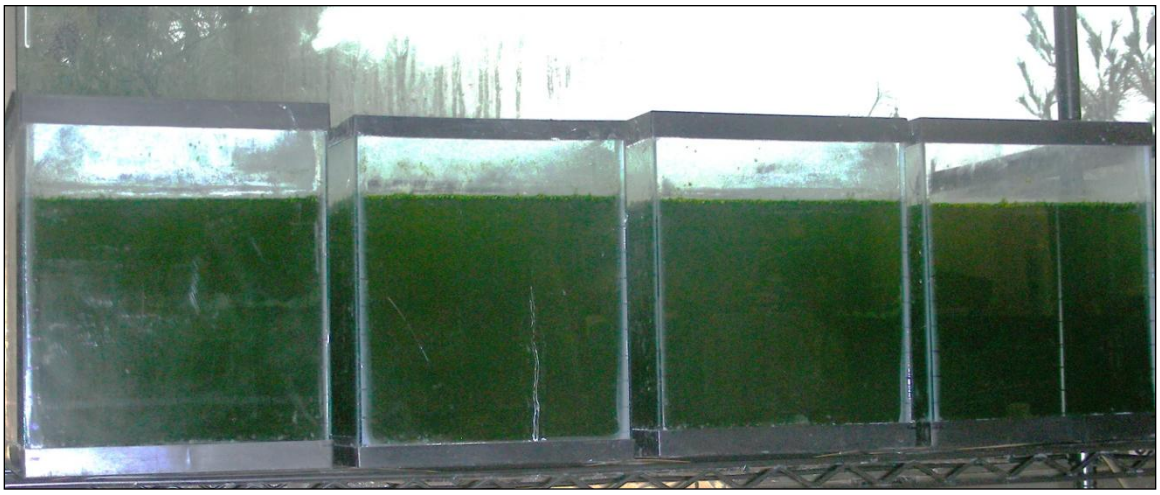

Figure 2.2. *Scenedesmus* algae grown in a tank.

Cell concentrations (C, mg/L) were indirectly measured by the absorbance of the cell suspension at 600 nm. A straight line calibration of absorbance versus dry weight (C, mg/L) was obtained (Figure 2.3).

2.3. DETERMINATION OF NITROGEN AND PHOSPHORUS CONTENT IN WATER

Two methods used to determine the nitrogen and phosphorus levels in water were modified from “standard methods for the examination of water and wastewater” (103).

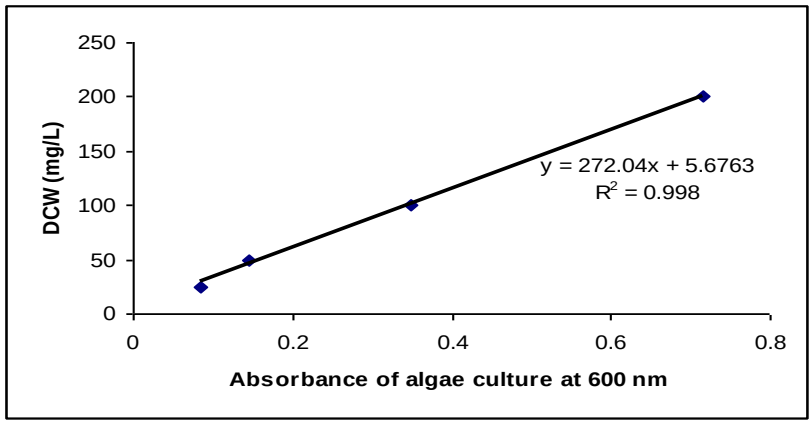

Figure 2.3. Dry cell weight determination of algae from absorbance.

First, 100 mL of the algae culture sample was collected in a plastic container and immediately filtered using a 47-mm-diameter, 1.0 µm-pore-size Whatman glass microfiber filter GF/B (Whatman Inc., New Jersey) followed by a 0.45 µm-pore-size Whatman cellulose nitrate membrane filter (Whatman Inc., New Jersey). The filtrate was then used as a sample to determine the nitrogen and phosphorus content of the water.

2.3.1. Determination of Nitrogen Content in Water by Phenate Method. An intensely blue compound, indophenol, was formed by the reaction of ammonia, hypochlorite, and phenol catalyzed by sodium nitroprusside.

The nitrogen present in the filtered water was converted to ammonia by distillation. First, 50 mL of algae water, 200 mL of deionized water, 1 mL of dechlorinating reagent (0.025 M sodium thiosulphate solution), and 25 mL of borate buffer (sodium tetraborate solution) were heated in a distillation flask with a few boiling chips. Distilled ammonia was then collected into 0.04 N H_2SO_4. Distillate of at least 200 mL was collected and then diluted to 500 mL with distilled water. The nitrogen content in the distillate was determined by phenate method.

Distilled water (25 mL), a phenol solution (1 mL), sodium nitroprusside (1 mL), and the oxidizing solution (2.5 mL) were added to a 50-mL Erlenmeyer flask. The samples were covered with a plastic wrap, and the color was allowed to develop at room temperature (22º to 27ºC) in dark for at least 1 hour. Absorbance was measured with the spectrophotometer at 640 nm. Finally, 25 mL of distillate was taken and the same reagents were added to determine absorbance.

The standards were prepared by diluting stock ammonium solution in the concentration range of 0 to 0.2 mg/mL. The calibration curve was obtained by plotting the various concentrations of standard ammonium solution with absorbance to determine the nitrogen concentration in the distillate.

2.3.2. Determination of Phosphorus Content in Water by Vanadomolybdo Phosphoric Acid Colorimetric Method. In a dilute orthophosphate solution, ammonium molybdate reacts under acidic conditions to form a heteropoly acid, molybdo phosphoric acid. Yellow vanadomolybdo phosphoric acid forms in the presence of vanadium. The intensity of yellow color is proportional to the phosphate concentration.

A 25 mL algae culture sample was added to 10 mL of a vanadate-molybdate reagent and diluted to the mark with distilled water in a 50 mL volumetric flask.

Standard phosphate solutions were prepared in the concentration range of 0 to 1 mg/mL and their absorbance was measured in the spectrophotometer. Absorbance was determined using a spectrophotometer at 470 nm. The calibration curve was obtained by plotting various concentrations of standard phosphate solution with absorbance to determine the phosphorus concentration in water as shown in the Figure 2.4. The samples for analysis were taken in triplicate for every determination.

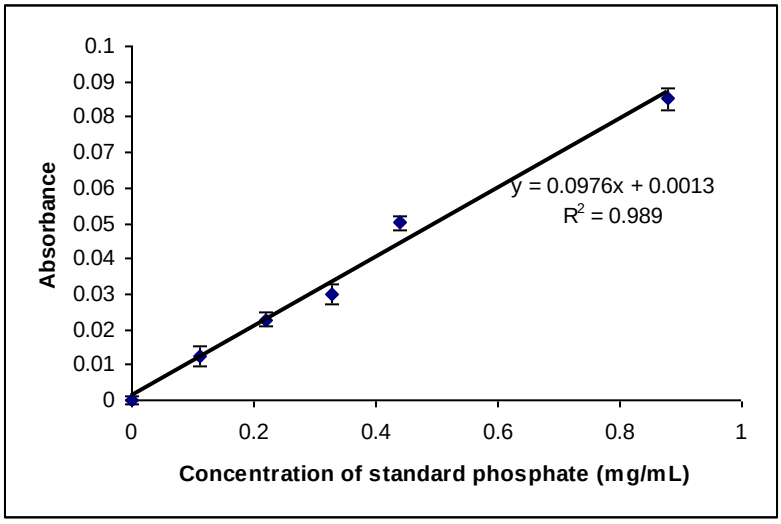

Figure 2.4. Calibration curve for phosphorus determination in water using vanadomolybdo acid colorimetric method.

2.4. GROWTH OF ALGAE WITH RECYCLED WATER FROM CHITOSAN FLOCCULATION PROCESS

Flocculation is one of the most commonly used methods for harvesting algae. The algae cells aggregate to form an algal floc on the addition of a flocculant. Flocculation using chitosan was found to be effective for the separation of algae particles from suspension. The chitosan solution was prepared for flocculating algae by mixing 100 mg of chitosan powder with 0.1 M HCl solution.

Water after flocculation using chitosan was saved and used for the subsequent cultivation of algae. The objective of this study was to evaluate the growth of algae in the recycled water after chitosan flocculation process.

Two liters of algae culture were added to each of two jars, one containing 1.7 L of fresh water and another containing 1.7 L of chitosan-flocculated water. Miracle-Gro fertilizer (2 g) was also added to both the fresh water and chitosan water. The flocculated

water used in this experiment was taken from a separate jar from the top after chitosan solution of concentration 1 mg/L in algae culture has been added.

The OD of the algae was determined daily by taking a 1 mL sample from the top of the culture and measuring it in a spectrophotometer at a wavelength of 600 nm. The pH of the algal culture was also measured. Algae cultures of both the fresh water and chitosan water in the jars were harvested at the end of day 18, first by flocculation, then using the centrifuge (Sorvall products, Newton, Connecticut) at 2700 rpm. The algae were then freeze dried (Ilshin Lab Company Ltd., Europe) to determine the weight of dry biomass.

2.5. EFFECT OF ATRAZINE ON *SCENEDESMUS*

Atrazine is the most commonly used herbicide in the United States. It is detected in the aquatic systems through agricultural farm runoff. It could present a problem if that wastewater is utilized in the cultivation of microalgae due to its potential toxicity. It was necessary to examine the effects of atrazine on algae. *Scenedesmus* algae were exposed to various concentrations of atrazine to determine the tolerance and inhibition levels of the atrazine.

Three liters of *Scenedesmus* culture each was set up in seven jars, to which 0.5 mL of F/2 media A and B were added. The nitrogen and phosphorus concentration in the F/2 media were 15 mg/L and 1 mg/L, respectively. Algae cultures with atrazine concentrations at 1, 5, 20, 100, 500 and 1000 μg/L were prepared. A blank control without atrazine was also set up. A 1 mL sample of algae was collected daily after mixing the contents of the jar thoroughly, and the OD was determined using the

spectrophotometer at a wavelength of 600 nm. The OD was determined in three replicate samples. pH of the algal culture was also measured.

2.6. HARVESTING OF ALGAE

Harvesting of algae is a major challenge in algal treatment systems and industrial-scale processing of microalgae for biodiesel production. It is associated with high operational costs and energy requirement. The harvesting process should be made more efficient with higher recovery to commercialize microalgae in biodiesel production.

Three methods of harvesting were tested to determine the one that was best considering the frequency of harvesting, human cost and algae dry mass obtained after harvesting. Optimum conditions for maximum yield of algae were also determined by varying the removal rates in harvesting.

Scenedesmus algae were grown in three 8-gallon tanks. The tanks were constantly aerated using air stones. The OD of the algae cultures was measured daily using a spectrophotometer. The pH of the algae culture was also measured.

The three tanks were harvested under varying dewatering conditions. From each tank, 90%, 50%, and 10% of the total volume of algae cultures was removed. Same volume of water was added to replace the algae cultures. The nutrient media was added every three days. The algae cultures were harvested again in the same percentage volumes after the initial OD had been reached.

Chitosan was used as a bioflocculant to flocculate the algae cultures. To every liter of algae, 1 mL of 1 mg/L chitosan solution was added. The algae biomass settled by gravity sedimentation after the addition of flocculant, and water from the top was

removed by decanting. The flocculated wet algae were centrifuged to remove water at a speed of 2700 rpm for 20 minutes. The biomass settled at the bottom, and again, the top layer consisting of water was easily removed. The algae from the centrifuge were frozen at -30°C and subjected to freeze drying to remove the remaining water. Freeze drying is a lengthy process, requiring an average of 3 to 4 days. After drying, the algae take the form of a dry powder.

2.7. GROWTH OF ALGAE WITH SOLUBLE CARBONATES

Carbon dioxide is a major limiting substrate for photosynthetic carbon assimilation in plants and other photosynthetic organisms. Algae can utilize carbon dioxide from the atmosphere, industrial flue gases, and in the form of soluble carbonates (45). This work tested the growth of algae with sodium carbonate and sodium bicarbonate.

Three liters of *Scenedesmus* culture each was set up in three jars. On day 0 and day 14, 0.5 mL of F/2 media A and B was also added to the algae culture. The nitrogen and phosphorus concentration in the F/2 media were 15 mg/L and 1 mg/L, respectively. The pH of the algal culture was measured. Carbon dioxide was added to the algae culture using air stones when the pH reached 9 and stopped when the pH dropped to around 6. Sodium carbonate (2.4 g) and sodium bicarbonate (1.9 g) were added to the algae culture at pH 6. A blank control was maintained separately with no bases added to compare the effect of the bases on algae growth. Next, 1 mL of algae was sampled by mixing the jar thoroughly, and OD was determined daily using the spectrophotometer at a wavelength of 600 nm. The OD was determined in three replicate samples.

3. RESULTS AND DISCUSSION

3.1. MICROALGAL BIOREMEDIATION OF NUTRIENTS IN WASTEWATER

The main nutrients, nitrogen and phosphorus, required for microalgae growth can be utilized from various wastewaters generated by different sources. These wastewaters could be agricultural wastewater, concentrated animal feed operations and municipal wastewater. These wastewaters cannot be discharged to the water bodies untreated as they can cause eutrophication and harmful algal blooms. Algae are proposed to remove the nitrogen and phosphorus components of the waste contributing to wastewater treatment. The release of oxygen due to microalgae photosynthesis and increased pH in the algal treatment of wastewater makes this process more efficient and a safe method to remove the nutrients from wastewater. Algal wastewater treatment and biofuel production greatly minimizes the freshwater requirement and the fertilizers for algae cultivation.

3.1.1. Nutrient Remediation to Study Phosphorus Removal. Phosphorus is considered as a main limiting nutrient for phytoplanktons in fresh water ecosystems. Microalgae require phosphorus as an essential element for growth. Phosphorus is needed for synthesis of cellular constituents such as phospholipids, nucleotides, and nucleic acids (12). The uptake of phosphorus and growth of *Scenedesmus* in batch culture under natural sunlight was studied. The concentration of phosphorus in water obtained by filtering the algae culture was measured daily after 40 mL of F/2 media was added to the 8-gallon of culture. F/2 media contained 8.5 mg/L phosphorus in addition to the phosphorus already contained in the algae culture. The variation of phosphorus concentration with time for 6

days of batch operation of *Scenedesmus* algae is depicted in the Figure 3.1. The phosphorus concentration decreased as the OD of algae increased. The pH of the algae also increased as the algae grew (Figure 3.2).

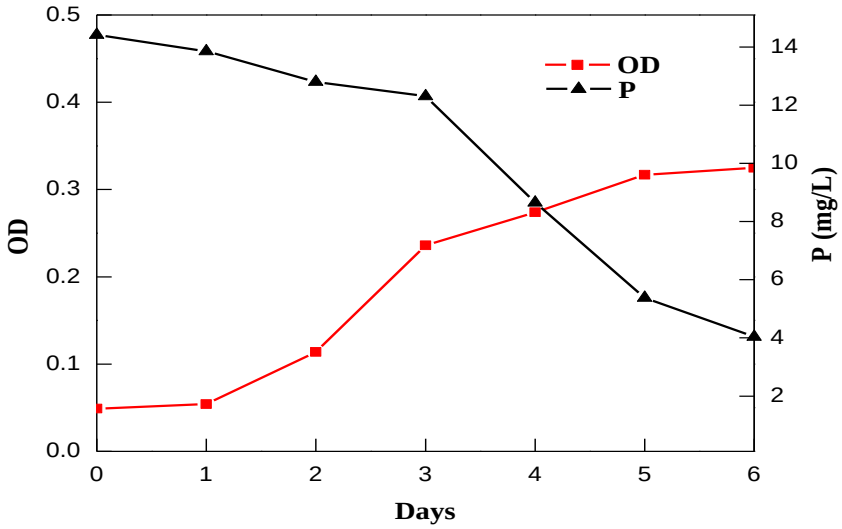

Figure 3.1. Change in phosphorus concentration and OD of algae culture over time before CO_2 was supplied in the batch culture of *Scenedesmus*.

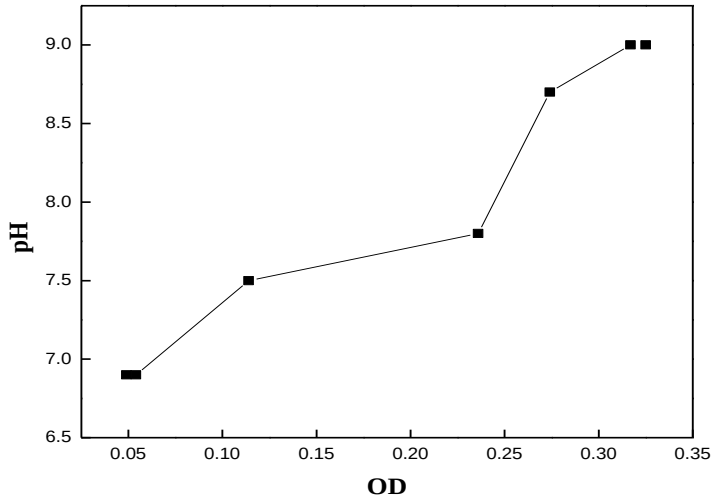

Figure 3.2. Change in OD and pH of algae culture over time before CO_2 was supplied.

The initial phosphorus concentration of the algae on the first day of the batch culture was 14 mg/L. The final phosphorus concentration after 6 days was 4 mg/L with 71% removal efficiency for phosphorus in the batch culture of *Scenedesmus* (Figure 3.1). The yield of algae biomass was calculated based on the consumption of phosphorus. The yield of *Scenedesmus* algae in the batch culture was 0.3 mg biomass μmol^{-1} phosphorus.

The phosphorus removal in this study was lower compared to some of the other studies. Martinez et al. achieved over 97% phosphorus removal by *Scenedesmus obliquus* with the phosphorus concentration of 11.8 mg/L when the algae cultures were subjected to continuous illumination using fluorescent lamps (104). Kim et al. observed over 83% removal of phosphorus by *Scenedesmus* in fermented swine wastewater with phosphorus concentration of 120 mg/L (105). Light could be the main limiting factor deciding the phosphorus uptake by algae. Algae cultures become denser and light limited at higher O.D.'s. There are various factors that contribute to the phosphorus removal in algae. These include the initial nutrient concentration, nitrogen/phosphorus ratio, light/dark cycle or algae species (106).

Carbon dioxide was also supplied to the algae culture through bubble stone in the tank. Algae were grown until their pH reached 9, and CO_2 was supplied on day 7 when pH had been reduced to 5.4 (Figure 3.3). The phosphorus consumption by algae was then determined.

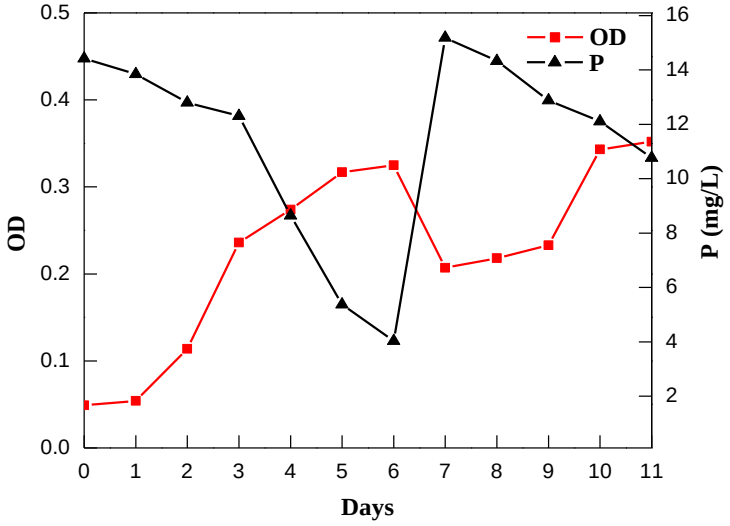

Figure 3.3. Change in phosphorus concentration and OD of algae culture over time after CO_2 was supplied.

The phosphorus concentration increased on day 7 as shown in the Figures 3.3 and 3.4 when carbon dioxide was supplied to the algae culture. This was likely due to the formation of apatite. Algae were grown using tap water, which was expected to be hard and contain calcium. Apatite, a calcium phosphate precipitate, was formed until the pH level reached 9 and then released after carbon dioxide was supplied when the pH had dropped to 5.4. Apatite [$Ca_5 (PO_4)_3 OH$] is a phosphorus mineral referred to as hydroxy apatite. Conversion of insoluble forms of phosphorus such as calcium phosphate [$CaHPO_4$] is mediated by microorganisms. The solubility of phosphates depends on the pH value of the water. The solubility of phosphate increases at low pH levels when dissolved phosphorus is $H_2PO_4^-$, and it decreases at high pH levels when dissolved phosphorus is HPO_4^{2-} and apatite forms (107).

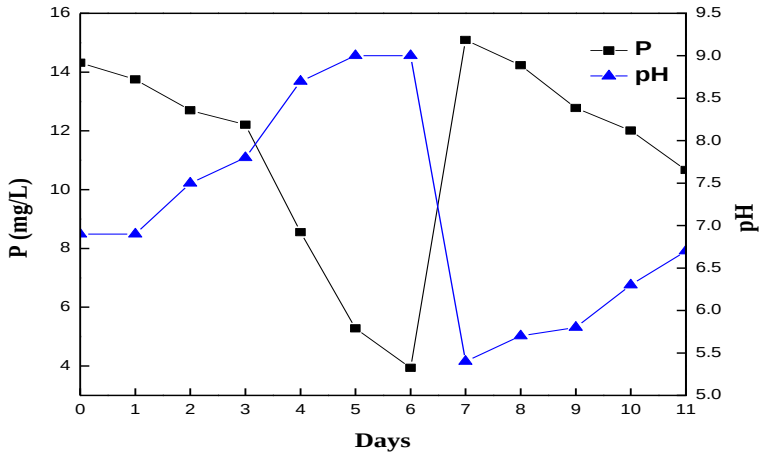

Figure 3.4. Change in phosphorus concentration with pH of algae culture after CO_2 was supplied.

The phosphorus in water filtered from the algae culture was also measured daily when Miracle-Gro was supplied to the 8 gallon of culture. Miracle-Gro contained 13.4 mg/L phosphorus in addition to the phosphorus already contained in the algae culture. On day 5, CO_2 was supplied, and the pH level decreased to 7.4 (Figure 3.5).

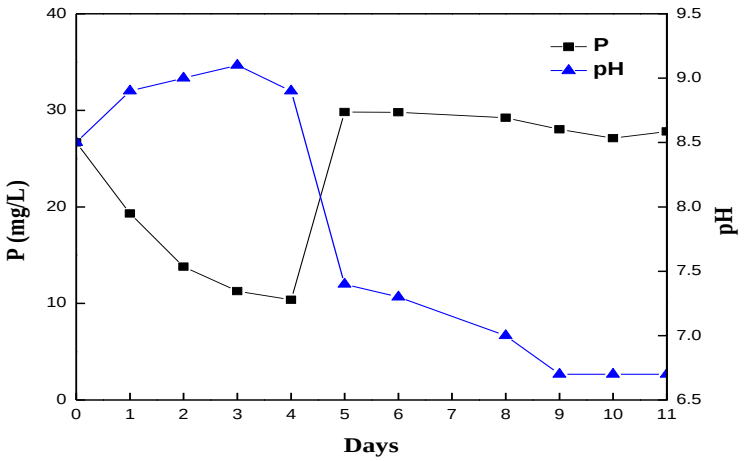

Figure 3.5. Change in phosphorus concentration and pH of algae culture over time when Miracle-Gro media was supplied.

The same result was observed when Miracle-Gro was supplied to the algae cultures. The calcium complex with phosphate was released at a lower pH when CO_2 was supplied.

3.1.2. Growth of Algae with Recycled Water from Chitosan Flocculation Process. Harvesting of microalgae by flocculation process is less economical and a spontaneous process. Flocculation is considered to be more favorable compared to other harvesting techniques because it offers a possibility of treating large quantities of algae culture and suitable for different algae species. This process when used before harvesting techniques like centrifugation or filtration could contribute to efficient recovery of algae. Flocculation using chitosan was found to be effective for the separation of algae particles from suspension.

Water after flocculation process was saved and used for the subsequent cultivation of algae. Water being a valuable resource was recycled and used again to grow microalgae. The objective of this study was to evaluate the growth of algae in the recycled water after chitosan flocculation process.

The growth curve for both fresh and chitosan water is shown in the Figure 3.6. Algae grown with fresh water had a higher growth curve than that with chitosan water. Chitosan tends to flocculate the algae. Most of the algae settled at the bottom, thus preventing light from reaching the bottom of the jar in chitosan water. Fresh water was exposed to more light, and had higher OD than chitosan water. The OD and pH followed similar trends as shown in the Figures 3.6 and 3.7. Fresh water had higher OD and pH levels than chitosan water. Chitosan water has lower pH than fresh water, likely due to its positive charge of the amine group in its structure and pKa value of 6.2 (108). The dry

weight of algae from fresh water was 0.95 g, and that of algae from chitosan water was 0.66 g. Fresh water consistently yielded higher algae biomass than chitosan water. Algae grown using chitosan water showed 30% less growth. Chitosan-flocculated water can be used to grow algae. There was 30% decrease in the biomass yield on the algae grown with chitosan water after flocculation.

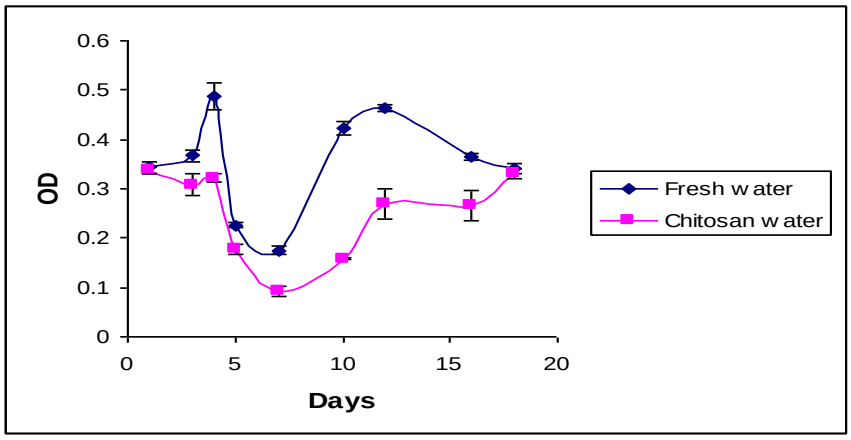

Figure 3.6. Algae grown with fresh water and recycled water after chitosan flocculation.

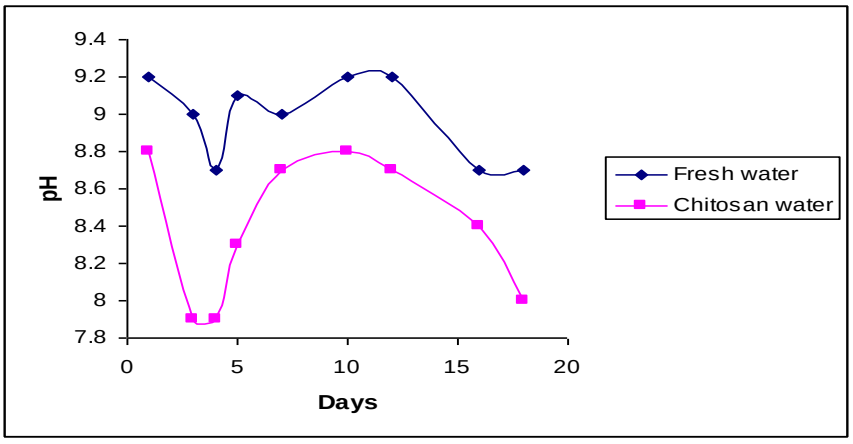

Figure 3.7. Change in pH of algae culture grown with fresh water and chitosan water recycled from flocculation process over time.

3.1.3. Effect of Atrazine on *Scenedesmus*. Modern agriculture has led to an enormous increase in the use of pesticides, fungicides, herbicides, etc. Atrazine is one of the most commonly used herbicide in the United States to protect the crops from weeds. It is often detected in the lakes and streams through agricultural run-off from the fields at levels that pose a threat to nontarget aquatic organisms. Its effects on algae are particularly of concern as they are the primary producers. Algae exposed to atrazine are found to cause acute or chronic toxicity and direct and indirect effects on the community. Acute effects are shown to cause death of algae. Chronic effects can cause a decrease in algae reproduction rates, disturbances to the community by affecting higher trophic levels (109).

The presence of herbicides such as atrazine in the aquatic systems can also present a problem if that wastewater is utilized in the cultivation of microalgae due to its potential toxicity. Therefore, it becomes necessary to examine the effects of atrazine on the algal community. The objective of this study was to find the maximum tolerance levels of atrazine in the *Scenedesmus* algae. Algae were exposed to various concentrations of atrazine as shown in the Figure 3.8.

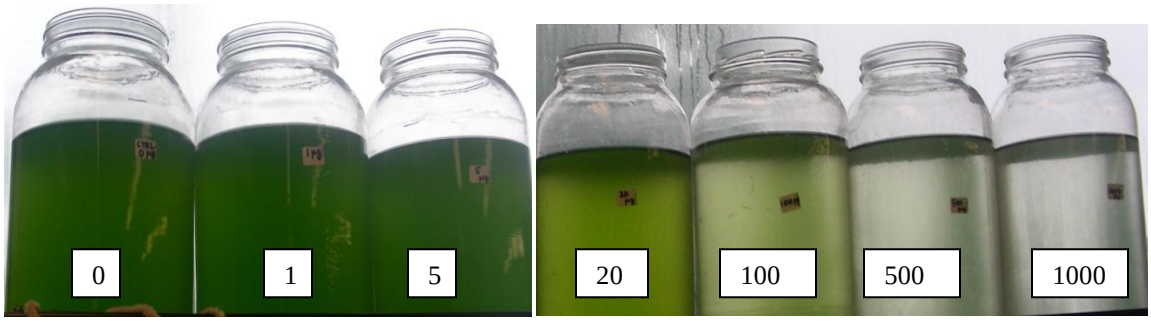

Figure 3.8. *Scenedesmus* algae exposed to various concentrations of atrazine in μg/L in the same time.

Concentrations of atrazine above 5 µg/L inhibit the growth of *Scenedesmus*, as shown in the Figure 3.9. This is confirmed by the color of algae, as shown in the Figure 3.8. Algae are dark green until concentrations 5 µg/L. The intensity of the green color decreases gradually as concentrations of atrazine increase, with no color at concentrations of 500 µg/L and 1000 µg/L. As expected, these concentrations show more inhibition than 20 µg/L and 100 µg/L. The pH values of algae culture also dropped as the concentrations of atrazine were increased from 20 µg/L to 1000 µg/L as shown in the Figure 3.10. Atrazine concentrations of 1 µg/L and 5 µg/L did not inhibit the growth of *Scenedesmus*. These results accord with those of with Behra et al. except that 20 µg/L atrazine was also not inhibiting *Scenedesmus subspicatus* (81).

Acute toxicity of a chemical is determined as the median effective concentration (EC50). It is the concentration of the chemical that reduce the growth of algae by 50%. These values are useful to exactly determine the concentration of chemicals that would inhibit algal growth (110). The 24 hour EC50 values of atrazine for *Scenedesmus obliquus* were reported to be between 38 and 57 µg/L (111). The EC50 value for *Scenedesmus subspicatus* was 21.5 µg/L (112).

Different species of fresh water algae exhibit different responses to atrazine exposure. Wurster explained that the variation in algal sensitivity towards atrazine were their due to the difference in the ability of algae to “resist pollutant stress” (113). Minimum concentrations of atrazine to affect photosynthesis and growth rate of phytoplankton range from as low as 1 µg/L for communities to 1000 µg/L for single species.

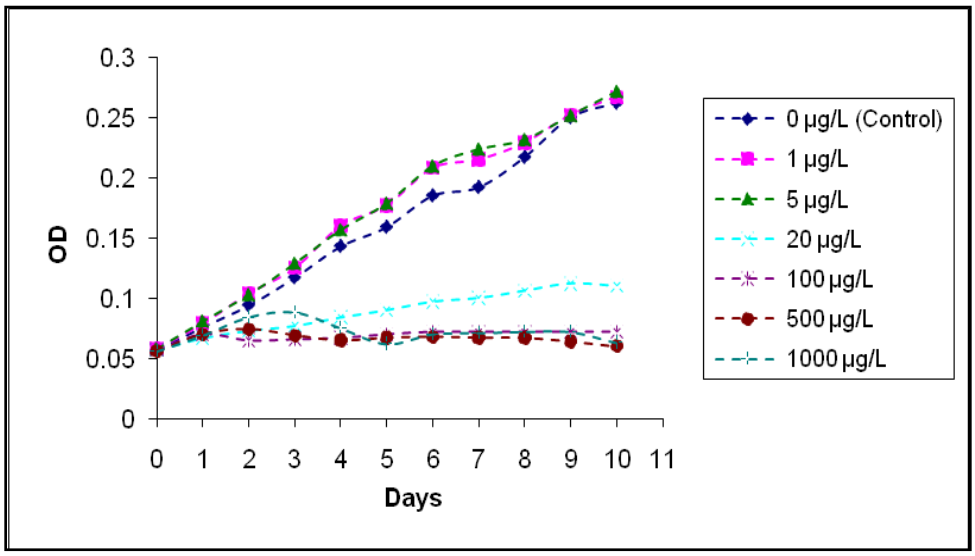

Figure 3.9. Algae cultures exposed to various concentrations of atrazine in µg/L.

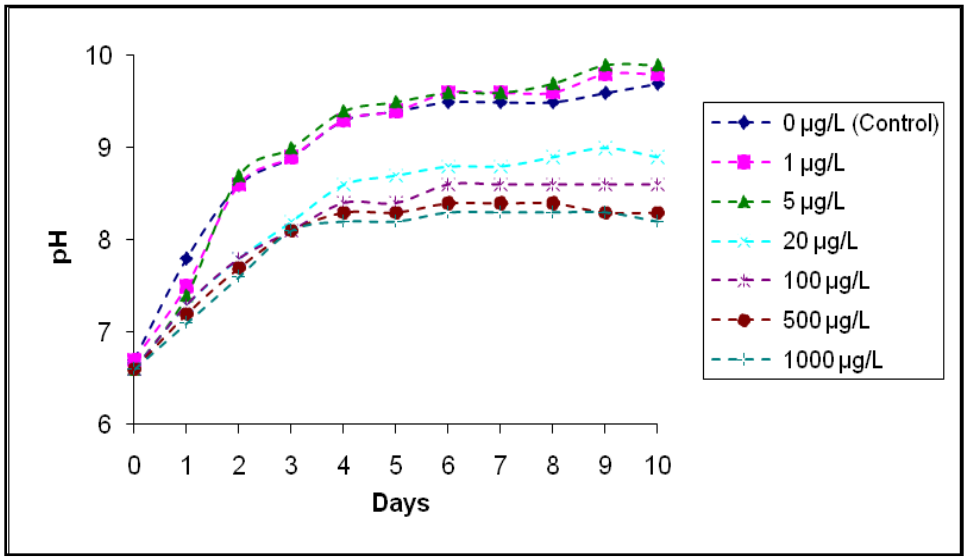

Figure 3.10. pH of algae cultures exposed to various concentrations of atrazine in µg/L for 10 days.

3.1.4. Harvesting of Algae. The dilute nature of the microalgal cultures makes recovery of the cells very difficult. Harvesting or dewatering of microalgal cultures is associated with high operational costs and energy requirement. The technique employed

for harvesting will depend upon the microalgae species and the desired product. The harvesting process should be made more efficient with higher recovery and less energy requirements.

Harvesting by flocculation before further techniques like centrifuging, flotation, filtration would contribute to higher recovery of algal cultures. Flocculation by cationic bioflocculants like chitosan was found to be effective for the separation of algae particles from suspension. Algae biomass is finally recovered after gravity sedimentation, centrifuging and freeze drying.

Three methods of harvesting were tested to determine the one which requires the least labor while producing the most algal dry biomass. Algae were harvested by removing 90%, 50%, and 10% of total volume of algae cultures. The biomass yield obtained on harvesting different volumes of algae cultures are shown in the Table 3.1.

Table 3.1. Biomass yield on harvesting different volumes of algae cultures.

Percentage volume removal of algae cultures	Biomass yield (mg/L/day)
90	29
50	24
10	29

The 90% harvest took 8 days to reach the initial OD as shown in the Figure 3.11. The 50% harvest took 6 days to reach the initial OD as shown in the Figure 3.12. The

10% harvest took only 2 days as the initial OD was reached fastly because of lesser algae removal as shown in the Figure 3.13. The biomass yields obtained by harvesting 90% and 10% were 29 mg/L/day. Although they have the same yield, 90% harvest took only 2 harvests and the 10% took around 11 harvests in a given time period. The 50% harvest took 3 harvests. It is the labor cost which decides the efficient way of harvesting. 90% harvest required less labor or energy cost as the frequency of harvesting was very less compared to 50% and 10% harvests. 90% volume harvest yielded most consistent growth rates and the best labor cost to yield ratio. The 90% harvest was consistent with less labor cost and the best considering the frequency of harvesting.

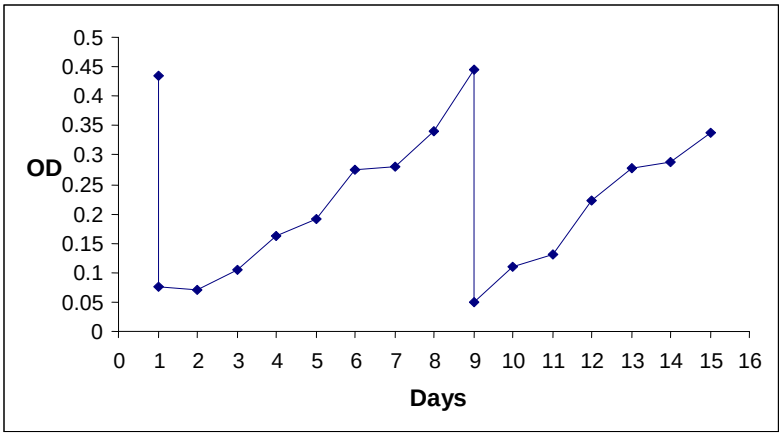

Figure 3.11. 90% volume harvesting algae growth curve showing daily OD.

Harvesting or removal of algae from algal-based wastewater treatment process is also a challenging process. The algae should be removed as the nitrogen and phosphorus are consumed in the wastewater. The 90% harvest would be the most ideal harvesting condition considering less labor and energy cost and minimum frequency of harvesting.

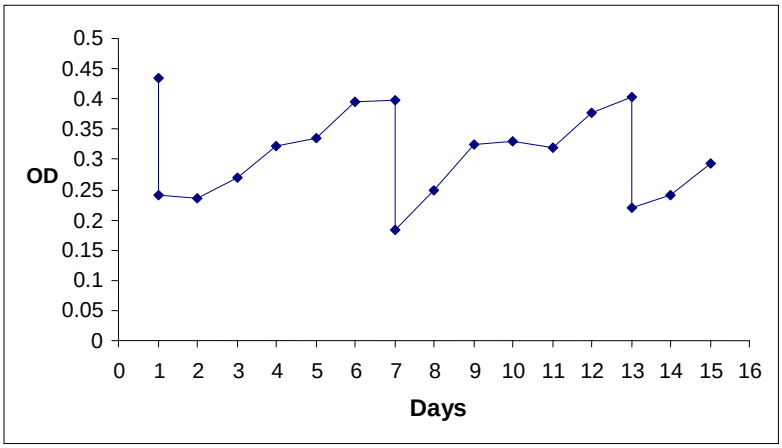

Figure 3.12. 50% volume harvesting algae growth curve showing daily OD.

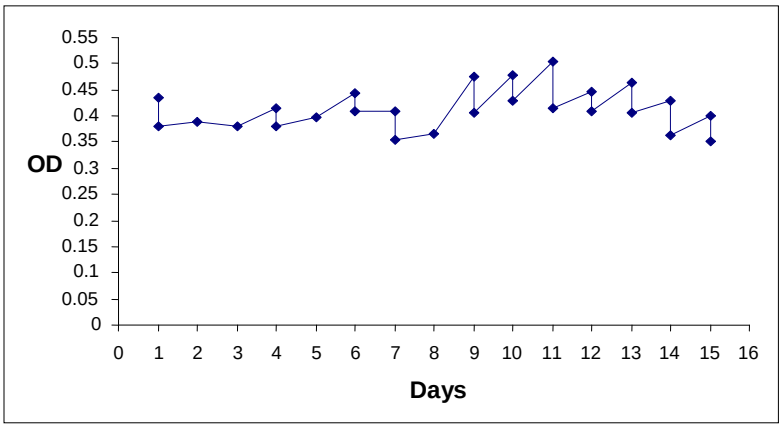

Figure 3.13. 10% volume harvesting algae growth curve showing daily OD.

There are different techniques available for harvesting or dewatering of microalgal cultures. The harvesting process should achieve a product with tolerable quality and require moderate costs for operation and maintenance. The technique should also be optimized to give higher yield and lower labor costs.

3.2. MODELING OF NUTRIENT CONSUMPTION BY ALGAE

The nutrient assimilation rates are calculated based on algal uptake stoichiometry. Redfield ratio is a universal ratio representing the stoichiometry of carbon, nitrogen, and phosphorus at balanced algae growth (114). These elemental ratios of algae are found to species-specific and vary considerably from the Redfield ratio (115). The average atomic ratio of *Scenedesmus* was 233:30:1 according to this reaction (116).

$$233\ CO_2 + 30\ NO^-_3 + HPO^{2-}_4 + 122\ H_20 + 18\ H^+ \rightarrow C_{233}H_{263}O_{406}N_{30}P_1 + 138\ O_2 \qquad (1)$$

The phosphorus removal is the difference in phosphorus concentration of water on 0^{th} and 6^{th} day of batch culture of *Scenedesmus*. The phosphorus removal by algae and the atomic ratio was used to calculate the nitrogen and carbon dioxide uptake by algae. The theoretical yield of algae was also determined based on the red field ratio. The experimental yield was calculated from the dry cell weight when *Scenedesmus* algae culture of 8 gallon was supplied with F/2 media containing 123 mg/L nitrogen and 8.5 mg/L phosphorus in addition to the phosphorus contained in the algae culture.

Phosphorus removed by algae after 6 days in the batch culture of *Scenedesmus* was 10.38 mg/L. The nitrogen and carbon dioxide removal rates obtained for the batch culture of *Scenedesmus* were 141 mg/L and 3436 mg/L, respectively. The theoretical yield of algae was 3354 mg/L. The experimental yield of algae was 75 mg/L after 6 days. The large difference between the theoretical and experimental yields of algae could be due to the phosphorus utilization by algae. Lodi et al. explained that only a portion of phosphate taken up by algae was used for growth under light limitation conditions (117). Phosphorus could be removed by both biological and chemical mechanisms. It must have

been assimilated into biomass during the growth phase and chemical precipitation occurred when the biomass reached certain density. Phosphorus was removed due to the precipitation of insoluble phosphates at high pH during growth in addition to its consumption by algae. The rate of algae growth may also have reduced due to light shading under limiting conditions.

Rodolfi et al. (38) obtained the yield of *Scenedesmus* as 210 mg/L/day. The algae was cultivated in a nutrient rich medium supplied with air/CO_2 (95/5, v/v) at a temperature of 25ºC, under continuous illumination provided by daylight fluorescent tubes. The main factors which determine the algae growth yield are the light/dark ratio, turbulence, nutrients including CO_2 supply (118).

Mixing is an important factor that could increase the algae yield in this study. Light is uniformly distributed throughout the reactor by mixing. It prevents algal sedimentation also. There is an increased rate of transfer of the nutrients between the algae cells and their growth media. The algal biomass productivity and photosynthetic efficiency could be increased further by mixing along with increased light/dark frequencies (118).

Open systems and closed systems are the two designs for intensive production of algal biomass. The photobioreactors are expensive than the open ponds, but are less prevalent to contamination and provide a controlled environment for algae growth. Grobbelaar et al. concluded that closed systems achieve higher biomass concentrations than open systems. Closed systems also have high light utilization efficiencies and lower respiratory losses (118).

The yield of algae can be increased when the nutrients nitrogen and phosphorus are efficiently utilized from the growth medium. Nitrogen and phosphorus utilization by algae depends on various factors such as the media composition, initial nutrient concentration of the media, light intensity, mixing, nitrogen/phosphorus ratio and light/dark cycle. Light limitation may be an important reason for low nutrient removal efficiencies at high nutrient concentrations (106).

3.2.1. Kinetic Model for Growth of *Scenedesmus* in a Batch Reactor. A kinetic model was developed to describe the growth and phosphorus consumption of the microalga *Scenedesmus* in batch cultures. The growth kinetics of algae was determined on the basis of the external phosphorus concentration in the algae cultures. The Monod kinetics were modified as shown in the equation (2) and used to determine the specific growth rate and biomass/substrate yield (71).

$$\mu = \frac{\mu_{m1} S + \mu_{m2} K_S}{K_S + S} \qquad (2)$$

where μ_{m1} = maximum specific rate (h^{-1})

μ_{m2} = specific growth rate in the absence of phosphorus from culture medium (h^{-1})

K_s = the substrate concentration at which the specific growth rate is equal to the semisum of the rates μ_{m1} and μ_{m2} (µM)

S = phosphorus concentration (µM)

This modified Monod model assumed that the growth rate of an alga is dependent on the concentration of a particular limiting nutrient. The specific growth rate (μ) during the exponential growth phase was obtained using the equation (3). The growth of *Scenedesmus* and the phosphorus concentrations during the batch culture is shown in the Figure 3.14.

$$\ln C = \ln C_o + \mu t \qquad (3)$$

where C and C_o are the biomass and initial biomass concentration, respectively in mg/L.

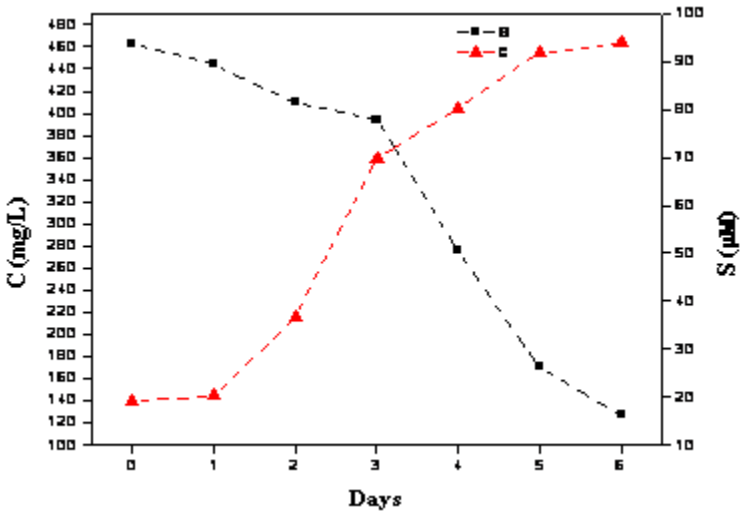

Figure 3.14. Growth and phosphorus concentrations during the batch culture of *Scenedesmus*.

Experimental results reported by Sancho et al. (71) were used to calibrate the model. The parameters μ_{m1} of 0.0466 h^{-1}, μ_{m2} of 0.0256 h^{-1} and K_s of 0.2 µM were used from the literature. The parameters for the model were estimated following a curve fitting process using the batch experimental data of algae growth. The values of μ_{m1} and μ_{m2} obtained by curve fitting method were 6 h^{-1} and 0 h^{-1}, respectively.

$$\mu = \frac{\mu_{m1} C_t S_t}{K_S + S_t} + \frac{\mu_{m2} C_t K_s}{K_s + S_t} \quad (4)$$

The "predicted" specific growth rate of the algae was calculated by using the equation (4). There was a difference between the observed and predicted values of specific growth rate as shown in the Figure 3.15 likely due to experimental differences. However, the trend of the specific growth rate was similar to the model represented by equation (5). Light could be one of the reasons limiting the batch growth of microalgae.

Algae cultures become denser gradually and they block light from reaching deep into the reactor. Mixing is the next factor that could expose the algae cultures throughout the reactor to moderate light intensity and improve the light absorption (119). Growth rates of algae were also found to be higher in aerated than in non-aerated cultures (120).

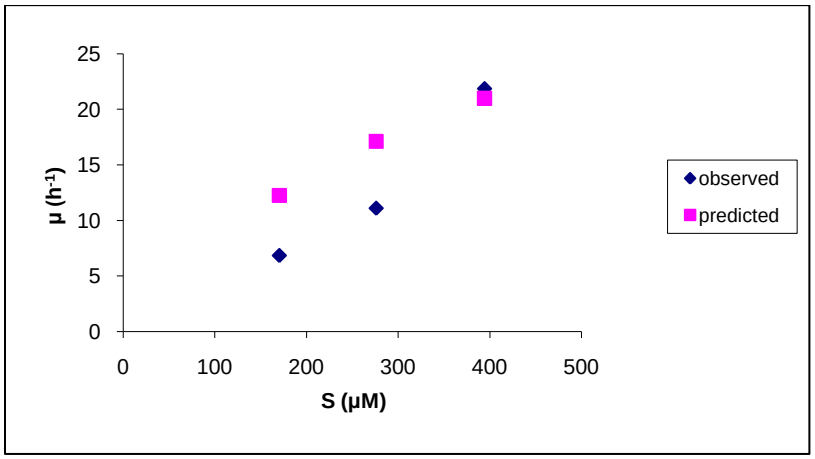

Figure 3.15. Change in phosphorus concentration with predicted and observed values of specific growth rate in the batch kinetics of *Scenedesmus*.

The day length is an important factor that can affect the growth rate of algae. Algae grown in light/dark cycles than continuous light could contribute to increased biomass production. The dark phase of algae growth is crucial for many biochemical processes. Dark phase is required in photosynthesis for two reactions. ATP and NADPH produced in the light dependent phase are used in the dark phase for growth and metabolism. Some key enzymes involved in photosynthesis and CO_2 fixation are inactive during the illumination (121). The cofactor $NADP^+$ required in the light phase is also produced in the dark phase.

Chlorophyceaen algae were observed with cell divisions during the dark conditions. The frequency of cell divisions was higher in the dark phase than the illuminated phase under cell equilibrium conditions. The photoperiod cycle with the light duration of between 12 and 15 hours is preferred to maintain the equilibrium in metabolic processes. This is considered as an optimum ratio to minimize the energy and biomass production in the large scale algae production units (121).

Algae are cultivated in autotrophic, heterotrophic and mixotrophic conditions. The cultivation mode also has a wide influence on the utilization of light and biomass production. Yang et al. found that only 1.5% of the supplied energy was transformed into autotrophic algal cultures. The biomass yield in the autotrophic culture was the lowest compared to other modes of cultivation because of the low absorption of light (122).

3.2.2. Biomass/Phosphorus Yield. The biomass yield obtained in the batch culture ($C-C_o$, mg/L) against the amount of phosphorus consumed given by the equation (5) showed a linear relationship as shown in the Figure 3.16.

$$C-C_o = Y_p (S_0-S) \quad (5)$$

where Y_p is biomass/phosphorus yield (mg μmol^{-1}).

The value of Y_p was calculated by linear regression (R^2=0.9511): This is the yield in the exponential phase.

$$Y_p = 0.3 \text{ mg } \mu mol^{-1} \quad (6)$$

The values obtained, though lower, are of the same order of the magnitude as those determined by Nyholm (123) for *Chlorella pyrenoidosa* (Y_p=0.97) and *Selenastrum capricornutum* (Y_p=0.89). The limiting factors for *Scenedesmus* growth could be irradiance, mixing and duration of the day light cycle.

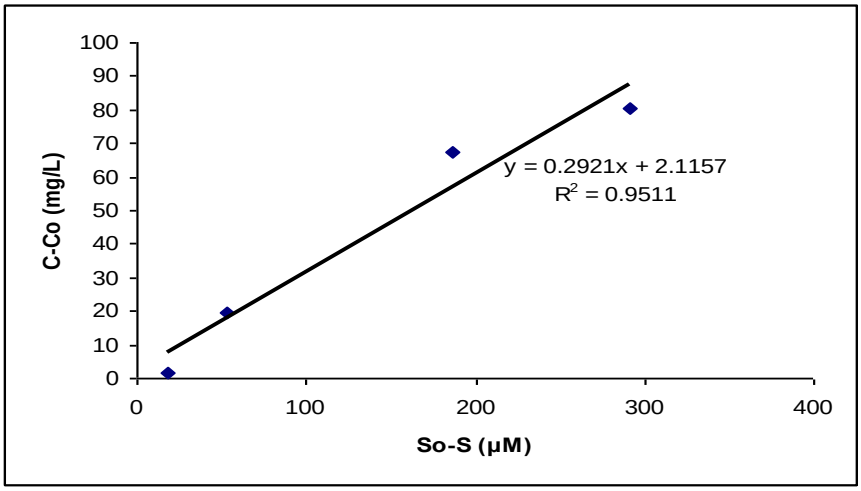

Figure 3.16. Relationship between biomass formed (C-C_0) and phosphorus consumed (S_0-S) in the batch culture of *Scenedesmus*.

3.3. MICROALGAL SEQUESTRATION OF CARBON DIOXIDE IN FLUE GAS

The combustion of fossil fuels generates carbon dioxide, a major greenhouse gas that is considered as a threat because of its potential to cause global warming. It becomes necessary to develop cost effective sequestration techniques. Microalgae are particularly considered for biofixation because of their ability to grow fast and fix greater amounts of carbon dioxide. The biomitigation of carbon dioxide and other flue gases by microalgae have significantly gained interest in reducing the emissions from coal-fired power plants. The algae biomass thus produced by capturing carbon can be used in generating valuable products such as fuel, animal feed and fertilizer.

3.3.1. Growth of Algae with Soluble Carbonates. Dissolved inorganic carbon, such as free CO_2 and bicarbonate, are carbon sources in the photoautotrophic growth of microalgae. Microalgae have higher photosynthetic efficiency than terrestrial plants because of the active transport system of inorganic carbon through an enzyme carbonic

anhydrase (99). This carbon concentrating mechanism enables algae to utilize carbon dioxide in various forms. Microalgae thus utilize carbon dioxide in the form of soluble carbonates. The ability of algae to grow with carbonate salts was investigated. Algae were grown with both sodium carbonate and sodium bicarbonate (Figure 3.17).

Figure 3.17. *Scenedesmus* algae grown with sodium carbonate (0.8 g/L) and sodium bicarbonate (0.63 g/L).

Algae were fed with carbonates and carbon dioxide on days 10 and 23 (Figure 3.18). The pH drop on days 10 and 23 are shown in Figure 3.19. The pH of algae culture with sodium carbonate was slightly higher by 0.5 than that with sodium bicarbonate after day 23. Algae grown with sodium bicarbonate had a higher growth by 0.131 difference of O.D. than with sodium carbonate on day 24. Algae grown with sodium bicarbonate translated to the increase in algae biomass of 177 mg/L and 255 mg/L on day 24 compared to sodium carbonate and fresh water, respectively, after the carbonates and carbon dioxide were supplied on day 23.

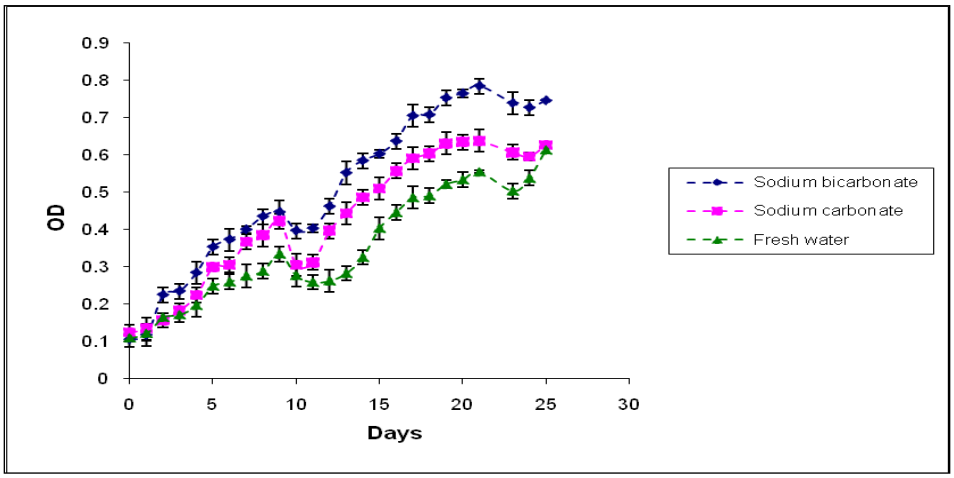

Figure 3.18. Growth curve of algae with sodium carbonate and sodium bicarbonate.

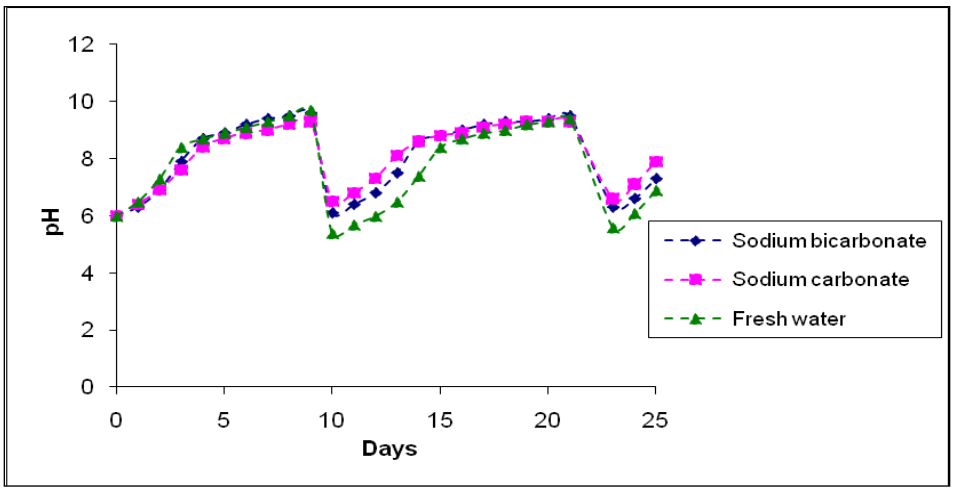

Figure 3.19. Change in pH of algae culture fed with carbonates and CO_2.

The addition of sodium bicarbonate to the culture medium enhanced the growth of algae. *Monoraphidium*, an order of the green alga belonging to *chlorococcales*, increased its growth rate 5 times as the bicarbonate concentration increased from 2 to 30 mM (6). *Chlorella vulgaris* grown with sodium bicarbonate had a higher growth rate than

compared to utilizing carbon dioxide from air. This alga showed highest growth rate at a concentration of 15.3 ppm of bicarbonate (124). Green algae *Chlorococcum littorale* also had higher growth rates with bicarbonate HCO_3^- than CO_2 (125). Bicarbonate provided more dissolved carbon and increased the rate of photosynthesis.

CO_2 is the readily absorbed form of carbon though algae can also utilize carbonate (CO_3^-) and bicarbonate (HCO_3^-). CO_2 is transported across the plasma membrane and algae cells incorporate it in the form of HCO_3^-. The enzyme carbonic anhydrase is responsible for the conversion of HCO_3^- to CO_2 (126). This enzyme also helps in the inorganic carbon uptake at alkaline pH and low CO_2 concentration in the growth culture (127).

The activity of the enzyme carbonic anhydrase depends on the amount of CO_2 present in the algae culture. The enzyme is more active in algae cultures grown in air than that supplemented with CO_2. Algae culture grown in air was able to incorporate larger dissolved inorganic carbon in their cells because of the activity of the enzyme carbonic anhydrase (128). This enzyme is also produced in larger amounts by many microalgae when grown under limited CO_2 conditions (129). Many unicellular microalgae have the ability to absorb increased amounts of dissolved inorganic carbon when transferred from high to low CO_2 conditions because of the activity of this enzyme (130).

Scenedesmus was found to have another mechanism to accumulate bicarbonate at higher pH from 7 to 11. Conversion from HCO_3^- to CO_2 was also possible by an "alkaline HCO_3^- transporter". This transporter aided in the incorporation of HCO_3^- from the medium to the CO_2 in the chloroplast of the algae cell. *Scenedesmus* were able to grow at alkaline pH upto 11 owing to the activity of this bicarbonate transporter (131).

3.3.2. Algal Sequestration of Carbon Dioxide from Coal Fired Power Plant. Carbon dioxide is a major greenhouse gas with a great potential to cause global warming. It is produced by both stationary and mobile sources. Power plants are the major sources of CO_2 and release 5.7 giga tones of carbon dioxide per year (132). Microalgae offer a natural way to recycle the carbon dioxide from the flue gas and thus help in reducing the effects of global warming and climate change.

Microalgal biomass is composed of 45% to 50% carbon based on dry weight measurements (133). The high carbon content of microalgae makes it suitable for storing carbon. CO_2 present is flue gas can significantly raise the growth rates of microalgae. Microalgae can be engineered in open ponds or photobioreactors to maximize CO_2 conversion to biomass thereby sequestering carbon and also producing a biofuel.

The selection of microalgae is the most important factor in the biomitigation of carbon dioxide from flue gases generated by power plants. The algae should have high growth and CO_2 utilization rates. The other suitable characteristics for carbon dioxide biofixation are their ability to tolerate SO_x and NO_x, and thrive in mass cultures without contamination. Lastly, the algae should be chosen also considering the harvesting process. Algae with autoflocculation characteristics simplify the harvesting step and minimize the energy and cost in the downstream processing of algae production (134).

Algae are isolated from the water bodies near to the power plants so that they are already adapted to the flue gases generated and environmental conditions of that area. Microalgae *Chlorella vulgaris* and *Scenedesmus obliquus* isolated from waste treatment ponds near a power plant showed tolerance to high CO_2 levels and were ideal for biofixation (135). They were able to grow in culture media with 18% (v/v) CO_2.

Biological carbon sequestration using microalgae offers several advantages over geological and ocean sequestration systems. Algae can sequester carbon dioxide directly and the costs of separation of CO_2 gas are avoided. Microalgae production systems can be located near the power plant: this does not require huge costs on transportation of CO_2. The advantages of using microalgae biofixation processes are: their ability to grow on flue gas, higher carbon fixation rates than other plants and their potential to grow in wastewaters thereby minimizing the fresh water needs (2).

The Solvay process can be modified to convert CO_2 from fossil fuel power plants to bicarbonates. The carbon dioxide gas is passed through brine solution with ammonia as a catalyst under alkaline conditions to produce sodium bicarbonate according to this chemical reaction (136).

$$CO_2 + NaCl + NH_3 + H_2O \rightarrow NaHCO_3 \downarrow + NH_4Cl \quad (7)$$

Sodium bicarbonate was a better carbon source for *Scenedesmus* than sodium carbonate. Carbon dioxide can be utilized in the form of carbonate salts using Solvay process. These salts can be used as a carbon source for algae growth when the power plant is not located near the algae pond. Algae can thus be used for biofixation of CO_2 in industrial flue gases.

The Central Electric Cooperative power plant in Jefferson City, Missouri at Chamois (Figure 3.20) is involved in a carbon capture research project by growing algae. It is a collaboration project done by a team of researchers from Lincoln University in Jefferson City and Missouri University of Science and Technology in Rolla in association with the Chamois power plant. Algae feeds on carbon dioxide emitted by power plants

that burn fossil fuels and may help address the global change. Algae are cultivated in five 2500 gallon pools near the power plant as shown in the Figure 3.21. The main flue gas stream at the top of the furnace is tapped with a four inch pipe and a small portion of the gas is diverted, which is run through a cooler, controlled for temperature and pressure, and then run through water stored in the pools. The algae strains were isolated and collected from water sources local to the Chamois plant.

Figure 3.20. Chamois power plant at Central Electric Cooperative at Jefferson City, Missouri.

Figure 3.21. Flue gas from power plant captured by five 2500 gallon algae pools.

4. CONCLUSIONS

Microalgae are valuable resources to the environment that offer a solution to both dwindling oil supplies of the world and environmental pollution. The broader aspect of this research was to explore the potential of microalgae in the bioremediation of nutrients in wastewater and sequestration of carbon dioxide from flue gas. The nitrogen and phosphorus for algal growth are utilized from wastewater in tertiary treatment and produce biomass. Carbon dioxide required for photosynthesis of microalgae is utilized from coal fired power plants thus helping in reducing the effects of global warming. The specific conclusions of this research were:

- The yield of *Scenedesmus* algae was 0.3 mg biomass μmol^{-1} phosphorus in batch cultures.
- There was a 30% decrease in the algae biomass obtained from growing algae with recycled water after flocculation using chitosan biopolymer.
- *Scenedesmus* algae were not inhibited of atrazine concentration upto 5 ppb.
- Harvesting of algae on a 90% volume yielded higher biomass with less labor cost and minimum frequency of removing water.
- *Scenedesmus* algae produced higher biomass with sodium bicarbonate as carbon source.

5. RECOMMENDATIONS

The following recommendations are made for future research:

- There are various steps to be taken for the implementation of the proposed system to operate economically on a full scale. A pilot plant needs to be established to study the algae growth and nutrient removal from wastewaters. Municipal wastewaters can be used to grow algae after the tertiary treatment when most of the BOD is already removed. This would minimize the contamination by bacteria and facilitate the growth of algae.

- Wastewater generated by different sources could be tested for algae growth. The presence of fertilizer (nitrogen and phosphorus) in the agricultural field run-off waters promotes growth whereas the pesticides, fungicides and herbicides may be detrimental to growth. It becomes necessary to determine the tolerance of these chemicals to algae. The other possible sources that could be tested for algae cultivation are water from concentrated animal feed operations and industrial wastewaters.

- Heterotrophic cultivation of algae using organic compounds offers a wide possibility of using industrial wastewaters to grow algae. The bioremediation of heavy metals from wastewaters can also be studied. These metals are required for various metabolic functions in algae.

- The combustion gases emitted by boilers can also be considered as a source of carbon dioxide to algae in addition to major sources like power plants. The water sources in the vicinity of the power plant could be used for algae growth. The algae isolated from these sources adapt well to the flue gas and tolerate the environmental conditions.

- The isolation of algae from various aquatic environments is essential for both nutrient removal and carbon dioxide biofixation. The lipid content and fatty acids of the algae should also be analyzed to find their suitability in biodiesel production. Genetic engineering of algae is also required to increase their growth and oil productivity.

- The development of effective harvesting techniques has always been a challenge in the commercial processing of microalgae. Optimum harvesting method should be selected based on a particular algae strain that would minimize the energy requirements. Bioflocculation using chitosan combined with magnetic separation technologies could be investigated.

BIBLIOGRAPHY

1. Chisti, Y. (2007) Biodiesel from microalgae. *Biotechnology Adv*. **25**: 294–306.

2. Schenk, P.M.; Thomas-Hall, S.R.; Stephens, E.; Marx, U.C.; Mussgnug, J.H.; Posten, C.; Kruse, O.; Hankamer, B. (2008) Second Generation Biofuels: High-Efficiency Microalgae for Biodiesel Production *Bioenergy. Res*. **1**: 20–43.

3. Brown, M.R.; Jeffrey, S.W.; Volkman, J.K.; Dunstan, G.A. (1997) Nutritional properties of microalgae for mariculture. *Aquaculture*. **151**: 315-331.

4. Becker, E.W. (2004) Large scale cultivation. In: Microalgae – Biotechnology and Microbiology. Cambridge University press, Cambridge. 63-171.

5. Tornabene, T.G.; Holzer G.; Lien, S.; Burris, N. (1983) Lipid composition of the nitrogen starved green alga *Neochloris oleabundans*. *Enzyme Microb. Technol.* **5**:435–440.

6. Sheehan, J.; Dunahay, T.; Benemann, J.; Roessler, P. (1998) "A look back at the US Department of energy's aquatic species program biodiesel from algae." National Renewable Energy Laboratory, Golden, Colorado.

7. Gouveia, L; Oliveira.A.C. (2008) Microalgae as a raw material for biofuels production *J Ind Microbiol Biotechnol*. **36**: 269-274.

8. Hu, Q.; Sommerfeld, M.; Jarvis, E; Ghirardi, M.; Posewitz, M.; Seibert, M.; Darzins, A. (2008) Microalgal triacylglycerols as feedstocks for biofuel production.: perspectives and advances. *The Plant Journal* **54**: 621–639.

9. Richmond, A. (2004) Basic Culturing Techniques & Downstream processing of cell-mass and products. In: Handbook of Microalgal culture-Biotechnology & Applied phycology. Blackwell publishing, Oxford, UK. 40-55 & 215-252.

10. Kirst, G. (1977) Ion composition of unicellular marine and freshwater algae, with special reference to Platymonas subcordiformis cultivated in media with different osmotic strengths. Oecologia **28**:177–189.

11. Sandnes, J.M.; Kallqvist, T.; Wenner, D.; Gislerod H.R. (2005) Combined influence of light and temperature on growth rates of *Nannochloropsis oceanica*: linking cellular responses to large-scale biomass production. *Journal of Applied Phycology* **17**: 515–525.

12. Richmond, A. (2004) Environmental effects on cell composition. In: Handbook of Microalgal culture-Biotechnology & Applied phycology. Blackwell publishing, Oxford, UK. 83-93.

13. Pulz, O. (2001) Photobioreactors: production systems for phototrophic microorganisms. *Appl. Microbiol. Biotechnol*. **57**:287–293.

14. Barbosa, B.; Albrecht, M.; Wijffels, R. (2003) Hydrodynamic stress and lethal events in sparged microalgae cultures. *Biotechnol. Bioeng.* **83**:112–120.

15. Pulz, O. (2001) Photobioreactors: production systems for phototrophic microorganisms. *Appl. Microbiol. Biotechnol.* **57**:287–293.

16. Carlozzi, P. (2003) Dilution of solar radiation through "culture lamination" in photobioreactor rows facing south north: a way to improve the efficiency of light utilization. *Biotechnol. Bioeng.* **81**:305–315.

17. Molina Grima EM, Fernandez FGA et al (1999) Photobioreactors: light regime, mass transfer, and scaleup. *J. Biotechnol.* **70**:231–247.

18. Janssen, M.; Slenders, P.; Tramper, J. (2001) Photosynthetic efficiency of Dunaliella tertiolecta under short light/dark cycles. *Enzyme Microb Tech* **29**:298**–**305.

19. Eriksen, N.T. (2008) The technology of microalgal culturing. *Biotechnol. Lett*. **30**: 1525–1536.

20. Garcia Camacho, F.; Molina Grima, E.; Miron, A.S.; Pascual, V.G.; Chisti, Y. (2001) Carboxymethyl cellulose protects algal cells against hydrodynamic stress. *Enzyme Microb. Technol.* **29**:602–610.

21. Gudin, C.; Thepenier, C. (1986) Bioconversion of solar energy into organic chemicals by microalgae. *Adv. Biotechnol. Process.* **6**: 73–110.

22. Uduman, N.; Qi, Y.; Danquah, M.K.; Forde, G.M.; Hoadley, A. (2010) Dewatering of microalgal cultures: A major bottleneck to algae-based fuels. *Journal of renewable and sustainable energy*. **2**:701-715.

23. Grima, E. M.; Belarbi, E.H.; Fernandez, F.G.; Medina, R; Chisti, Y. (2003) Recovery of micro algal biomass and metabolites: process options and economics. *Biotechnology Advances*. **20**: 491–515.

24. Petrusevski, B.; Bolier, G.; Van Breemen, A. N.; Alaerts, G.J. (2000) Tangential flow filtration: A method to concentrate freshwater algae. *Water Research* **29**: 1419-1424.

25. Rossignol, N.; Vandanjon, L; Jaouen, P.; Quemeneur, F. (1999) Membrane technology for the continuous separation microalgae: culture medium: compared performances of cross-flow microfiltration and ultrafiltration *Aquacultural Engineering*. **20**: 191–208.

26. Chen, Y.M.; Liu. J.C.; Hsu Ju, Y. (1998) Flotation removal of algae from water. Colloids and Surfaces *Biointerfaces*. **12**: 49-55.

27. Danquah, M.K.; Gladman, B; Moheimani, N; Forde, G.M. (2009) Microalgal growth characteristics and subsequent influence on dewatering efficiency. *Chemical Engineering Journal*. **151**: 73-78.

28. Golueke, C.G.; Oswald W.J. (1965) Harvesting and processing sewage grown planktonic algae. *J Water Pollution Control Federation*. **37**: 471– 98.

29. Tenney, M.W.; Echelberger, W.F.; Schuessler, R.G.; Pavoni, J.L. (1969); Algal flocculation with synthetic organic polyelectrolytes. *Appl Bacteriol*. **18**: 965–71.

30. Bilanovic, D.; Shelef, G.; Sukenik, A. (1988) Flocculation of microalgae with cationic polymers: effects of medium salinity. *Biomass* **17**: 65–76.

31. Chisti, Y. Shear sensitivity. In: Flickinger MC, Drew SW, editors. Encyclopedia of bioprocess technology: fermentation, biocatalysis, and bioseparation, vol. 5. New York: Wiley; (1999) 2379–406.

32. Kaya V.M.; Picard, G. (1996) Stability of chitosan gel as entrapment matrix of viable Scenedesmus bicellularis cells immobilized on screens for tertiary treatment of wastewater. *Bioresource Technology*. **56**: 147– 55.

33. Spoehr, H.A.; Milner, H.W. (1949) The chemical composition of Chlorella: Effect of environmental conditions. *Plant Physiology*. **24**: 120–149.

34. Khotimchenko, S.V., Yakovleva, I.M. (2005) Lipid composition of the red alga Tichocarpus crinitus exposed to different levels of photon irradiance. *Phytochemistry* **66**: 73–79.

35. Renaud, S.M.; Thinh, L.V.; Lambrinidis, G.; Parry, D.L. (2002) Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. *Aquaculture*. **211**:195–214.

36. Liu, Z.Y.; Wang, G.C.; Zhou, B.C. (2008) Effect of iron on growth and lipid accumulation in Chlorella vulgaris. *Bioresour. Technol.* **99**: 4717–4722.

37. Roessler, P.G. (1990) Environmental control of glycero lipid metabolism in microalgae: Commercial implications and future research directions. *J Phycol*. **26**: 393–399.

38. Rodolfi, L.; Zittelli, G.; Bassi, N.; Padovani, G.; Biondi, N.; Bonini, G.; Tredici, M (2009) Microalgae for Oil: Strain Selection, Induction of Lipid Synthesis and Outdoor Mass Cultivation in a Low-Cost Photobioreactor *Biotechnology and Bioengineering*. **102**: 100-112.

39. Hsieh, C.H.; Wu, W.T. (2009) Cultivation of microalgae for oil production with a cultivation strategy of urea limitation. *Bioresource technology*.**17**: 3921-6.

40. Li, Y.; Horsman, M.; Wang, B.; Wu, N.; Lan, C.Q. (2008) Effects of nitrogen sources on cell growth and lipid accumulation of green alga Neochloris oleoabundans *Appl Microbiol Biotechnol.* **81**: 629–636.

41. Takagi, M.; Watanabe, K.; Yamaberi, K.; Yoshida, T. (2000) Limited feeding of potassium nitrate for intracellular lipid and triglyceride accumulation of Nannochloris sp. UTEX LB1999. *Appl. Microbiol. Biotechnol.* **54**: 112–117.

42. Amin, S. (2009) Review on biofuel oil and gas production processes from microalgae *Energy Conversion and Management*. **50**: 1834–1840.

43. McKendry, P. (2003) Energy production from biomass: conversion technologies. *Biores. Technol.* **83**: 47–54.

44. Goyal, HB; Seal, D; Saxena, R.C. (2008) Bio-fuels from thermochemical conversion of renewable resources: a review. *Renew Sustain Energy Rev.* **12**: 504**–**517.

45. Wang, B; Li, Y; Wu,N; Lan, C.Q. (2008) CO_2 bio-mitigation using microalgae *Appl Microbiol Biotechnol*. **79**: 707–718.

46. Sialve B., Bernet, N.; Bernard, O. (2009) Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable, *Biotechnol Adv*. **27**: 409-416.

47. Tornabene, T.G.; Holzer G.; Lien, S.; Burris, N. (1983) Lipid composition of the nitrogen starved green alga *Neochloris oleabundans*. *Enzyme Microb. Technol.* **5**:435–440.

48. Speece, R.E. Anaerobic biotechnology for industrial wastewaters. Nashville: Archae press; 1996

49. Becker, E.W. (2004) Chemical composition. In: Microalgae – Biotechnology and Microbiology. Cambridge University press, Cambridge. 177-195.

50. Spolaore, P.; Cassan C. J., Duran, E; Isambert, A. (2006) Commercial applications of microalgae A. *Journal of Bioscience and Bioengineering*. **101**: 87-96.

51. Iwamoto, H.: Industrial production of microalgal cell-mass and secondary products – major industrial species – *Chlorella*, p.255-263. In Richmond, A. (ed.), Handbook of microalgal culture. Blackwell, Oxford (2004).

52. Metting, F.B. (1996) Biodiversity and application of microalgae. *J. Ind. Microbiol.* **17**: 477-489.

53. Yamaguchi, K. (1997) Recent advances in microalgal bioscience in Japan, with special reference to utilization of biomass and metabolites: a review. *J. Appl. Phycol*. **8**: 487–502.

54. Liang, S.; Xueming, L.; Chen, F.; and Chen, Z. (2004) Current microalgal health food R&D activities in China. Hydrobiologia **512**: 45–48.

55. Muller-Feuga, A. (2000) The role of microalgae in aquaculture: situation and trends. *J. Appl. Phycol.* **12**: 527–534.

56. Borowitzka, M. A. (1997) Microalgae for aquaculture: opportunities and constraints. *J. Appl. Phycol.* **9**: 393–401.

57. Zhang, C.W., Zmora, O., Kopel, R., Richmond, A., (2001) An industrial-size flat plate glass reactor for mass production of *Nannochloropsis* sp. (Eustigmatophyceae). *Aquaculture* **195**: 35–49.

58. Stolz, P. and Obermayer, B. (2005) Manufacturing microalgae for skin care. Cosmetics Toiletries **120**: 99–106.

59. Del Campo, J. A.; Moreno, J.; Rodríguez, H.; Vargas, M. A.; Rivas, J.; and Guerrero, M.G. (2000). Carotenoid content of chlorophycean microalgae: factors determining lutein accumulation in Muriellopsis sp. (Chlorophyta). *J. Biotechnol.* **76**: 51–59.

60. Hoffman, J.P. (1998) Wastewater treatment with suspended and non suspended algae. *J. Phycol.* **34**: 757–763.

61. Gonzales, L.E., Canizares, R.O., Baena, S. (1997) Efficiency of ammonia and phosphorus removal from a Colombian agroindustrial wastewater by the microlagae *Chlorealla vulgaris* and *Scenedesmus dimorphus*. *Bioresource Technol.* **60**: 259–262.

62. Martinez, M.E., Sanchez, S., Jimenez, J.M., El Yousfi, F., Munoz, L. (2000) Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*. *Bioresource Technol.* **73**: 263–272.

63. Olguın, E.J.; Galicia, S.; Mercado, G.; Perez, T. (2003) Annual productivity of *Spirulina* (*Arthrospira*) and nutrient removal in a pig wastewater recycle process under tropical conditions. *J. Appl. Phycol.* **15**: 249–257.

64. Tchobanoglous, G.; Burton, F.L.; Stensel, H.D. (2003) Constituents in waste water. In: Wastewater Engineering -Treatment and reuse. 4th ed. Metcalf & Eddy, Inc. Tata McGraw-Hill Publishing Co. Ltd. 27-64.

65. Kim, S.B.; Lee, S.J.; Kim, C.K.; Kwon, G.S.; Yoon, B.D.; Oh, H.M. (1998) Selection of microalgae for advanced treatment of swine wastewater and optimization of treatment condition *Korean Journal of Applied Microbiology and Biotechnology*. **26**: 76-82.

66. Travieso, L.; Benitez, F.; Sanchez, E.; Borja, R.; Leon, M.; Raposo, F.; Rincon, B. (2008) Assessment of a microalgae pond for post-treatment of the effluent from an anaerobic fixed bed reactor treating distillery wastewater. *Environmental Technology*. **29**: 985-992.

67. Hodaifa, G.; Martinez, E. and Sanchez, S. (2008) Use of industrial wastewater from olive-oil extraction for biomass production of *Scenedesmus obliquus*. *Bioresource Technology*. **99**: 1111-1117.

68. Morris, I. Nitrogen assimilation and protein synthesis, in: W.D.P. Stewart (Ed.), Algal Physiology and Biochemistry, University of California Press, California, 1974, pp. 583–610.

69. Kull, A. (1962) *Physiology and Biochemistry of Algae,* ed. R. A. Lewin. Academic Press, New York. 211-229.

70. Powell, N.; Shilton, A.N.; Pratt, S.; Chisti, Y. (2008) Factors Influencing Luxury Uptake of Phosphorus by Microalgae in Waste Stabilization Ponds. *Environ. Sci. Technol*.**, 42**: 5958–5962.

71. Sancho, M.E.; Jimenez Castillo, J. M.; Yousfi, F. (1997) Influence of phosphorus concentration on the growth kinetics and stoichiometry of the microalga *Scenedesmus obliquus. Process Biochemistry*. **32**: 657-664.

72. Garcia, J.; Mujeriego, R.; Hernandez, M. (2000) High rate algal pond operating strategies for urban wastewater nitrogen removal *Journal of Applied Phycology*. **12**: 331–339.

73. Olguin E.J.; Galicia, S.; Mercado, G.; Perez, T. (2003) Annual productivity of Spirulina (Arthrospira) and nutrient removal in a pig wastewater recycling process, under tropical conditions. *J. Appl. Phycol.* **15**:249–57.

74. Mallick, N. (2002) Biotechnological potential of immobilized algae for wastewater N, P and metal removal: A review *BioMetals*. **15**: 377–390.

75. Brouers, M.; Dejong, H.; Shi, D.J.; Hall, DO. (1989) Immobilized cells: An appraisal of the methods and applications of cell immobilization techniques. In: Cresswell, RC, Rees, TAV and Shah, N, eds. *Algae and Cyanobacterial Biotechnology*. New York: Longman Scientific and Technical Pub; 272–290.

76. Travieso, L.; Benitez, F.; Weiland, P.; Sanchez, E.; Dupeyron, R. ; Dominguez, A.R. (1996) Effect of immobilization on microalgae for nutrient removal in wastewater treatments. *Bioresource Technol*. **55**: 181–186.

77. Tam, N.F.Y.; Wong, Y.S. (2000) Effect of immobilized microalgal bead concentrations on wastewater nutrient removal. *Environ. Pollut*. **107**:145–151.

78. Bashan, L.E.; Hernandez, J.P.; Morey, T; Bashan,Y. (2004) Microalgae growth-promoting bacteria as ‘‘helpers’’ for microalgae: a novel approach for removing ammonium and phosphorus from municipal wastewater. *Water Research*. **38**: 466–474.

79. Weiner, J.A.; DeLorenzo, M.E.; Fulton, M.H. (2007) Atrazine induced species-specific alterations in the subcellular content of microalgal cells. *Pesticide Biochemistry and Physiology* **87**: 47–53.

80. Weiner, J.A.; DeLorenzo, M.E.; Fulton, M.H. (2004) Relationship between uptake capacity and differential toxicity of the herbicide atrazine in selected microalgal species. *Aquatic Toxicology* **68**: 121–128.

81. Behra, R.; Genoni, G. P; Joseph, A.L. (1999) Effect of Atrazine on Growth, Photosynthesis, and Between-Strain Variability in *Scenedesmus subspicatus* (Chlorophyceae). *Arch. Environ. Contam. Toxicol.* **37**: 36–41.

82. DeNoyelles, F.; Kettle, D.; Sinn, D.E. (1982) The responses of plankton communities in experimental ponds to atrazine, the most heavily used pesticide in the United States. *Ecology* **63**:1285–1293.

83. Hersh, C.M.; Crumpton, W.G. (1987) Determination of growth rate depression of some green algae by atrazine. *Bull Environ Contam Toxicol* **39**:1041–1048.

84. Lampert, W.; Flechner, W.; Pott, E.; Schober, U.; Storkel, K.U. (1989) Herbicide effects on planktonic systems of different complexity. *Hydrobiologica* **189**:415–424.

85. Solomon, K.R.; Baker, D.B.; Richards, R.P.; Dixon, K.R.; Klaine, S.J.; La Point, T.W.; Kendall, R.J.; Weisskopf, C.P.; Giddings, J.M.; Giesy, J.P.; Hall, L.W.; Williams, W.M. (1996) Ecological risk assessment of atrazine in North American surface waters. *Environ Toxicol Chem* **15**:31–76.

86. H.H. Omar (2002) Bioremoval of zinc ions by Scenedesmus obliquus and Scenedesmus quadricauda and its effect on growth and metabolism. *International Biodeterioration & Biodegradation*. **50**: 95 – 100.

87. Yu,R.Q.; Wang, W.X. (2004) Bio kinetics of cadmium, selenium, and zinc in freshwater alga *Scenedesmus obliquus* under different phosphorus and nitrogen conditions and metal transfer to *Daphnia magna*. *Environmental Pollution*. **129**: 443–456.

88. Shehata, S.A.; Badr, S.A. (1980) Growth response of scenedesmus to different concentrations of copper, cadmium, nickel, zinc and lead. *Environment International*. **4**: 431–434.

89. Chen, F.; Johns, MR. (1991) Effect of C/N ratio and aeration on the fatty acid composition of heterotrophic Chlorella sorokiniana. *Appl. Phycol.* **3**:203–9.

90. Wu, Q.Y.; Yin, S.; Sheng, G.; Fu, J. (1994) New discoveries in study on hydrocarbons from thermal degradation of heterotrophically yellowing algae. Sci China (B) **37**:326–35.

91. Huang, G.; Chen, F.; Wei, D.; Zhang, X; Chen, G. (2010) Biodiesel production by micro algal biotechnology. *Applied Energy*. **87**: 38-46.

92. Xu, H.; Miao, X.L.; Wu, Q.Y. (2006) High quality biodiesel production from a microalga Chlorella protothecoides by heterotrophic growth in fermenters. *J. Biotechnol.* **126**:499–507.

93. Xue, F.; Zhang, X.; Luo, H.; Tan, T. (2006) A new method for preparing raw material for biodiesel production. *Process Biochem.* **41**:1699–702.

94. Bilanovic, D.; Andargatchew, A; Kroeger, T.; Shelef, G. (2009) Freshwater and marine microalgae sequestering of CO_2 at different C and N concentrations – Response surface methodology analysis. *Energy Conversion and Management*. **50**: 262–267.

95. Sakai, N.; Sakamoto, Y.; Kishimoto, N.; Chihara, M.; Karube, I. (1995) Chlorella strains from hot springs tolerant to high temperature and high CO_2. *Energy Convers. Manag.* **36**:693–696.

96. Maeda K, Owada M, Kimura N, Omata K, Karube I (1995) CO2 fixation from the flue gas on coal-fired thermal power plant by microalgae. *Energy Convers. Manag.* **36**:717–720.

97. Matsumoto, H.; Hamasaki, A.; Sioji, N.; Ikuta, Y. (1997) Influence of CO_2, SO_2 and NO in flue gas on microalgae productivity. *J Chem Eng Japan* **30**:620–6.

98. Emma Huertas, I.; Colman, B.; Espie G.S.; Lubian, L.M. (2000) Active transport of CO_2 by three species of marine microalgae. *J Phycol.* **36**:314–320.

99. Azov, Y. (1982) Effect of pH on Inorganic Carbon Uptake in Algal Cultures. *Applied and environmental microbiology*. **43**: 1300-1306.

100. Sobczuk, T.M.; Camacho, F.G.; Rubio, F.C.; Fernandez, F.G.; Grima, E.M. (2000) Carbon dioxide uptake efficiency by outdoor microalgal cultures in tubular airlift photobioreactors. *Biotechnology and Bio engineering*. **67**: 465-475.

101. Chinnasamy, S.; Ramakrishnan, B.; Bhatnagar, A.; Das, K.C. (2009) Biomass Production Potential of a Wastewater Alga *Chlorella vulgaris* ARC 1 under Elevated Levels of CO_2 and Temperature. *Int. J. Mol. Sci*. **10**: 518-532.

102. FitzGerald, G.P.; Rohlich, G.A. (1962) Biological removal of nutrients from treated sewage: Laboratory experiments. *Verh. Int. Verein. Theor. Angew Limol. XV*, 597-608.

103. Eaton. A.D.; Clesceri.L.S.; Greenberg, A.E.; American Public Health Association, American Water Works Association and Water Engineering Federation (1995) Standard methods for the examination of water and waste water.19th ed. APHA publication, Washington DC, US. 4.75-4.82 & 4.106-4.112.

104. Martinez, M.E.; Sanchez, S.; Jimenez, J.M.; El Yousfi, F.; Munoz, L. (2000) Nitrogen and phosphorus removal from urban wastewater by the microalga Scenedesmus obliquus. *Bioresource Technology* **73:** 263-272.

105. Kim, M.K.; Park, J.W.; Park, C.S.; Kim, S.J.; Jeune, K.H.; Chang, M.U.; Acreman, J. (2007) Enhanced production of Scenedesmus spp. (green microalgae) using a new medium containing fermented swine wastewater. *Bioresource Technology* **98:** 2220–2228.

106. Aslan,S; Kapdan,I.K. (2006) Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecological Engineering*. **28**: 64-70.

107. Isac, L.; Dumitrescu, L.; dragan, D.; Manciulea, I.; Tica, R. (2003) The phosphorus biogeochemical cycle in Brasov district lakes. *Ovidius University Annals of Chemistry*. **14**: 24-27.

108. Rabea, E.I.; Badawy, M.E.; Stevens, C.V.; Smagghe, G.; Steurbaut, W. (2003) Chitosan as Antimicrobial Agent: Applications and Mode of Action. *Biomacromolecules.*

109. Fleeger, J.W.; Carman, K.R.; Nisbet, R.M. (2003) Indirect effects of contaminants in aquatic ecosystems. *The Science of the Total Environment* **317**: 207–233.

110. Anton, F.A.; Laborda, E.; Laborda, P. (1993) Acute toxicity of technical captan to algae and fish. *Bull. Environ. Contamin. Toxicol.* **50**:392-399.

111. Larsen, D.P.; deNoyelles, Jr. F.; Stay, F.; Shiroyama. T. (1986) Comparisons of single-species, microcosm and experimental pond responses to atrazine exposure. *Environ. Toxicol. Chem.* **5**:179-190.

112. Reinhold, D.; Hofner, W.; Kohler, W. (1994) Influence of Cu, Cd and atrazine on the metabolism of the unicellular green alga *Scenedesmus subspicatus. Z. Pflanzenernahr. Bodenk.* **157**: 145–50.

113. Wurster, C.F. (1968) DDT reduced photosynthesis by marine phytoplankton. *Science* **159**: 339–351.

114. Schnoor, J.L. (1996). Eutrophication of lakes. In: Environmental Modeling – Fate and transport of pollutants in water, air and soil. John Wiley & Sons, US.185-194.

115. Rhee, G.Y. (1978) Effects of N:P atomic ratios and nitrate limitation on algae growth, cell composition and nitrate uptake. *Limnol. Oceanogr.* **23**: 10-25.

116. Rothhaupt, K. O. (1995) Algal nutrient limitation affects rotifer growth rate but not ingestion rate. *Limnol. Oceanogr.* **40**: 1201-1208.

117. Lodi, A.; Binaghi, L.; Solisio, Converti, A.; Del Borghi, M. (2003) Nitrate and phosphate removal by *Spirulina platensis*. *J Ind. Microbiol. Biotechnol*. **30**: 656–660.

118. Grobbelaar. J.U. (2000) Physiological and technological considerations for optimising mass algal cultures. *Journal of Applied Phycology*. **12**: 201–206.

119. Rocha, J.; Garcia, J.; and Henriques, M. (2003) Growth aspects of the marine microalga *Nannochloropsis gaditana*. *Biomolecular engineering.* **20**: 237-242.

120. Perez, E.B.; Pina, I.C.; Rodriguez, L.P. (2008) Kinetic model for growth of *Phaeodactylum tricornutum* in intensive culture photobioreactor. *Biochemical Engineering Journal.* **40**: 520–525.

121. Bouterfas, R.; Belkoura, M.; Dauta, A. (2006) The effects of irradiance and photoperiod on the growth rate of three freshwater green algae isolated from a eutrophic lake. *Limnetic*. **25**: 647-656.

122. Yang, C.; Hua, Q.; Shimizu, K. (2000) Energetics and carbon metabolism during growth of microalgal cells under photoautotrophic, mixotrophic and cycliclight-autotrophic/dark-heterotrophic conditions. *Biochemical Engineering*. **6**: 87– 102.

123. Nyholm, N. (1977) Kinetics of phosphate limited algal growth. *Biotechnology and Bioengineering*. **19**: 467-492.

124. Jeong, M.L.; Gillis, J.M.; Hwang, J.Y. (2003) Carbon Dioxide Mitigation by Microalgal Photosynthesis *Bull. Korean Chem. Soc.* ***24:*** 1763.

125. Ota, M.; Kato, Y.; Watanabe, H.; Watanabe, M.; Sato, Y.; Smith Jr, R.L.; Inomata, H. (2009) Effect of Inorganic Carbon on Photoautotrophic Growth of Microalga Chlorococcum littorale. *Biotechnology Progress* **25:**492-498.

126. Badger, M.R.; Price, G.D. (1994) The CO_2 concentrating mechanism in cyanobacteria and green algae. *Physiol. Plant.* **84**: 606-615.

127. Moroney, J.V.; Husic, H.D.; Tolbert, N.E. (1985) Effects of carbonic anhydrase inhibitors on inorganic carbon accumulation by *Chlamydomonas reinhardtii*. *Plant J.* **9**:819-827.

128. Aizawa, K.; Miyachi, S. (2006) Carbonic anhydrase and CO_2 concentrating mechanisms in microalgae and cyanobacteria. *FEMS Microbiology letters*. **39**:215-233.

129. Raven, J.A. (1997) Inorganic carbon acquisition by marine autotrophs. .*Adv Bot Res.* **27:** 85–209.

130. Miyachi, S.; Iwasaki, I.; Shiraiwa, Y. (2003) Historical perspective on microalgal and cyanobacterial acclimation to low- and extremely high-CO_2 conditions *Photosynthesis Research* **77**: 139–153.

131. Thielmann, J.; Tolbert, N.E.; Goyal, A.; Senger, H. (1990) Two Systems forConcentrating CO_2 and Bicarbonate during Photosynthesis by *Scenedesmus*. *Plant Physiol.* **92**: 622-629.

132. Kadam, K.L. (2001) Microalgae Production from Power Plant Flue Gas: Environmental Implications on a Life Cycle Basis. National Renewable Energy Laboratory, technical report.

133. Schlesinger.W.H. (1991) Biogeochemistry: An analysis of global change. 2nd ed. Academic press, California, US.

134. Brennan, L.; Owende, P. (2010) Biofuels from microalgae - A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews.* **14**: 557–577.

135. De Morais, M.G.; Costa, J.A.V. (2007) Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. *Energy Conversion and Management*. **48**:2169–73.

136. Huang. H. P.; Shi,Y.; Li, W.; and Chang, S.G. (2001) Dual Alkali Approaches for the Capture and Separation of CO_2. *Energy & Fuels.* **15**: 263-268.

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