DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

EFFECT OF OPERATING PARAMETERS ON NUTRIENT REMOVAL BY.*C.VULGARIS* IN CONTINUOUSLY OPERATED PHOTOBIOREACTOR SYSTEM

by

Hülya AYDIN

October, 2007

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EFFECT OF OPERATING PARAMETERS ON NUTRIENT REMOVAL BY.*C.VULGARIS* IN CONTINUOUSLY OPERATED PHOTOBIOREACTOR SYSTEM

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M.Sc THESIS EXAMINATION RESULT FORM

We certify that we have read this thesis and **"EFFECT OF OPERATING PARAMETERS ON NUTRIENT REMOVAL BY*C.VULGARIS* IN CONTINUOUSLY OPERATED PHOTOBIOREACTOR SYSTEM"** completed by **HÜLYA AYDIN** under supervision of **ASSOC. PROF. DR. İLGİ K. KAPDAN** and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

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Hülya AYDIN

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ABSTRACT

Nitrogen and phosphorus removal from synthetic wastewater by *C. vulgaris* was investigated in this thesis. The study has two parts as fed-batch flask experiments and continuously operated immobilized photobioreactor system. The experiments were conducted at neutral pH and at room temperature with artificial illumination.

Effects of initial ammonia nitrogen concentrations and N/P ratio on nitrogen and phosphorus removal were evaluated in the fed-batch experiments. The results indicated that the maximum removal efficiency was obtained with minimum ammonia nitrogen concentration and N/P ratio.

The photobioreactor system was continuously operated at different nitrogen/phosphorus ratio and light intensity. The optimum conditions for maximum NH_4-N removal were determined as minimum light intensity and minimum N/P ratio.

Keywords: Microalga**,** *Chlorella vulgaris*, nutrient removal, nitrogen, phosphorus, wastewater treatment.

C.VULGARIS İLE SÜREKLİ İŞLETİLEN FOTOBİYOREAKTÖR SİSTEMİNDE İŞLETİM PARAMETRELERİNİN NÜTRİENT GİDERİMİNE ETKİSİ

ÖZ

Bu tezin kapsamında *Chlorella vulgaris* ile sentetik atıksulardan azot ve fosfor giderimi incelendi. Çalışma yarı kesikli flask deneyleri ve sürekli işletilen baglı büyüme fotobiyolojik reaktor sistemi deneyleri olmak üzere iki kısımdan oluşmaktadır. Deneyler oda sıcaklıgında, nötral pH'da ve yapay ışıkta yürütülmüştür.

Azot/fosfor oranı ve giriş azot konsantrasyonlarının azot ve fosfor giderimi üzerine etkileri değerlendirilmiştir. Bulunan sonuçların işaretinde, maksimum azot giderim verimi düşük azot derişiminde ve azot/fosfor oranında elde edilmiştir.

Fotobiyolojik reaktor sistemi sürekli koşullarda farklı azot/fosfor oranlarında ve farklı ışık şiddetinde işletilmiştir. Optimum şartlarda maksimum azot giderimi düşük ışık şiddetinde tayin edilmiştir. Maksimum giderim verimi düşük azot/fosfor oranında elde edilmiştir.

Anahtar Kelimeler: Mikroalk**,** *Chlorella vulgaris,* nutrient giderimi, azot, fosfor, atıksu arıtımı.

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CHAPTER ONE
INTRODUCTION

1.1 Literature Review

Main supplies of eutrophication are nitrogen and phosphorus that human motions as agricultural practices, urbanization, industrialization and other alterations produce. Dangerous environmental problems and algal blooms can be a reason of eutrophication which is known as the most critical water quality problem all over the Earth. Biological nutrient removal systems for averting eutrophication widely can be differentiated as bacterial nutrient removal and algal nutrient removal.

1.1.1 Bacterial nutrient removal

Issued particular scientific researches about nitrogen and phosphorus removal structured on biological processes and various combinations of anaerobic, aerobic and anoxic zones (Bardenpho, A_2O, UCT and modifications included). In the other hand, biological and chemical methods were applied in combination also. Bardenpho process for nitrogen and phosphorus removal makes use of the sequences of anaerobic, anoxic and aerobic actions. During that process nitrogen is moved off by nitrification-denitrification, while phosphorus is moved off by wasting sludge from system. In addition to it, sequencing batch reactor (SBR) operations plays a big role in COD/BOD and phosphate removal. In order to succeed nitrogen removal by nitrification and denitrification in addition to COD and phosphate removal, the modifications of sequencing batch operation are practiced. Inside same reactor (Kargi & Uygur, 2003) the SBR anaerobic, anoxic and oxic phases are placed in the SBR operations. Moreover, constructed wetlands and immobilize nitrifiers are potentially useful to achieve nitrogen and phosphorus removal from wastewaters (Picard et al., 2005).

1.1.2 Algal Nutrient Removal

Various algal species are very potential by the means of biotechnology for generation of many beneficial products. For example, feed in aquaculture and biological treatment of wastewaters, animal feed, biofertilizer and food. Current stuidies show that how algae for production of variety compounds such as, polysaccharides, lipids, carotenoids, pigments, vitamins, sterols, enzymes, antibiotics, pharmaceuticals and other fine chemicals as well as hydrogen, hydrocarbons and other biofuels can be potentially usefull.

Microalgae have a valuable potential to fix carbon dioxide that makes green house effect. Due to higher photosynthetic rates per unit biomass, photosynthetic microorganisms are expected to play a wider role than higher plants in photosynthetic carbon dioxide fixation

Treatment of wastewater by Algae is provided by two mechanisms. The first mechanism is carried by producing of oxygen melted in wastewater and used by bacteria to oxidize wastes. In the other mechanism, like ammonia for its own biomass generation the algae exercise nutrients through water. Due to the rapid increasing growth resulting in production of biomass and consumption of nutrients from the wastes, algae are very beneficial for waste treatment. Containing ecological variety, microalgae are able to exert different mediums. In this scope, cultivation issues can be solved in multiple approaches. Some inputs as carbon dioxide, water, light and mineral salts or other nutrient sources (N, P) are needed during process of algal biomass production. Potentially algae is very advantageous to decrease and/or ionize odorous unites as hydrogen sulfide, ammonia.

1.1.2.1 Algal Treatment Systems

Both open and closed systems are eligible for production of algae. While considering open systems they are divided into artificial ponds or containers and natural systems (lakes, lagoons, ponds). Closed systems can be handled under below groups:

a) closed container based systems (tanks, hanging plastic bags).

b) vertical or horizontal tubular systems (glass, plastic, and bags),

c) ultrathin immobilized configuration systems,

d) inclined or horizontal flate-plate systems,

Open systems have several difficulties for obtaining high productivity which can be regarded as an obstacle. Extravagant evaporative loses, diffusion of CO_2 to the atmosphere, light intensity during a day, different temperatures are considerable problems. As contamination arises open ponds need broad surface place. While some disadvantages are removed by covering ponds, better control of temperature, light intensity, better control of gas transfer and broader surface area-to-volume ratio will be carried by enclosed systems.

By preserving algae genetics off the contamination and decreasing the chance of parasite infestation the practice of closed photobioreactors (PBRs) raise commercial algal biomass production. Known as initial generation of closed PBRs enhance of simple container based systems ,afterwards encountered with critical limitations for efficient supply of light by maximizing the illumination surface-to-volume ratio of the reaction. Therefore, the underwater lighting has some technical approaches (submersed lambs or light diffusing optical fibers).

Due to the light distribution and appropriate flow vertical arrangements of horizontal running plates or tubes are chosen (Ogbonna &Tanaka, 1997; Pulz, 2001). Pls see Advantages and disadvantages of closed and open systems are given in Table 1.1.

Table 1.1 Advantages and disadvantages of open and closed algal cultivation plants (Pulz, 2001).

Parameter	**Open Ponds(raceways)**	**Closed systems (PBR_S)**
Contamination risk	Extremely high	Low
Space required	High	Low
Water losses	Extremely high	Almost none
CO_2-losses	High	Almost none
Biomass quality	Not susceptible	Susceptible
Variability as to cultivatable species	Not given,cultivation possibilities are resticted to a few algal varieties	High, nearly all microalgal varieties may be cultivated
Flexibility of production	Change of production between the possible varieties nearly impossible	Change of production without any problems
Raproducibility of production parameters	Not given,depending on exterior conditions	Possible within certain tolerances
Process control	Not given	Given
Standardization	Not possible	Possible
Weather dependence	Absolute,production impossible during rain	Insignificant because closed configurations allow production also during bad weather
Period until net production is reached after start or interruptions	Long,approx. 6-8 weeks	Relatively short , approx. 2-4 weeks
Biomass concentration during production	Low, approx. 0.1-0.2 g/l	High, approx. 2-8 g/l
Efficiency of treatment processes	Low, time consuming, large volume flows due to low concentrations.	High,short time,relatively small volume flows

1.1.2.2 Important Design Parameters for Photobioreactors

Several environmental characteristics as light intensity, CO_2/O_2 balance, temperature, salinity, nutrients, pH value and turbulence are the fundaments for the usage of algae for wastewater treatment.

1.1.2.2.1 Growth Index: Designing and optimizing photobioreactors have wide range of features, many of which are offered by mathematical modeling of photosynthetic cell growth. Obviously, growth parameters in growth kinetics and photosynthetic cell growth models regarded as specific rates being used in the process of exponential growth. The cell growth thanks to its strong effect on to culture productivity would be a positive index for process design and optimization during a growth. Research conducted by Ogbonna & Tanaka (1997) shows us the relevant importance of the exponential and linear growth rates during light-limited batch cultivation of photosynthetic cells practicing different types and sizes of photobioreactors as a first step in the photobioreactor design. Cultivation process of *Chlorella pyrenoidosa* C-212 and *Spirulina platensis* M-135 reveals about negative relevance among the particular growth rates and the linear growth rates or between the specific growth rates and the final cell concentrations. They also developed the mathematical approach to explain that the different growth phases existing during the light-limited batch cultivation. In respect to this model the exponential growth phase under various conditions is shorter than the linear growth phase. Therefore, the specific growth rate is worse than the linear growth rate.

1.1.2.2.2 Light inside the photobioreactor: The photoautotrophic life is mainly limited by the light factor. Positive outcome of the illumination intensities is the proportionality of the photosynthesis rate to light intensity. But, photosynthetic receptor system can be damaged within a several minutes by very high illumination intensities. Most suitable measure of photobioreactor performance is the sum of the light energy supplier per unit volume. Studies by Ogbonna & Tanaka provide that increase in depth of the photobioreactors is a reason of decrease in linear growth rates.

Considering these outcomes which indicate the light distribution inside the reactor in the rational design and scale-up of photosynthetic processes, is a must. Shallow photobioreactor has more homogenous light distribution that deep photobioreactor. Therefore, surface area per unit volume can be quoted as a vital design parameter. To achieve efficient photobioreactor having a high surface area-to-volume ratio in cultivation ponds reserving the pond as shallow as possible can be very effective. Nevertheless, several scale-up problems still remain which can be stated as limiting tube diameters and panel depths.

1.1.2.2.3 CO_2/O_2 Balance: In order to perform photosynthesis algae need an inorganic carbon source as other autotrophically growing plant. Therefore, in algal cultures CO_2 has to be sourced while increasing O_2 has to be elimininated until reaching inhibitory concentrations. Oxygen can trigger a problem in algal cultures of high cell densities not just for the reason of the limitation of the rate of photosynthesis, but also compatible energy, oxygen radicals may develop during the respiratory gas exchange and cause toxic effects on cells due to membrane damage. The lack of concentration rate (only 0.03 %) of the natural CO_2 in air is not sufficient for sustaining growth and high productivity. Chemical analyses show that 50% of algal biomass composed from carbon, which states that production of 1 kg of algae needs 1,8 kg of carbon dioxide. So, it is required to enter some CO_2 .Costliness of pure carbon dioxide, make alternative and cheaper sources of this gas from industrial combustion process, diesel engines, cement plants or fermentation be examined. Thus it would be better to place algal ponds near such plants.

1.1.2.2.4 Temperature: Between the features that have an impact on the growth of microalgae and photosynthetic reactions in algal medium, temperature can be referred as a major one. While carbon dioxide or light is limiting for photosynthesis effective role of temperature is enormous. As temperature decreases, photosynthesis becomes less efficient. At high temperatures it can deteriorate suspension cultures by the difference in decrease of CO_2 and O_2 solubility.

1.1.2.2.5 Salinity, nutrients and pH value: One of the vital features in algal cultivation is the pH. Affecting solubility of carbon dioxide and minerals in the medium, metabolism of the algae directly or indirectly is influenced by it. pH of algal cultures is affected by some factors as amount of dissolved CO_2 solubility, composition and buffering capacity of medium and metabolic activity of algal cells. Raise of ammonia stripping and precipitation of calcium phosphate is possible in alkaline pH. The lack of nutrients will be a reason of disorder in metabolism and disproportionate production of intermediates of photosynthesis. Physiological reactions and productivity problem will be the reasons of declinations from the salinity and optimum osmotical conditions.

1.1.2.2.6 Turbulence: Photosynthetic reactions having a vital effect on light distribution in algal medium present an important parameter called as turbulence.

1.1.2.2.7 Mixing: Known as a major parameter for photobioreactors, mixing is used in keeping cells in suspension, distributing the nutrients, generating heat in the reactor, improving CO_2 transfer into the reactor, stripping the photosyntheticaly generated O_2, improving mass transfer among cells and the liquid milieu and facilitating the movement of cells in and out of the illuminated part of the reactor. Lack of cells, mobility and filamentous features makes much of photosynthetic cells breakable and sensitive to shear stress. Therefore, reserving the hydrodynamic stress in lower levels should be preferred.

1.1.2.2.8 Contamination: Lowness of contamination by heterotrophic microorganisms will depend on deficiency of organic carbon source. As contamination by other photoautotrophs is a critical topic sterilization of the new photobioreactor is required.

1.1.3 Literature on Algal Nutrient Removal

Bitch, Yaziz & Baktı using high rate algal pond (HRAP) for nitrogen removal of agro-industrial wastewater examined the combination of *Chlorella vulgaris* and waterhyacinth (*Eichhornia crassipes*). Duration of each run was 20 days with the ambient temperature averaging at 32°C amid the experiment. 500 mg/lt COD, 42.1 mg/lt NH_4-N, 1.33 mg/lt NO_3-N, 0.61 mg/lt NO_2-N was added to the wastewater. In normal HRAP system the N removal efficiency was approximate to 80%.Normal HRAP had achieved 23% worse removal than integrated system did. Assimilation by the algae (55%) and waterhyacinth (10%) now calculated for about 65% of the total N removal could be embraced by the nitrogen removal in combined system. Calculated for 15%, ammonia volatilization after nitrification-denitrification amounted about 20%. Furthermore, no extra need presents for suspended solid removal in integrated treatment system.

Craggs, McAuley & Smith (1997) examined the wastewater nutrient removal by marine microalgae grown on a corrugated raceway. A sewage outfall site in Scotland was a place from which two marine microalgal isolates were picked up (SA91B33, *Phaeodactylum tricornutum* and SA91CY1, *Oscillatoria sp*.). Diluted 1:1 with sterile seawater, wastewater was gradually included to raceway algal cultures grown under ambient conditions. Amount of efficient concentratations were about 497.7 mmol NH_4-N m^{-3} and 76.2 mmol PO_4-P m^{-3} . 100% of ammonium and orthophosphate was prolonged to be removed from both species. Nutrient removal not only provided by invasion and assimilation into algal biomass where pH of raceway effluents stayed up (>9.5). There we can assume that violitalization of ammonium and precipitation of orthophosphate can be possible. The end of the study demonstrated that two isolates could preserve unialgal culture during summer and autumn situations. Not affected by diurnal cycle removal of nutrients was accomplished and continual. It is pointed that the system does not need mixing and may permit simple mechanical harvesting of the algal biomass by using the adherent properties of these isolates. In this study proposed issue is about potential use of microalgae for nutrient removal.

Olguin, Galicia, Angulo-Guerrero & Hernandez (2001) researched the impact of flux and nitrogen lack on the chemical composition of *Spirulina Arthrospira* grown on complex medium containing sea-water supplemented with anaerobic effluents from digested pig waste. Bench raceways in batch culture conditions were provided by the experiments. They examined two different light flux of 66(lower) or 144 μmol photon $m^{-2}s^{-1}$ (higher). Growth kinetics was degreed in complex medium and in modified Zarrouk medium as a control medium during cultivation at two light fluxes. Biomass concentration was alike in two media when cultivated at 66 μmol photon $m^{-2}s^{-1}$ at the end of 12 days. The first ammonia concentration of medium was 1 mM, incubated at 32°C and under nun-pH control regime. Therefore, it was estimated to get big amounts of ammonia stripping and by consumption could become nitrogen deficient after several days. Not regarding the the light intensity, Protein content of the biomass in the complex medium was completely lower. Nevertheless, biomass from the complex medium was enriched in total lipids (28.6 %) at lower light flux.

Spirulina Arthrospira was evaluated under its annual productivity and its ability to abolish nutrients in outdoor raceways treating anaerobic effluents from pig wastewater under tropical conditions. The temperature of maintained pH at 9.5±0.2 was in the range of 29-34 °C and the average light intensity in the range of 476-1784 μmol photon $m^{-2}s^{-1}$. The productivity was importantly more in 1998 with a pond depth of 0.10 m than 0.065 m for batch cultures. In autumn 2000 or winter 2001 the average productivity in semi-continuous cultures was lower than summer 1999, when the depth was 0.15 and 0.20 m (14.4 and 15.1 g $m^{-2}d^{-1}$ in range). The common protein content of the semi-continuous cultures was 48.9% ash-free dry weight.

Based on the depth of the culture and the season NH_4-N removal was in the range 84-96 % and P removal in the range of 72-87 % (Olguin, Galicia, Mercado & Perez, 2003). Travieso, Benitez, Weiland, Sanchez, Dupeyron & Dominguez (1996) conducted some experimental studies on nutrient removal from raw sewage and pre-treated cattle-manure by immobilization of microalgae. Internal immobilization with sodium alginate and k-carrageen and external immobilization with using polystyrene

and polyurethane foams were used to evaluate the immobilization capacities of *Chlorella vulgaris, Chlorella kessleri* and *Scenedesmus quadricauda*.. Good results with raw sewage were obtained after the use sodium alginate pellets of *Chlorella vulgaris* and *Chlorella Kessleri* (Ammonia-N=31±5, O-Phosphate=6±2 mg/lt). Utilization by *Chlorella vulgaris* in an expanded-bed when the columns operated under natural light caused higher efficiencies.By use of *Chlorella vulgaris* the maximum orthophosphate removal (72 %) was achieved, while it was 60 % in the *kessleri* expanded column. Natural light raised the nutrient removal compared with the artificial light in respect to results. Because of the dark color of this substrate, internal immobilization and the use of an upflow pattern could not succeed with pretreated cattle manure (Ammonia-N=237±43, O-Phosphate=34±3 mg/lt, diluted 1:4 with tab water). Nevertheless, in the range of 0.5-2.5 lt/day obtained results were good at hydraulic loadings using downflow columns. Nutrient removal efficiency was decreased as hydraulic loading increased. The ammonia nitrogen removal changed from 64 to 48 % during the state of *Chlorella* column. Taking into account the low cost of this material compared with sodium alginate and k-carrageenan, external immobilization with polyurethane foam is expected to have a positive outcomes in respect to results.

The effect of immobilized microalgal bead concentrations on simulated settled domestic wastewater nutrient removal was studied by Tam & Wong (2000). *Chlorella vulgaris,* a unicellular green microalga, was entrapped in calcium alginate beads. bead concentration (12 beads ml^{-1}, equivalent to 1:3 algal beads: wastewater, v/v).

Absolutely higher nutrient reduction was spotted in bioreactors containing algal beads than the blank alginate beads. By treatment in bioreactor having optimal during 24 h complete removal of NH_4^+-N and around 95% $PO_4^{3—}$P was succeeded. Low (around 4 beads ml^{-1}) and high (>15 beads ml^{-1}) algal bead concentrations had significantly lower $NH4^+$-N removal. During the study, the (NO_2^-+NO_3^-)-N concentrations in all reactors were not more than 0.25 mg/lt. However, the effect of bead concentration on phosphate removal, was not so apparent. Moreover, according to algal cells the immobilization matrix, the alkaline pH was established and aeration of wastewater also supported the removal of nutrients from wastewater.

De-Bashan, Moreno, Hernandez & Bashan (2002) provided research on nutrient removal from synthetic wastewater by *Chlorella vulgaris* coimmobilized in alginate beads with the microalgae growth-promoting bacterium *Azospirillum brasilense*. The experiments was conducted under semi-continuous, continuous and batch conditions. Removal of ammonium (100%, 2 days incubation) and soluble phosphorus ions (83%, 2 days incubation) was significantly increased by coimmobilized culture under semi-continuous conditions compared to immobilization of the microalgae alone (NH_4^+-N removal=85%, PO_4^{3-}-P removal=33%). Nutrient removal was more efficient in semi-continuous culture than continuous (NH_4^+-N removal=91%) or batch (NH_4^+-N removal=93%) cultures. In current study it is recommended that coimmobilization of a microalgae with microalgae growth-promoting bacteria is able to maintain as a tool in planning novel wastewater treatments.

J. K. Kim, Lee, Kim & Moon (1999) made a characterıstics of denitrifying photosynthetic bacteria, *Rhodopseudomonas palustris*, isolated from photosynthetic sludge. Growth parameters were featured in anaerobic flask culture. Optimum pH, temperature, and illumination intensity were 5.5, 31°C and 5000 lux in range. The highest particular growth rate and gas production rate by denitrification were 0,095 h^{-1} and 0.2 ml N_2 h^{-1}, respectively.

As the late-log phase and a small amount of nitrite was aggregated at the end of the culture finished then dissimilatory nitrate reduction to nitrogen gas by the isolate started. Highest number assigned to viable cells was 14×10^{8} cells ml^{-1} with 1.07 gl^{-1} of dry-cell weight and the maximum concentration of bacteriochlorophyll *a* was 0.17 OD_{775} g^{-1} of dry-cell weight.

K. Lee & Lee (2001) used *Chlorella kessleri* on batch culture cases to research effect of light/dark cycles on wastewater treatment. Research reveals that nitrate removal efficiency under the continuous light is slightly higher than that under L/D cycles while illumination of L/D (12h/12h) cycles offers better efficiency in the removal of organic carbon and phosphorus per increased biomass. By the end three days, nitrate was eliminated to 136.5 and 154.1 mg NO_3N/lt from 168.1 mg NO_3^- N/lt under continuous illumination and under diurnal cycles, respectively. Results that was obtained from experiment proved that if density algal cultures use optimized photobioreactors with diurnal cycles, energy will be saved and organic carbon sources removal will be improved.

Gitelson, Qiuang & Richmond (1996) in their research studied the features of the photic zone and light penetration depth in cultures with ultrahigh cell densities of the *Spirulina platensis.* Scientists implementing high-spectral-resolution radiometric measurements determined the effect of underwater optical properties on the penetration depth of photosyntheticaly active radiation, the inherent optical properties of algal suspensions (i.e. absorption and scattering coefficients), and also apparent optical properties (i.e. the reflectance and the vertical attenuation coefficient of downwelling irradiance). Vertical attenuation coefficient was used to predict the depth of the light penetration into a reactor having an ultrahigh cell density (chlorophyll concentration, up to 300.000 mg/m^3). Being just a small fraction of the reactor volume in ultrahigh-cell-density cultures, the photic volume has a great difference for the blue and red light compared with the green light. Under these unique circumstances a photosynthetic role for green light is recommended.

An, Sim, Lee & Kim (2003) tested a laboratory-scale bubble column for hydrocarbon production from secondarily treated piggery wastewater. Experiments were carried out on the removal of nitrogen and phosphorus from piggery wastewater during growth of *Botryococcus braun,* including measurements of hydrocarbon formation by the alga. During experiment the test was conducted on initial nitrate (10-1020 mg/lt) and phosphorus (14 mg/lt) concentration on the optimum concentration range for a culture in wastewater.It is achieved to get high cell density with 510 mg NO_3-N/lt. The growth rate was not so dependent from initial phosphate concentration, but under conditions of phosphate deficiency. By the end, dry cell weights of 8.5 mg/lt and hydrocarbon level of 0.95 g/lt were obtained. Indication stated that nitrate (788 mg N/lt) removal was 80%. after 12 days.

Quantitative analysis of the daily cyclic variation of culture parameters was carried out by Fuentes et al (1999). They examined the outdoor continuous culture of *Porpyridium cruentum* in a tubular photobioreactor. Study reports covered the daily cyclic variation of oxygen generation rates, carbon consumption rates, photosynthetic activities, growth rates and biochemical composition of the biomass in the system. The test declared a linear relationship between the external irradiance and the verage irradiance inside the culture. Respectively, reduction in photosynthetic activity of the cells at noon and recovery in the afternoon was investigated. Therefore, as cells demonstrated short-term response of characteristics such as oxygen generation rate as well as carbon consumption rate with external and average irradiance it was proposed to use a model of photosynthesis rate considering photoinhibition. Mentioned model was applied for designating CO_2 requirements of the system and for the operation and scale up of tubular photobioreactor. As photosynthesis rates get higher, carbon losses get lower, ranging from 25% at noon to 100% during the night. Growth rate demonstrated parallel relationship with the daily mean average irradiance inside the culture. Also, linear relationship presented between the oxygen generation rate and the growth rate.

The cells demonstrated a long-term response of metabolic routes to mean daily culture conditions, according to biochemical composition of the biomass. Energy was stored and carbohydrates were consumed as illumination continued. While the fatty acid profile, as total fatty acid, was a function of growth of growth rate, fatty acid dry weight content reduced during the daylight period. There was also inspected a short-term variation of exopolysaccharides synthesis with solar irradiance, i.e. excretion of exopolysaccharides as a protection against adverse culture conditions was increased as the external irradiance got higher.

Quantitative assessment of factors affecting nutrient removal from ground water by Cyanobacteria was studied by Hu, Westerhoff & Vermaas (2000). Under labaratory conditions 3 cyanobacteria strains were tested in batch mode glass columns. The maximum nitrate uptake rate was shown by *Synechococcus sp*. Strain PCC 7942. However, all other species demonstrated fast removal of nitrate. Enhance of light intensity was proportional to the increase of the nitrate uptake rate. The cause of the highest growth and nitrate uptake rate was as an irradiance of 160 μmol of photons $m^{-2}s^{-1}$. Inoculums size (i.e., cell density), fixed nitrogen level in the cells and aeration rate, with vigorously aerated, nitrate sufficient cells in mid-logarithmic phase having the highest long-term nitrate uptake rate influenced the nitrate uptake rate. It was possible to obtain average nitrate uptake rates up to 0.05 mM NO_3-h^{-1} at a culture optical density at 730 nm of 0.5 to 1.0 over a 2 day-culture period. Current study obviously shows that one or more trace elements became limiting for long-term growth of *Synechococcus sp* after nitrate and phosphate.

As it is intended to achieve nutrient uptake and growth of the microalgae *Nannochloropsis Sp.* by Cattaneo et al (n.d) a tubular photobioreactor was used for them. Tubular photobioreactor was a place to research the ammonium nitrogen and phosphorus removal from sewage waters by phytoplankton microalgae. The system demonstrated that an ammonium daily removal output of 100%, while phosphorus uptake was about 86 % after launching period of about 10 days. 6 hours was enough for the complete nutrients consumption to be supplied with mean rates of 1.5 mg/h and 1 mg/h for N-NH_4 and P-PO_4. By the end of experiment decreasing of the algal

cells number, and, therefore, of the chlorophyll a concentration, served to decrease in the nutrients uptake velocity.

Laliberte, Lessard, Noue & Sylvestre (1997) studied the effect of phosphorus addition on nutrient removal from wastewater with the cyanobacterium *Phormidium bohneri* in outdoor 24-l triangular bioreactors. During experimentation various N/P ratios was used and for each of them higher outputs and productivities were obtained at the lowest N/P ratios after 90h. The biomass production of *P.bohneri* in water was enhanced as phosphates was added with an exception of enormously affecting removal of the inorganic ions ammonium, nitrate and phosphate.

Martinez Sancho, Jimnez Castillo & El Yousfi (1999) investigated removal of phosphorus by *Scenedesmus obliguus* in a continuous photobioreactor. The effect of light intensity was researched at different rates of dilution, under limited including saturating intensities of illumination. It is recommended to work at a high light intensity with the working dilution rate near the critical highest dilution rate for obtaining efficient removal of phosphorus from the liquid medium, in respect to experimental outcomes. Removal was a different from those of maximum biomass productivity at all light intensities, the former being displaced at higher dilution rates. Surface adsorption to the cells and the culture vessel assisted the removal of phosphate from the medium.

Dumas, Laliberte, Lessard & de la Noue (1998) studied Biotreatment of fish farm effluents the cyanobacterium *Phormidium bohneri* in photobioreactors. In experiment, according to retention time of 8.12 and 24 h, respectively, the completely mixed 70 lt photobioreactors were used. During one month period average ammonia nitrogen removal efficiency from rainbow trout (*Oncorhynchus mykiss*) effluent effluent was 82% and 85% for soluble orthophosphate.

As Rates of ammonia nitrogen removal were free from hydraulic loading rate the time of retention was not setting a limit to the uptake of ammonia by *P. bohneri*.

De-Bashan, L.E., Moreno, M., Hernandez, J.,& Bashan, Y. (2002) carried out laboratory-scale experiments for biological treatment of recalcitrant anaerobic industrial effluent (from ethanol and citric acid production). Chlorella vulgaris after its use as microalgae turned to macrophyte *Lemna minuscule.* Wash water make the recalcitrant wastewater be diluted to to 10% of the original concentration. At the beginning the ammonium ion concentration in the wastewater was 3-8 mg NH_4-N/lt. As five days passed, the level of ammonium ion removal from the wastewater by *C. vulgaris* ranged from 60% to 78%, with an average of 72%. The level of phosphorus ion removal from the treated wastewater ranged from 0% to 51%, with an average of 28%.The first concentration of phosphorus was 1.5-3.5 mg PO_4^{-3}/lt. after five days of incubation by 61% (from 3100 to 1200 mg/lt) vulgaris treatment decreased COD. A large population of *C Vulgaris* resulted in the dramatic elimination and sedimentation of microalga to the bottom of the reactors, after addition of macrophyte *L. minuscula* to treated wastewater to it.

By using olive mill wastewater Villasclaras, S.S., Sancho, M.E.M., Caballero, M.T.E, & Perez, A.D. (1996) investigated production of microalgae using olive mill wastewater. The effect of the aeration level and the composition of the culture medium were researched referring to the concentration of alpechin and KNO_3 added in a batch culture of the microalgae *Chlorella pyrenoidosa* and *Scenedesmus obliguus*. The highest specific growth rate and the biomass were stated as kinetic parameters. Achieved biomass composition was evaluated for chlorophyll and crude protein content as well as fatty acid composition corresponding to the lipid fraction. In order to obtain a balanced biomass composition in terms of proteins and lipids, the most available conditions covered an alphechin concentration of 10%, without the addition of nitrates and with an aeration level of 1 v/v min. The μ_m values reached with both microalgae were around 0.03 h-1, according to mentioned conditions.

Martinez Sancho, M.E., Jimenez Castillo, J.M., & El Yousfi, F. (1999) performed experimental researches on removal of phosphorus and nitrogen by freshwater alga *Scenedesmus obliguus* under various conditions of stirring and temperature. Chosen operating temperatures were 20°C, 25°C, 30°C, and 35°C respectively.Maximum and minimum levels chosen were allowed by the installation used.The maximum level which assured complete mixing. The minimum level was the lack of magnetic stirring. Submitted cultures to continual illumination for the duration of each experiment (8 days) were at mean intensity of 11 334 lux in the interior of the empty reactors.In the stirred cultures with the highest μ value being 0.0438 h^{-1} at 30°C , it was achieved to get the highest specific growth rate. Stirring enhanced biomass productivity in the linear growth phase with the optimum appearing at 25°C which was followed after exponential growth. It was not necessary to rich the maximum percentage of P elimination ($\%P_{max}$) for the evaluation of the influence of temperature. However, in the case of reduction the time required to obtain highest P removal percentage (t_{max}). The highest $\%P_{max}$ value as 98% for the shortest time period, t_{max}=94.33 h, was spotted in the culture with stirring at 25°C. The maximum highest percentage of ammonium removal ($\%N_{max}$), 100%, finished at the final culture time (t_f) of 188.33 h, in the stirred culture at 25°C. The culture obtained N/P ratio at 12.9.

1.2 Objective and Scope

The aforementioned studies could be clearly defined that algal cultivation is eligible for nutrient removal from wastewaters . Using of algae has been extensively studied for nutrient and COD removal from wastewaters under different operating conditions and reactor configurations. Recently, the large-scale cultivation of microalgae and the practical use of its biomass as a source of certain constituents have a great potential. By considering this fact, the main objective of this thesis is to investigate the nutrient removal performance of microalgae *Chlorella vulgaris* from synthetic wastewater in a fed-batch operated flasks and continuously operated immobilized photobioreactor. In the content of that objective, the scope of the study can be summarized as follows;

1. to investigate the effect of different initial NH_4-N concentration on nutrient removal performance of *Chlorella vulgaris* in fed-batch experiments,
2. to determine optimum nitrogen/phosphorus ratio on nutrient removal in fed-batch experiments,
3. to investigate the maxsimum ChA concentrations for effective effluent nutrient concentrations in fed-batch experiments,
4. to determine optimum ChA concentration on optimum biomass concentration in fed-batch experiments,
5. to evaluate the effect of initial nitrogen concentration on nutrient removal in continuously operated immobilized-cell photobioreactor,
6. determine optimum N/P ratio on nutrient removal in continuously operated immobilized-cell photobioreactor,

CHAPTER TWO
MATERIALS AND METHODS

2.1 Microbial Culture

The green alga *Chlorella vulgaris* was supplied by the Algae Collection of the Bioengineering Department of Ege University. The culture was cultivated in the laboratory of Dokuz Eylül University.

2.2 Wastewater Composition

Synthetic wastewater was used throughout the experiments add it was composed of; $MgSO_4.7H_2O$, 1000 mg/lt; $CaCl_2$, 84 mg/lt and 0.5 ml of trace elements. The trace elements solution was made up of H_3BO_3, 57 mg/lt; $FeSO_4.7H_2O$, 25 mg/lt; $ZnSO_4.7H_2O$, 44 mg/lt; $MnCl_2.4H_2O$, 7 mg/lt; MoO_3, 35 mg/lt; $CuSO_4.5H_2O$, 8 mg/lt; $Cu(NO_3)_2.6H_2O$, 2.5 mg/lt; Na_2EDTA, 250 mg/lt; and $NaHCO_3$, 2500 mg/lt. NH_4Cl and KH_2PO_4, were used as nitrogen and phosphorus sources, respectively. The concentrations of these nutrients were varied between 10-65 mg/lt for NH_4-N and 1.2-9.1 mg/lt for PO_4-P depending on the experimental conditions.

2.3 Experimental Set-up

2.3.1 Fed-Batch Experiments

The Fed-Batch treatment of synthetic wastewater was carried by using 5 lt reactors.. At the beginning of each series of experiments, 500 ml of culture medium was inoculated to flasks with a suspension of pre-cultured cells. The flasks were aerated to provide CO_2 and for mixing. The flasks were continuously illuminated from one side by using fluorescent lamps.

The pH was maintained at 6-7 value by adding of %5 potassium hydroxide (KOH) or %10 acetic acid (CH_3COOH) solutions when necessary. The experiments were conducted at room temperature.

2.3.1.1 Experimental Studies

Effect of operating parameters on *C. vulgaris* were investigated. System was loaded with fresh media at the beginning of each experimental condition and it was operated at 3 days. Temperature, pH, initial and effluent NH_4-N , PO_4-P, Cha , AKM concentrations of daily samples were monitored.

5000 ml flasks were operated fed-batch with synthetic wastewater at different initial NH_4-N, PO_4-P concentrations, N/P ratio in order to investigate the effect of those operating parameters on nutrient removal efficiency. The system was kept constant 20/4 light/dark cycle and 2780 lux light intensity at the outside and 1100 lux light intensity inside of the reactor.

2.3.2 Photobioreactor

The continuous treatment of synthetic wastewater was carried out in an immobilized photobioreactor system. The photobioreactor was made up of glass with dimensions of 30 cm height, 80 cm length and 15 cm width. The liquid volume in the reactor was 30 lt. Plastic non-commercial support materials were used for the immobilization of the organisms. The surface area of support material in reactor was around 2.8 m^2.Temperature was maintained at 26^oC with a heater. Schematic diagram of reactor is given in Figure 2.1.

Photobioreactor was inoculated with dens culture of *Chlorella vulgaris* at the beginning of the study. The system was operated at 3800 and 8400 lux light

intensity, but only 1650 and 3727 lux light intensity achieved in photobioreactor. The operation conditions were adjusted to 20/4 light/dark cycle, N/P ratio of 15/1. The initial NH_4-N concentration was varied between 20 and 60 mg/lt.

2.3.2.1 Experimental Studies

System was loaded with fresh media at the beginning of each experimental condition and it was operated at least 10 days until the system reached to almost steady state conditions. Temperature, pH, initial and effluent NH_4-N and PO_4-P concentrations of daily samples were monitored.

The system was operated at different N/P ratios and light intensities. In the first part of the continuous experiments, N/P ratio was changed between 4/1 and 15/1 by keeping NH_4-N concentration constant at $[NH_4\text{-}N]_o$ =20±3 mg/lt. The other parameters were adjusted as light intensity=2700 lux, L/D=20/4 and hydraulic retention time= 5 days. In the second part, Light intensity was 1650 lux at constant NH_4-N concentration of $[NH_4\text{-}N]_o$ =20±2 mg/lt. Finally, light intensity was increased to 3750 lux and ammonia nitrogen concentration was varied between from 20±2 mg/lt to 60±2 mg/lt. The other parameters were adjusted as N/P= 15/1, L/D= 20/4 and hydraulic retention time= 15 days.

2.4. Analytical Methods

2.4.1 Sampling

Daily samples withdrawn from system were centrifuged at 5000-6000 rpm until clear supernatant was obtained. Ammonia nitrogen, phosphorus, biomass and chlorophyll A concentrations were monitored on the samples throughout the experiments.

2.4.2 Chlorophyll A Analysis

To determine chlorophyll a content, 10 ml of algal suspension centrifuged at 5000-6000 rpm for 30 minute and supernatant was discarded. The cells were suspended in 3 ml of methanol and boiled for about 5 min in a water bath. The samples were cooled to room temperature and then the volume was made up to 5 ml by adding distilled water. The chlorophyll A concentration in the extract was calculated by reading the absorption (A) of the pigment extract in a spectrophotometer at the given wavelength against a solvent blank by using the following equation (Becker, E. W., 1994): Chlorophyll A = $(16.5 \times A_{665}) - (8.3 \times A_{650})$

2.4.3 Nitrogen and Phosphorus analysis

The concentration of ammonia nitrogen and phosphorus were measured in clear supernatant by colorimetric method by using APHA-AWWA-WPCF 1995. The number of Merck kits using for ammonium and phosphorus analysis were 1.14752 and 1.14842, respectively.

2.4.4 Biomass measurement

Initial and final algae biomass concentration for fed-batch experiments were determined by filtration of aliquots on pre-weighted filter (Whatman GF/C) which was dried at 105°C for 24 h and then weighed after cooled to room temperature in a desicator. ChA content in biomass varied between 1%-3%.

2.4.5 pH and light intensity measurement

pH values were measured by using WTW series 720 pH meter. Calibration of pH meter was carried out with standard solutions. . Light intensity was measured by a digital light meter, Luxeron LX-1108.

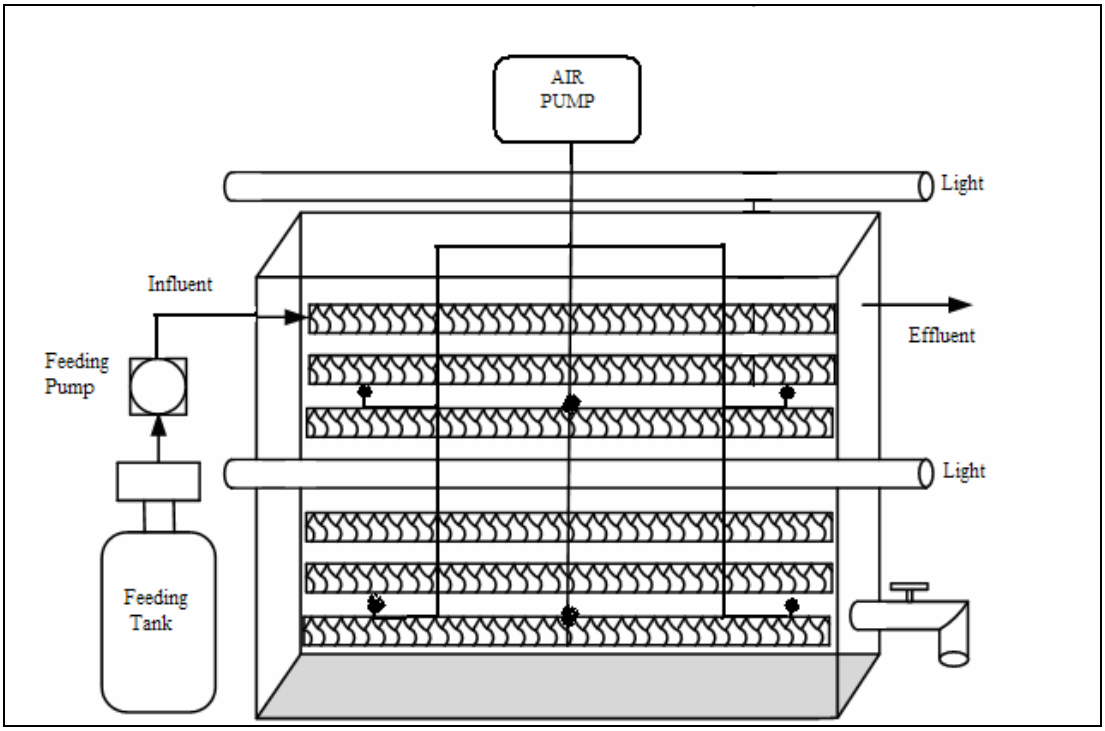

Figure 2.1 Schematic diagram of immobilized photobioreactor

CHAPTER THREE
RESULTS AND DISCUSSIONS

The study contains fed-batch and continuous experiments. In the fed-batch experiment, the effect of media composition was examined. In continuous experiments, the effects of two operation parameters, such as N/P ratio, light intensity on nutrient removal performance of *C. vulgaris* in an immobilized photobioreactor system were investigated.

3.1 Continuous Experiments in Immobilized Photobioreactor System

In the first part of the study, the nutrient removing performance of continuously operated immobilized photobioreactor system with the same organism was investigated. The system was operated at different N/P ratios (4/1-15/1) and light intensities (1650-3750).

3.1.1 Effect of N/P ratio on NH4-N Removal Performance of Continuous Immobilized Photobioreactor System

In order to examine the effect of N/P ratio on the nutrient removal performance of immobilized photobioreactor, six different N/P ratios between 4/1-15/1 were tested. A microalgae *Chlorella vulgaris* was used as dominant microalgal culture. Light intensity, L/D cycle and hydraulic retention time was kept constant at about 2700 lux, 20h/4h, 5.5 day, respectively. NH_4-N analyses were carried out daily samples taken from influent and effluent wastewater of the system.

Variation of effluent NH_4-N concentration and removal efficiency with time at 4/1 of the N/P ratio are given in Figure 3.1. As seen in the Figure, NH_4-N decreased gradually from 20 mg/L to 5.1 mg/L with 75% removal efficiency. In the last 3 days of operation, effluent NH_4-N concentration was almost stable at about 5 mg/L.

N/P ratio was adjusted to 6.7/1. Figure 3.2 indicates the variation of effluent NH_4-N concentration and NH_4-N removal efficiency with time. The effluent NH_4-N concentration decreased from 20 mg/L to 7.6 mg/L within 6 days of continuous operation. However, then a substantial decrease to around 3.8 mg/L in the effluent concentration was observed. As a result, the efficiency increased from 64% to 80%.

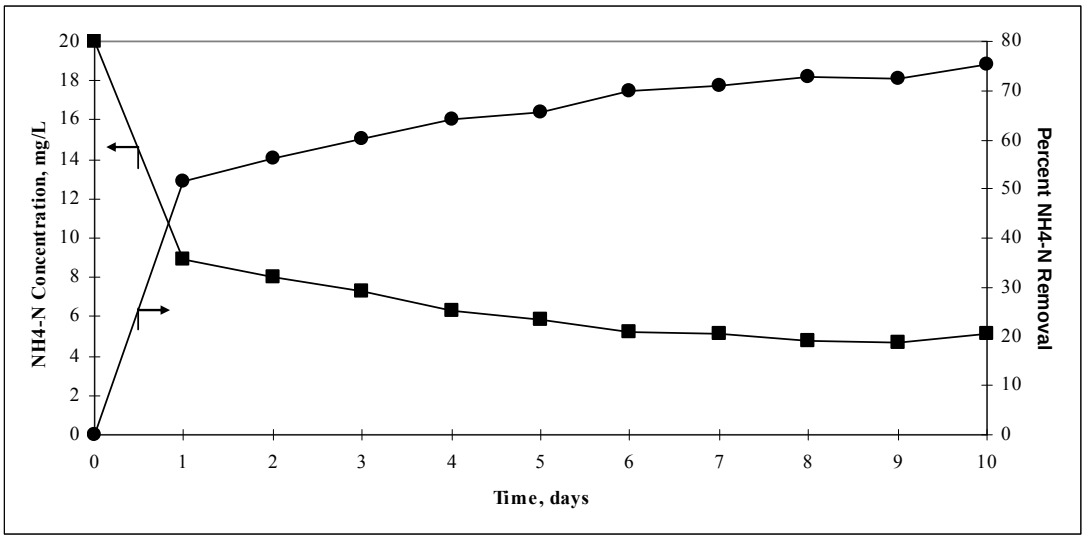

Figure 3.1 Variation of effluent NH_4-N concentration and removal efficiency with time at N/P=4/1 , $\boldsymbol{\theta}_H$ =5.5 days (—■—NH_4-N removal efficiency, —●— effluent NH_4-N concentration).

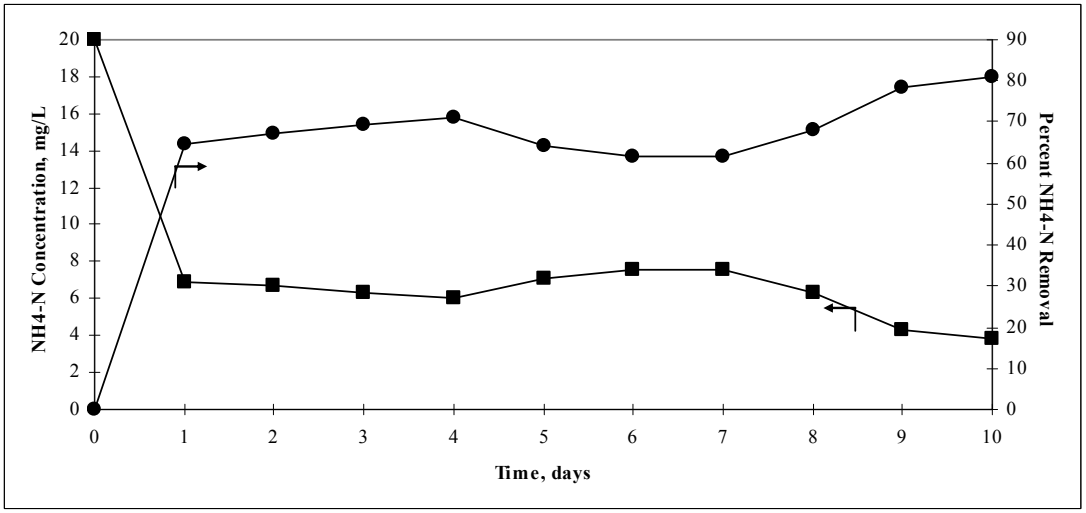

Figure 3.2 Variation of effluent NH_4-N concentration and removal efficiency with time at N/P=6.7/1 , $\boldsymbol{\theta}_H$ =5.5 days (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

N/P ratio was further increased to around 10/1. Figure 3.3 depicts variation of effluent NH_4-N concentration and NH_4-N removal efficiency with time at this initial N/P ratio. The removal efficiency was almost stable at 30% for the first 3 days of experiment. After that,NH_4-N decreased to 5.5 mg/L from 14.2 mg/L with 72.0 % NH_4-N removal efficiency in this operation period. These results indicate that, increasing the N/P ratio 10/1 provided a substantial improvement in NH_4-N removal performance of the system.

In order to investigate the effect of highest ratios, N/P ratio increased to 13/1 and the results are given in Figure 3.4. The concentration of ammonia nitrogen reduced from 20.4 mg/L to 4.4 mg/L in the first 6 days of continuous operation. Then no significant variation in effluent nitrogen concentration was observed. Under the steady state condition, the removal efficiency was about 91 %.

Finally, N/P was increased to 15/1. The performance of the system significantly increased. Effluent NH_4-N concentration varied around 3 mg/L for the first 6 days of continuous operation. For the last days of the operation, a further decrease in the effluent concentration was observed and it decreased to 0.9 mg/L. As a result 96% removal efficiency was obtained (Figure 3.5).

Figure 3.6 summarizes the effect of initial NH_4-N concentration on effluent NH_4-N concentration and NH_4-N removal efficiency. Effluent NH_4-N concentrations decreased from 5.1 mg/L to 0.9 mg/L with respect to increase in N/P ratio from 4/1 to 15/1 as expected. Depending on that variation in effluent NH_4-N, removal efficiency increased from 75% to 96%, respectively. These results indicate that, *Chlorella vulgaris* microalgal culture can efficiently remove nitrogen at high initial N/P ratio ([N/P]|> 12/1). Lower N/P ratios ([N/P]=4/1) significantly affect NH_4-N removal performance of the system resulting in decreasing in removal efficiency around 75% with effluent NH_4-N concentration of 6.8 mg/L. 96% removal efficiency around 15/1 ratio can be evaluated as high NH_4-N removal performance for the *C.vulgaris* immobilized photobioreactor.

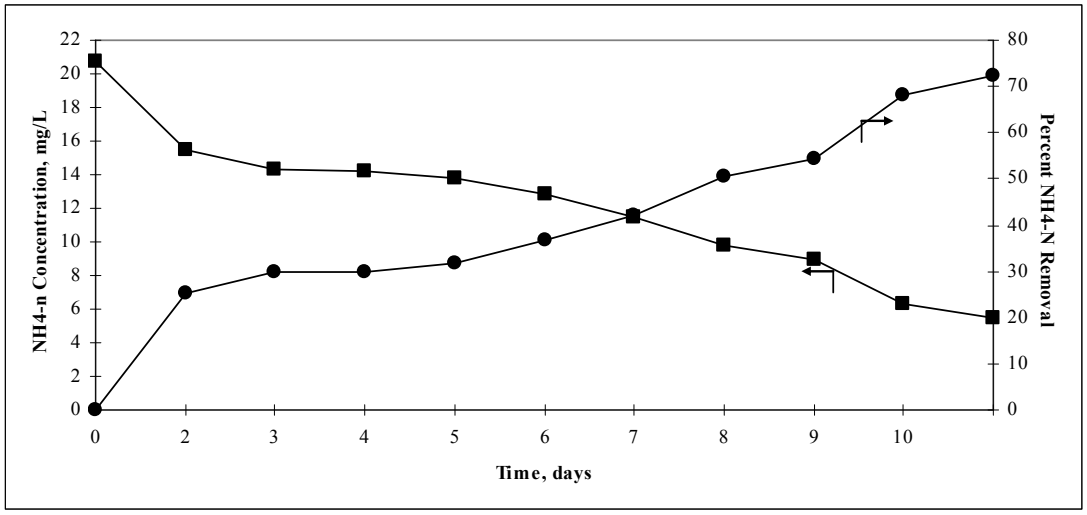

Figure 3.3 Variation of effluent NH_4-N concentration and removal efficiency with time at N/P=10/1 , θ_H =5.5 days (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

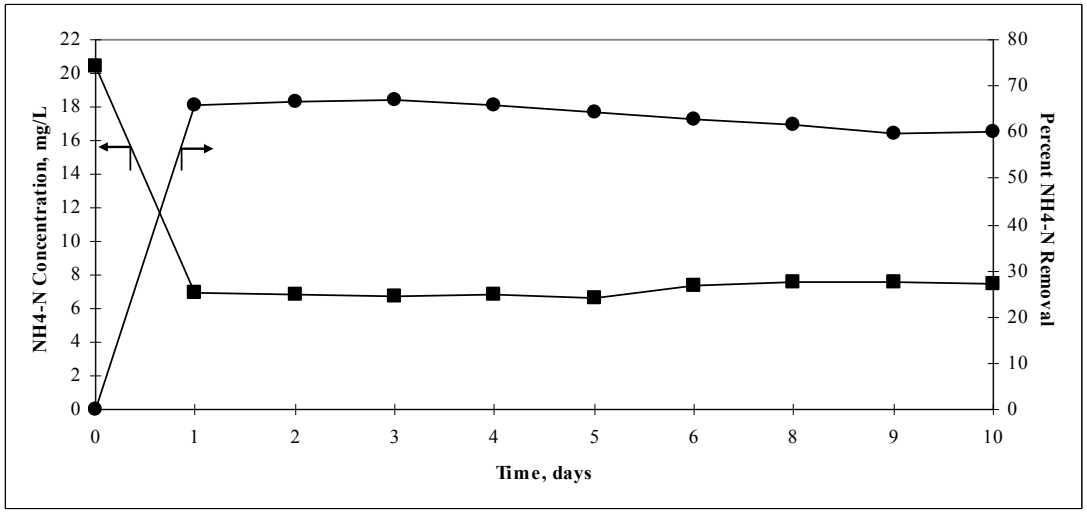

Figure 3.4 Variation of effluent NH_4-N concentration and removal efficiency with time at N/P=13/1 , θ_H =5.5 days (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

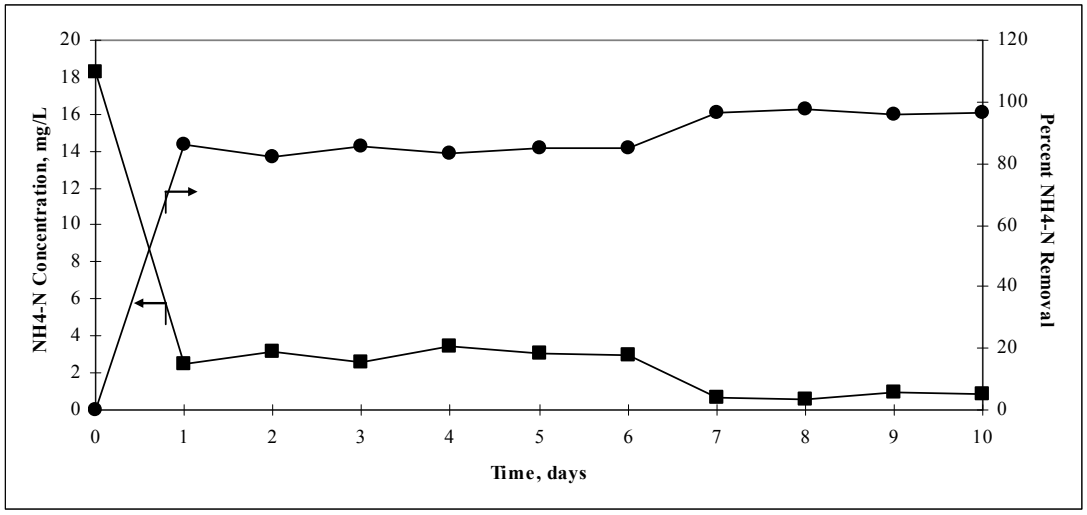

Figure 3.5 Variation of effluent NH_4-N concentration and removal efficiency with time at N/P=15/1 , θ_H =5.5 days (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

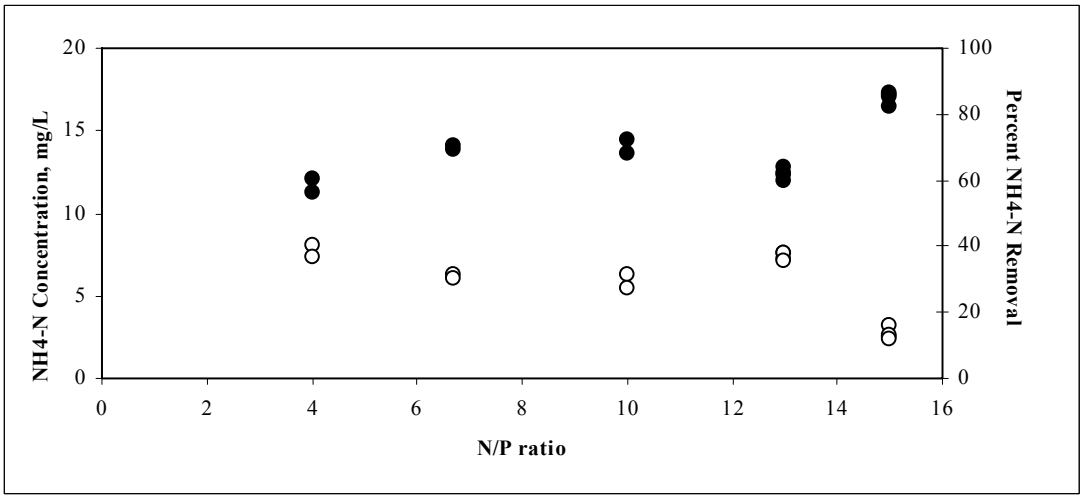

Figure 3.6 Variation of effluent NH_4-N concentration and removal efficiency with time between N/P=4 and N/P=15 , θ_H =5.5 days (●NH_4-N removal efficiency, ○ effluent NH_4-N concentration).

3.1.2 Effect of Light Intensity on NH4-N Removal Performance of Continuously Operated Immobilized Photobioreactor System

In order to examine the effect of light intensity on nutrient removal efficiency in continuous experiments with microalgae *Chlorella vulgaris*, light intensity was gradually increased from 1650 lux to 3727 lux. Although maximum NH_4-N removal was obtained at around $[NH_4\text{-}N]_o$=20 mg/L, the initial NH_4-N concentration was adjusted to between 20mg/L and 60 mg/L NH_4-N concentrations in order to evaluate the system performance at different nitrogen concenteration. The L/D cycle, HRT and N/P ratio were kept constant at 20/4, 5.7 days and 15, respectively. The variations of nutrient removal with time at different light intensities were given in Figures 3.7 to 3.14.

At the beginning of this part of experiment, the performance of *Chlorella vulgaris* at 1650 lux initial light intensity was examined. Variation of effluent NH_4-N and PO_4-P concentrations at 1650 lux light intensity are shown in Figure 3.7 and 3.8. Initial nutrient concentrations were $[NH_4\text{-}N]_o$=19.2 mg/L and $[PO_4\text{-}P]_o$=1.4 mg/L. PO_4-P decreased gradually from 1.4 mg/L to 0.1 mg/L with 93 % removal efficiency during the continuous operation. *Chlorella vulgaris* significantly removed NH_4-N from 19.2 mg/L to 2.1 mg/L with 89 % removal efficiency

Light intensity was increased to 3727 lux and the results are given in Figure 3.9 and 3.10. NH_4-N concentration decreased to 2.3 mg/L within the first 7 days of operation resulting in 89 % removal efficiency. However a slight increase to 3.1 mg/L in the effluent NH_4-N concentration was observed. Depending on this increase in concentration, NH_4-N removal efficiency decreased from 89 % to 85 %. PO_4-P removal was slower compared to NH_4-N removal. PO_4-P concentration decreased slowly from 1.5 mg/L to 0.3 mg/L with 80 % removal efficiency at the end of 10 days period.

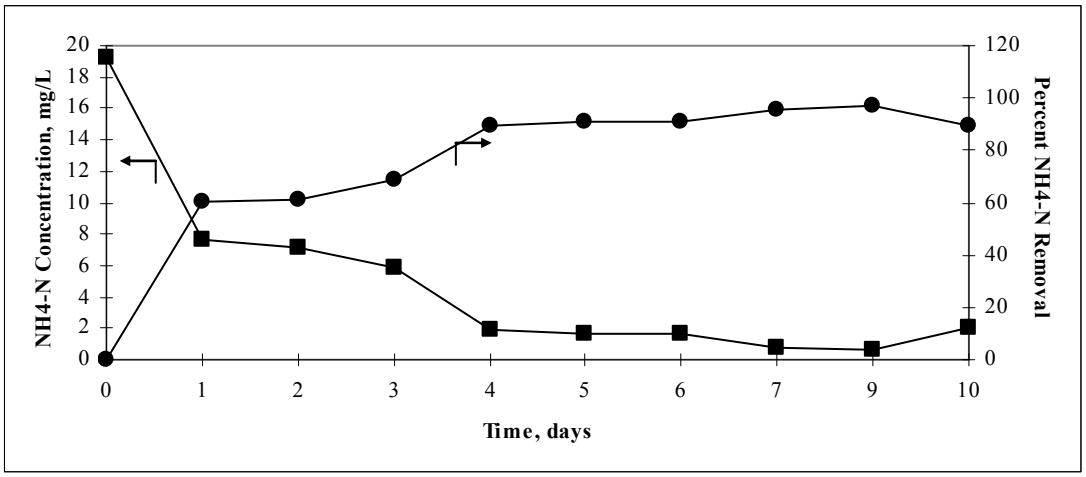

Figure 3.7 Variation of effluent NH_4-N concentration and removal efficiency with time at light intensity=1650 lux and NH_4-N=19.2 mg/L (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

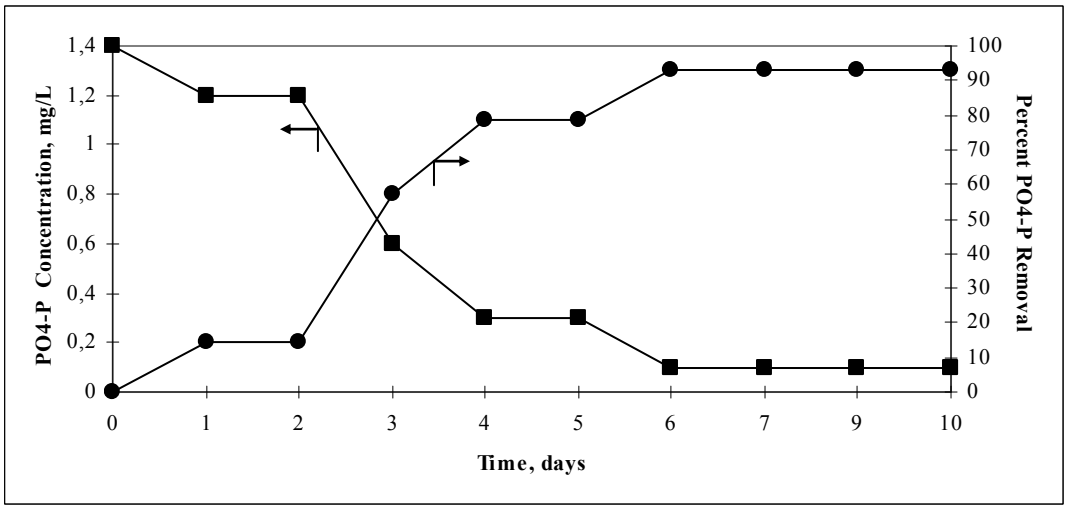

Figure 3.8 Variation of effluent PO_4-P concentration and removal efficiency with time at light intensity=1650 lux and NH_4-N=19.2 mg/L (—■— effluent PO_4-P concentration, —●— PO_4-P removal efficiency).

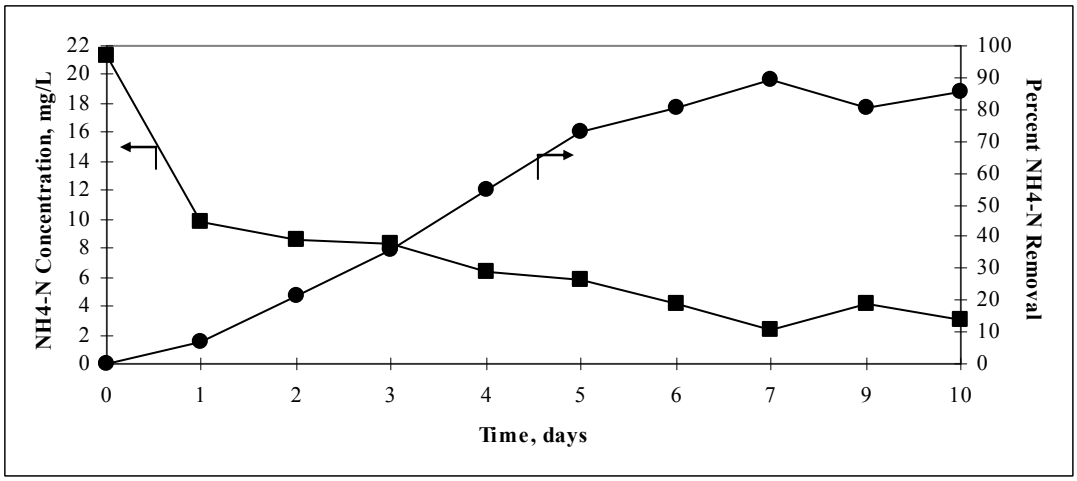

Figure 3.9 Variation of effluent NH_4-N concentration and removal efficiency with time at light intensity=3727 lux and NH_4-N=21.3 mg/L (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

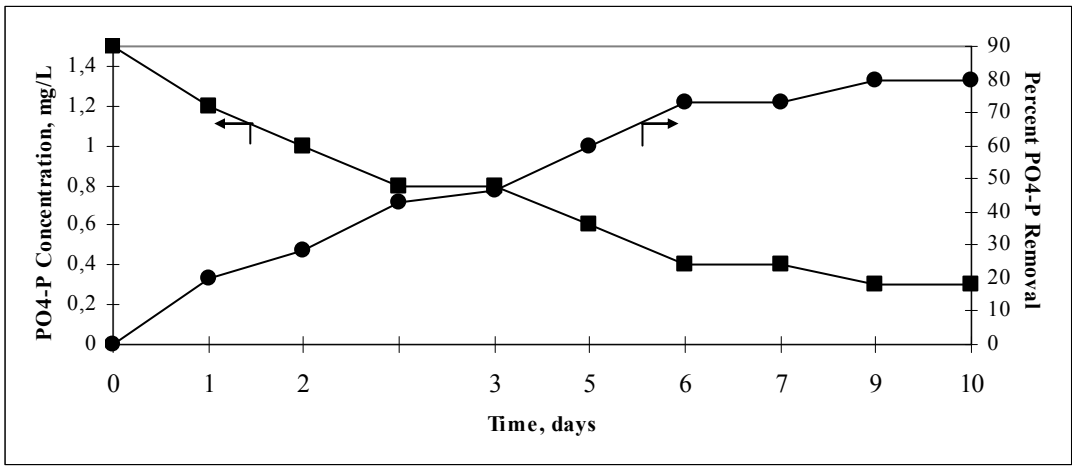

Figure 3.10 Variation of effluent PO_4-P concentration and removal efficiency with time at light intensity=3727 lux and NH_4-N=21.3 mg/L (—■— effluent PO_4-P concentration, —●— PO_4-P removal efficiency).

Influent NH_4-N concentration was further increased to 40 mg/L. Variation of effluent NH_4-N, PO_4-P concentration and NH_4-N, PO_4-P removal efficiencies at 3727 lux light intensity are shown in Figure 3.11 and 3.12, respectively. No significant NH_4-N removal, E=51 %, with effluent concentration of 19.2 mg/L was observed at 10^{th} days. PO_4-P decreased gradually from $[PO_4\text{-}P]_o$= 2.9 mg/L to $[PO_4\text{-}P]_e$=1.2 mg/L with 59 % removal efficiency.

The highest initial NH_4-N examined in this set of experiment was 60.8 mg/L. Results of this experiment are indicated in Figure 3.14 and 3.15. Initial NH_4-N, PO_4-P were $[NH_4\text{-}N]_o$=60.8 mg/L, $[PO_4\text{-}P]_o$=4.1 mg/L, respectively. NH_4-N and PO_4-P decreased gradually to $[NH_4\text{-}N]_e$=31.2 mg/L and $[PO_4\text{-}P]_e$=2.4 mg/L within 10 days. As a result final removal efficiencies were obtained as 49 % for NH_4-N and 44 % for PO_4-P.

Lower light intensities significantly affected continuous operation performance. Efficiencies were 89 %, 85 % at 1650 lux and 3727 lux light intensities, respectively for the initial concentration of 20 mg/L. However, NH_4-N removal efficiency decreased to 51 % with 3727 lux light intensity and 40 mg/L NH_4-N initial concentration. These results indicate that, for the maximum NH_4-N removal efficiency at 20±1 mg/L NH_4-N concentration with *Chlorella vulgaris*, no need to expose the culture to high light intensities. 1650 lux light intensity is enough to provide at least 85% removal f nitrogen. In conclusion, lower light intensity and initial NH_4-N concentration is the limiting factor for the nutrient removal by microalgae *C.vulgaris*.

Similar results were observed for PO_4-P removal. Decreasing light intensity enhanced the phosphorus removal by *C. vulgaris*. However, the effect of light intensity on phosphorus uptake was not as efficient in ammonia nitrogen. Significant phosphorus uptake was observed up to 1650 lux and the removal efficiency was around 93 %. Percent removal decreased from 80 % to 59 % with the increased in influent concentrations of 20 mg /L and 40 mg/L when light intensity 3727 lux, respectively.

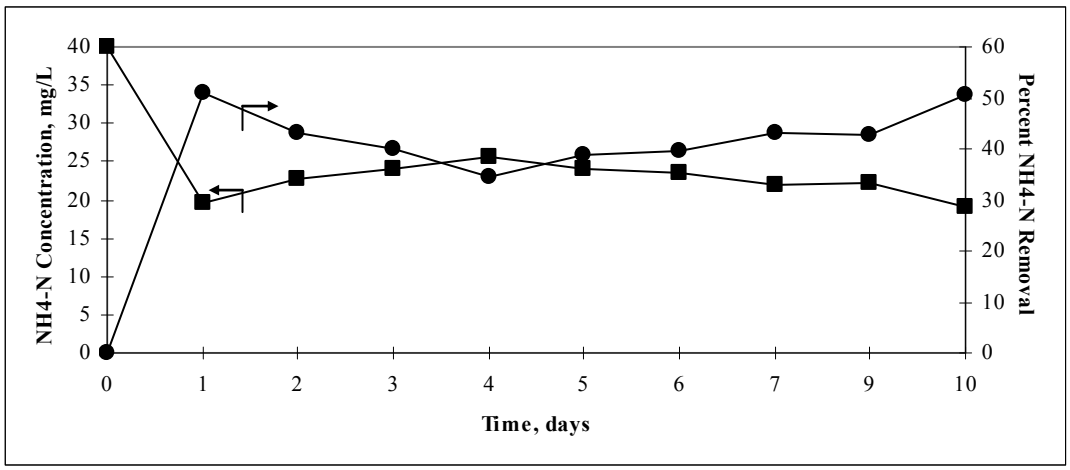

Figure 3.11 Variation of effluent NH_4-N concentration and removal efficiency with time at light intensity=3727 lux and NH_4-N=40 mg/L (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

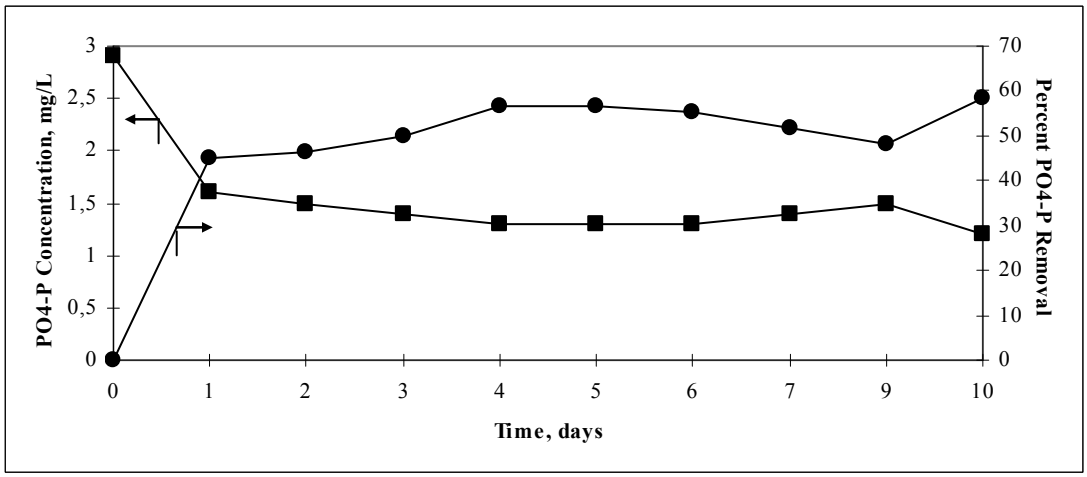

Figure 3.12 Variation of effluent PO_4-P concentration and removal efficiency with time at light intensity=3727 lux and NH_4-N=40 mg/L (—■— effluent PO_4-P concentration, —●— PO_4-P removal efficiency).

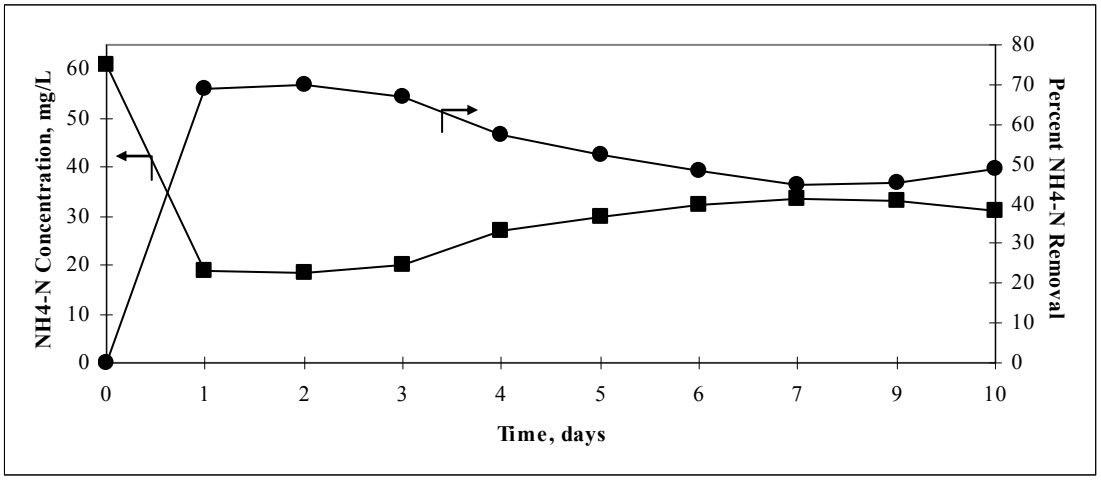

Figure 3.13 Variation of effluent NH_4-N concentration and removal efficiency with time at light intensity=3727 lux and NH_4-N=60.8 mg/L (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

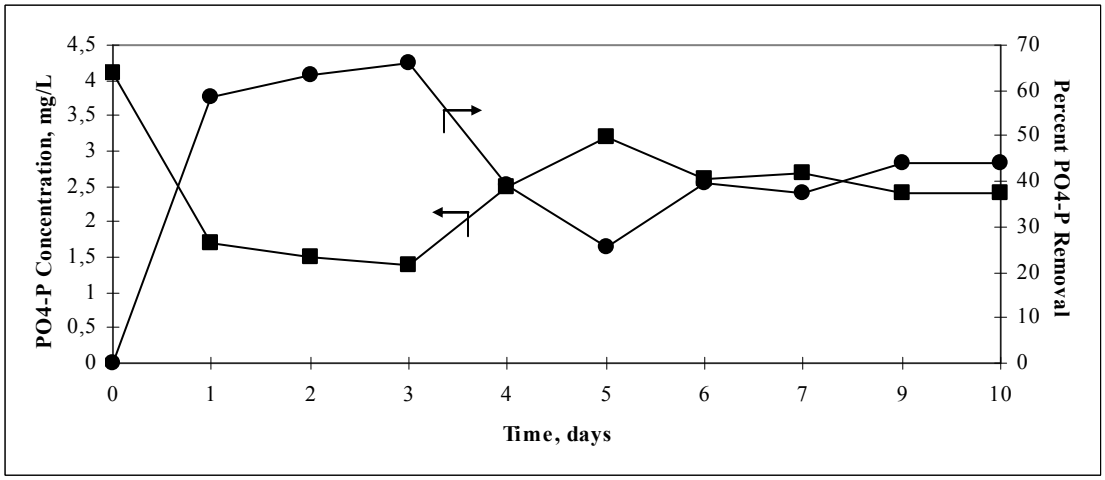

Figure 3.14 Variation of effluent PO_4-P concentration and removal efficiency with time at light intensity=3727 lux and NH_4-N=60.8 mg/L (—■— effluent PO_4-P concentration, —●— PO_4-P removal efficiency).

3.2 Fed-Batch Experiments

In the second part of study, the nutrient removing performance of fed-batch system with the same organism was investigated. The system was operated at different initial NH_4-N and N/P ratio. Initial NH_4-N concentration was varied between 12.6 mg/L and 32 mg/L while light intensity, L/D cycle and N/P ratio was 1100 lux, 20h/4h and 4/1 respectively, in the first part of the fed-batch experiments. In the second part, N/P ratio was changed between from 4.6/1 to 7.3/1 at constant initial NH_4-N concentration of $[NH_4\text{-}N]_o$ =42±2 mg/L. The other parameters were adjusted as light intensity=1100 lux, L/D=20/4 and sludge age= 3 days.

3.2.1 Effect of Initial NH_4-N Concentration on Nutrient Removal Performance and Chla, Biomass Concentration of the Fed-Batch System

Figure 3.15.a indicates the variation of effluent NH_4-N concentration and NH_4-N removal efficiency with time at initial NH_4-N concentration of $(NH_4\text{-}N)_o$=12.6 mg/L in the feeding tank. However, there was dilution in the system as it was feed. The theoretical concentrations of the nutrients in the system were calculated by considering this dilution with the operation. Percent removal and system performance was evaluated by taking the theoretical concentrations into consideration. So, the final theoretical concentration of ammonium nitrogen in the system was $(NH_4\text{-}N)_{teo}$= 11 mg/L. However, the final concentration in the system was obtained as 1.8 mg/L NH_4-N at the end of 3 days. As a result, 84 % removal efficiency for NH_4-N was obtained.

Variation of effluent PO_4-P concentration with time at $(PO_4\text{-}P)_o$= 3.2 mg/L initial nutrient concentration is depicted in Figure 3.15.b. At the end of the 3 days of fed-batch operation, the observed effluent concentration was $[PO_4\text{-}P]_e$=0.4 mg/L. If there were no algae in the system , the final concentration would be $(PO_4\text{-}P)_{teo}$= 2.3 mg/L. In the light of this result, it could be concluded that about 83% of the phosphate was removed by the algae.

Figure 3.15.c indicates the variation of theoretical Chla concentration and effluent Chla concentration with time. 11.2 mg/L Chla was the Chla concentration in the beginning of the operation. The final Chla concentration reached to $(Chla)_e$=10.3 mg/L at the end of 3 days. Although it seems that there was a decrease in Chla concentration, there is no reduction, instead production of Chla with feeding. Because, if there were no Chla production, the final concentration would be 1.4 mg/L with the effect of dilution at the end of operation with fed-batch feeding.

Figure 3.15.d indicates the variation of theoretical biomass concentration and effluent biomass concentration with time. The initial biomass concentration during start up was 770 mg/L. The calculated biomass concentration was 94 mg/L, however, the ob served one was 459 mg/L. So there was substantial growth of the algae in the system.

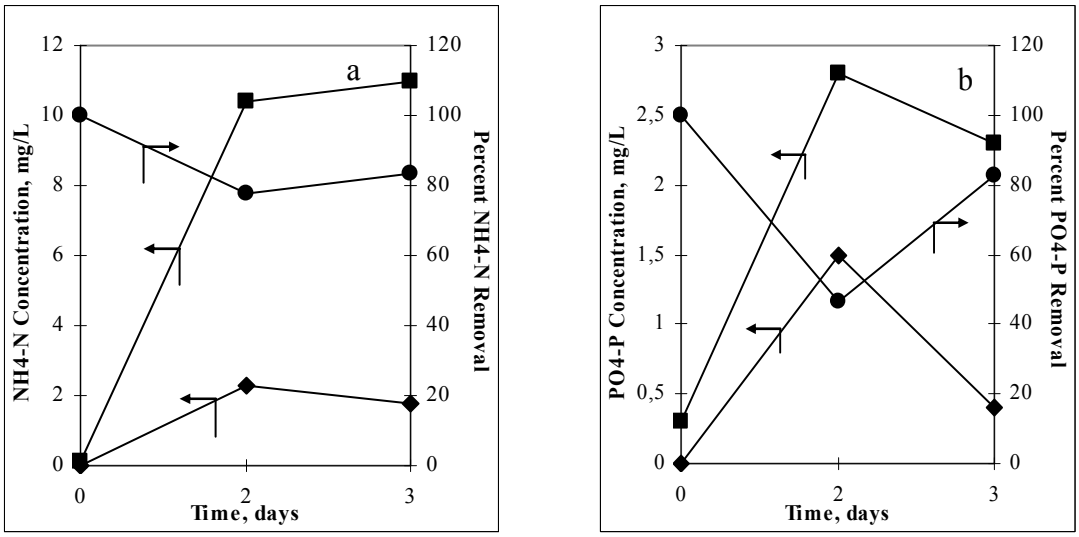

Figure 3.15 (a) Variation of effluent NH_4-N concentration, theoretical NH_4-N concentration and NH_4-N removal efficiency with time at $(NH_4\text{-}N)_o$=12.6 mg/L (—■— theoretical NH_4-N concentration, —♦— effluent NH_4-N concentration,—●— NH_4-N removal efficiency). (b) Variation of effluent PO_4-P concentration, theoretical PO_4-P concentration and PO_4-P removal efficiency with time at PO_4-P_o=3.2 mg/L (—■—theoretical PO_4-P concentration, —♦— effluent PO_4-P concentration, —●— PO_4-P removal efficiency)

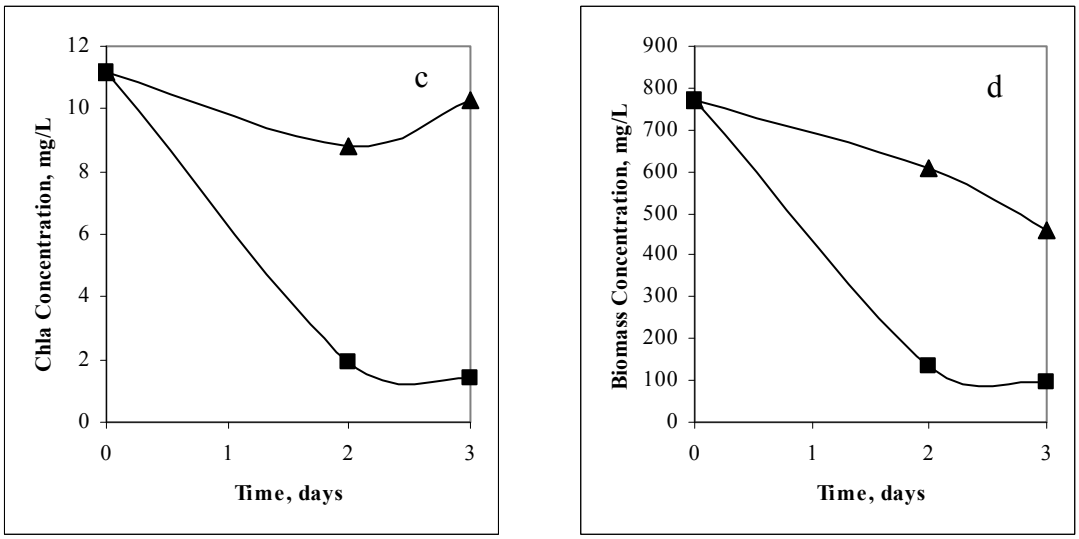

Figure 3.15 (c) Variation of theoretical Chla concentration and effluent Chla concentration with time at $(NH_4\text{-}N)_o$=12.6 mg/L (—■— theoretical Chla concentration, —▲— effluent Chla concentration) (d) Variation of biomass concentration and effluent biomass concentration with time at $(NH_4$-$N)_o$=12.6 mg/L (—■—theoretical biomass concentration, —▲— effluent biomass concentration)

Figure 3.16.a indicates the variation of effluent and theoretical NH_4-N and NH_4-N removal efficiency with time. Initial NH_4-N was adjusted to 22.4 mg/L. No significant removal in NH_4-N concentration was observed until 1st day of operation. NH_4-N decreased from 19.7 mg/L to 8.8 mg/L with 55 % NH_4-N removal efficiency between the 1st and 3rd days of operation period. Similarly, Figure 3.16.b shows the variation of removal efficiency for PO_4-P and theoretical and effluent PO_4-P with time for PO_4-P= 5.6 mg/L. There was no significant removal for PO_4-P until 1st day. After that, PO_4-P concentration decreased from 4.8 mg/L to 2.1 mg/L with 56 % removal efficiency at 3rd day of operation

Variation of theoretical and effluent Chla concentration with time at $(NH_4\text{-}N)_o$=22.4 mg/L is depicted in Figure 3.16.c. 15.5 mg/L Chla was the Chla concentration at the beginning of the operation. The final Chla concentration reached to $(Chla)_e$=8.5 mg/L at the end of 3 days. Although it seems that there was a decrease in Chla concentration, there is no reduction, ,instead production of Chla with feeding. Because, if there were no Chla production, the final concentration would be 1.9 mg/L with the effect of dilution at the end of operation with fed-batch feeding.

Figure 3.16.d indicates the variation of theoretical biomass concentration and effluent biomass concentration with time. The initial biomass concentration during start up was 671 mg/L mg/L. The calculated biomass concentration was 82 mg/L mg/L, however, the ob served one was 433 mg/L. So these results indicates that there was substantial growth of the algae in the system and the nutrients were up-taken by the newly growing algae cultures.

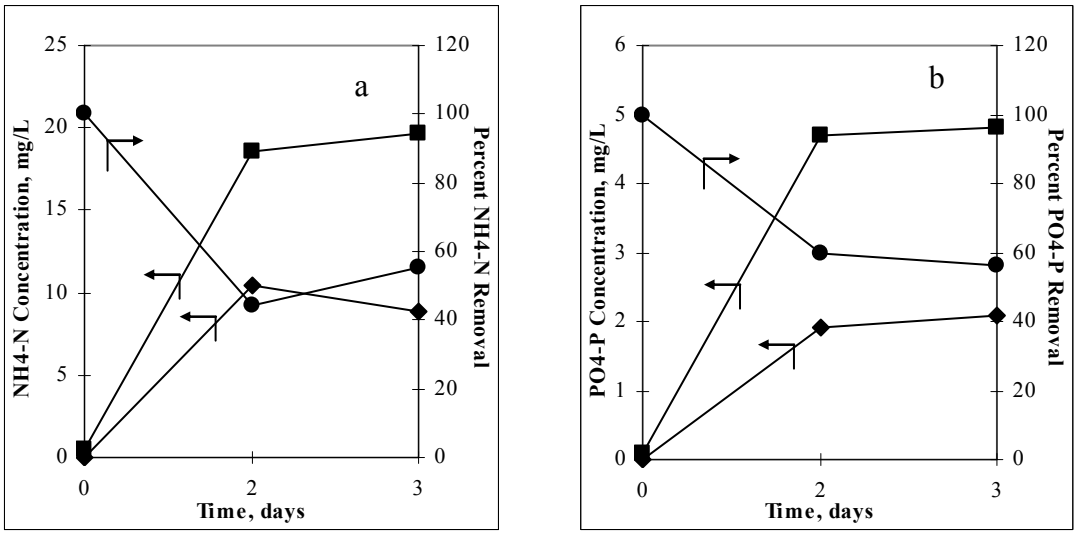

Figure 3.16 (a) Variation of effluent NH_4-N concentration, theoretical NH_4-N concentration and NH_4-N removal efficiency with time at $(NH_4\text{-}N)_o$=22.4 mg/L (—■— theoretical NH_4-N concentration, —♦— effluent NH_4-N concentration,—●— NH_4-N removal efficiency). (b) Variation of effluent PO_4-P concentration, theoretical PO_4-P concentration and PO_4-P removal efficiency with time at $PO_4\text{-}P_o$=5.6 mg/L (—■—theoretical PO_4-P concentration, —♦— effluent PO_4-P concentration, —●— PO_4-P removal efficiency)

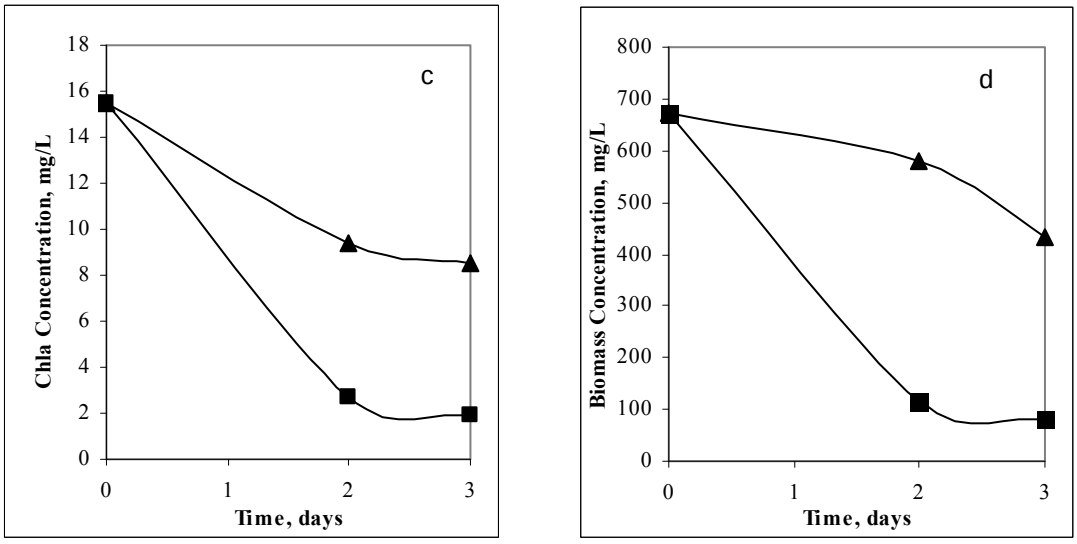

Figure 3.16 (c) Variation of theoretical Chla concentration and effluent Chla concentration with time at $(NH_4\text{-}N)_o$=22.4 mg/L (—■— theoretical Chla concentration, —▲— effluent Chla concentration,). (d) Variation of biomass concentration and effluent biomass concentration with time at $(NH_4\text{-}N)_o$=22.4 mg/L (—■—theoretical biomass concentration, —▲— effluent biomass concentration)

Figure 3.17.a indicates the variation of effluent and theoretical NH_4-N concentration and removal NH_4-N efficiency with time at $(NH_4\text{-}N)_o$= 32 mg/L. Figure 3.17.b shows the removal efficiency and effluent and theoretical PO_4-P concentration with time for $(PO_4\text{-}P)_o$= 8 mg/L. The final NH_4-N and PO_4-P concentrations were $(NH_4\text{-}N)_e$=10.6 mg/L $(PO_4\text{-}P)_e$=2.3 mg/L, respectively . No significant removal in NH_4-N concentration was observed until 2nd days of operation. NH_4-N dropped to gradually from 26.9 mg/L to 20.2 mg/L between the 0th and 2nd day of operation period. Similarly, there was no significant removal for PO_4-P until 2nd day. PO_4-P decreased gradually from 6.7 mg/L to 5.3 mg/L with 21 % removal efficiency between the 0th and 2nd day of operation period. After that, NH_4-N decreased slowly to 10.6 mg/L with 63 % removal efficiency and final PO_4-P removal efficiency was 68 % with $(PO_4\text{-}P)_e$= 2.3mg/L.

Figure 3.17.c depicts variation of theoretical and effluent Chla concentration with time at $(NH_4\text{-}N)_o$=32 mg/L. As seen from the figure, 13.7 mg/L Chla was beginning concentration at system. Although theoretical Chla concentration was calculated as 1.6 mg/L fed-batch feeding, the effluent Chla concentration was obtained as $(Chla)_e$= 4.9 mg/L at the end of 3 days. It means there was synthesis of the Chla in the algae cells.

Figure 3.17.d indicates the variation of theoretical Biomass concentration and effluent Biomass concentration with time. 740 mg/L biomass was beginning concentration at system .Although calculated biomass concentration was 90 mg/L at the end of fed-batch operation, the effluent biomass concentration was 397 mg/L at the end of 3 days. This result indicating that there was substantial growth of algae.

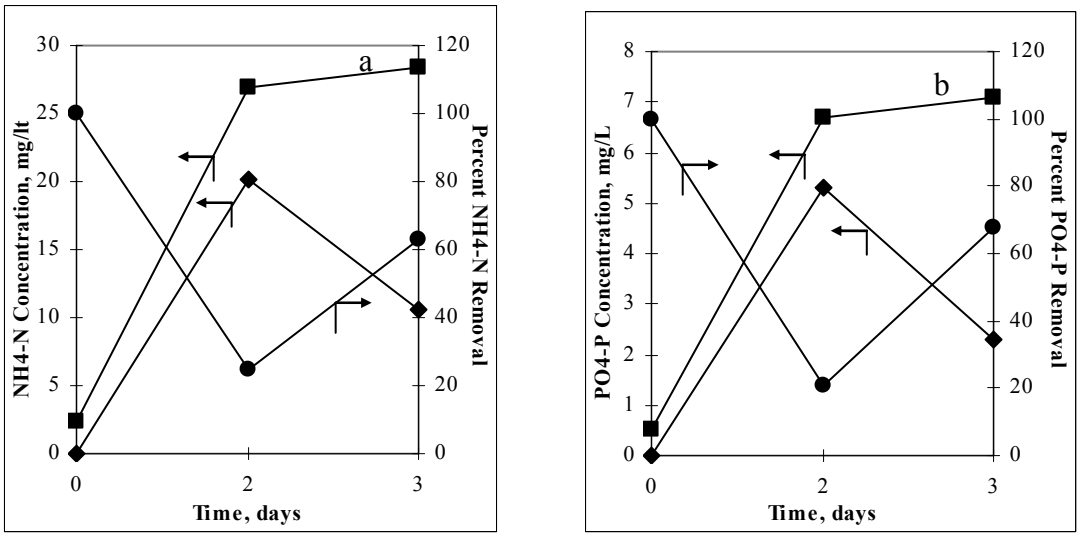

Figure 3.17 (a) Variation of effluent NH_4-N concentration, theoretical NH_4-N concentration and NH_4-N removal efficiency with time at $(NH_4\text{-}N)_o$=32 mg/L (—■— theoretical NH_4-N concentration, —♦— effluent NH_4-N concentration,—●— NH_4-N removal efficiency). (b) Variation of effluent PO_4-P concentration, theoretical PO_4-P concentration and PO_4-P removal efficiency with time at PO_4-P_o=8 mg/L (—■—theoretical PO_4-P concentration, —♦— effluent PO_4-P concentration, —●— PO_4-P removal efficiency)

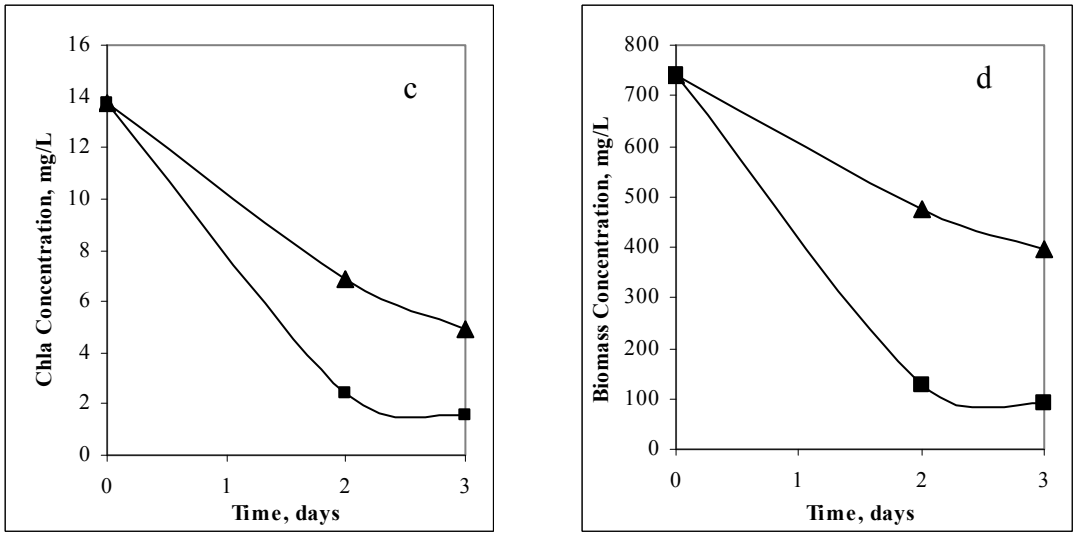

Figure 3.17 (c) Variation of theoretical Chla concentration and effluent Chla concentration with time at $(NH_4\text{-}N)_o$= 32 mg/L (—■— theoretical Chla concentration, —▲— effluent Chla concentration). (d) Variation of biomass concentration and effluent biomass concentration with time at $(NH_4\text{-}N)_o$= 32 mg/L (—■—theoretical biomass concentration, —▲— effluent biomass concentration)

3.2.2 Effect of N/P Ratio on Nutrient Removal Performance and Chla , Biomass Concentration of the Fed-Batch System

Another parameter that affects nutrient removal in any biological treatment system is N/P ratio. The limitation in one of the nutrient may affect the removal of the other nutrient. Therefore, fed-batch operation was conducted at different N/P ratios which was varied between 4.6/1- 7.3/1. NH_4-N concentration was kept constant around 42±3 mg/L while phosphate concentration was gradually decreased from 8.7 mg/L to 6.1 mg/L.

As the first part of this parameter, nutrient removal performance of *Chlorella vulgaris* at N/P=4.6 was examined. Variation of effluent and theoretical NH_4-N, PO_4-P concentrations with time at N/P=4.6 is shown in Figure 3.18.a and 3.18.b, respectively. Initial NH_4-N and PO_4-P were $(NH_4\text{-}N)_o$= 40 mg/L, $(PO_4\text{-}P)_o$= 8.7 mg/L, respectively. Although theoretical concentrations were 35.6 mg/L for ammonium nitrogen and 8.7 mg/L for PO_4-P, the observed concentrations in the effluent were 8.8 mg/L NH_4-N and 1.4 mg/L PO_4-P. As a results only 14 % of NH_4-N and 75 % of PO_4-P were removed at the end of operation. So the N/P=4.6 did not provide efficient nitrogen removal. Excess amount of phosphorus could have resulted in the limitation in the uptake of nitrogen. Therefore, low nitrogen removal was observed.

Figure 3.18.c depicts variation of theoretical and effluent Chla concentration with time at $(NH_4\text{-}N)_o$=40 mg/lt. The initial Chla concentration was 18.7 mg/L and it reduced to $(Chla)_e$= 8.7 mg/L at the end of 3 days. Although it seems there was no Chla synthesis in the algae, the substantial difference between observed and theoretical concentrations ($(Chla)_{teo}$= 2.3 mg/L) indicates that there was synthesis of Chla.

Figure 3.18.d indicates the variation of theoretical Biomass concentration and effluent Biomass concentration with time. The system was operated with 770 mg/L initial biomass concentration. The final biomass concentration was 484 mg/L at the end of 3 days of operation, although the theoretical one 94 mg/L.

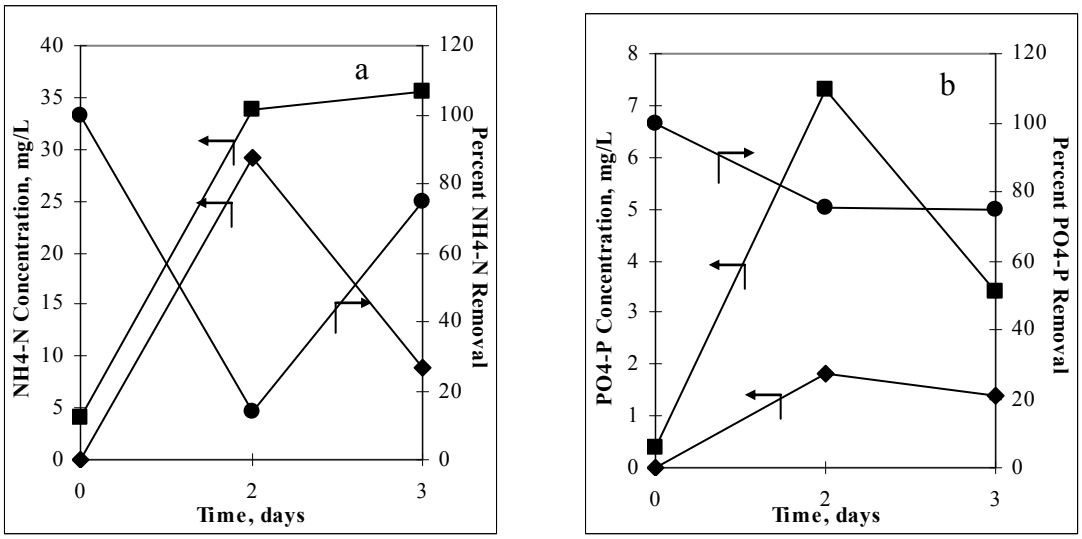

Figure 3.18 (a) Variation of effluent NH_4-N concentration, theoretical NH_4-N concentration and NH_4-N removal efficiency with time at N/P=4.6 (—■— theoretical NH_4-N concentration, —♦— effluent NH_4-N concentration,—●— NH_4-N removal efficiency). (b) Variation of effluent PO_4-P concentration, theoretical PO_4-P concentration and PO_4-P removal efficiency with time at N/P= 4.6 (—■—theoretical PO_4-P concentration, —♦— effluent PO_4-P concentration, —●— PO_4-P removal efficiency)

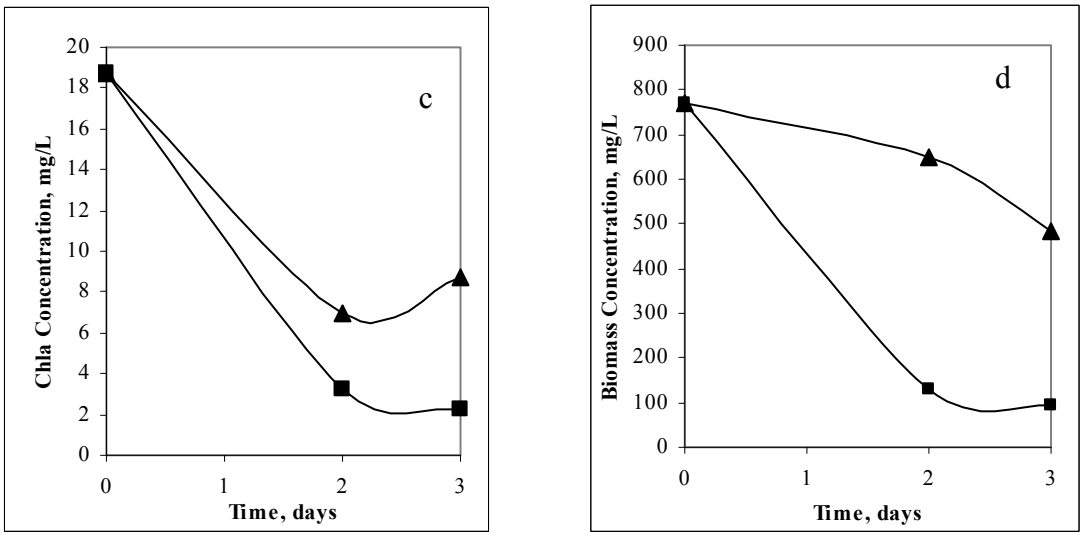

Figure 3.18 (c) Variation of theoretical Chla concentration and effluent Chla concentration with time at N/P= 4.6 (—■— theoretical Chla concentration, —▲— effluent Chla concentration). (d) Variation of biomass concentration and effluent biomass concentration with time at N/P= 4.6 (—■—theoretical biomass concentration, —▲— effluent biomass concentration).

Figure 3.19.a and Figure 3.19.b depict variation of effluent and theoretical NH_4-N, PO_4-P concentrations and nutrient removal efficiencies with time at N/P=6.6. Initial NH_4-N and PO_4-P concentrations were adjusted as $(NH_4\text{-}N)_o$=44 mg/L, $(PO_4\text{-}P)_o$= 6.7 mg/L, respectively. No significant removal of NH_4-N was observed until 2nd days of operation as seen in Figure 3.19.a. Effluent NH_4-N decreased to about 12.8 mg/L with 68 % NH_4-N removal efficiency. Similarly, s slow decrease in PO_4-P concentration was observed within 3 days of fed-batch operation. The effluent phosphorus concentration decreased to $(PO_4\text{-}P)_e$=2.8 mg/L and PO_4-P resulting in 65% removal efficiency.

Figure 3.19.c depicts variation of theoretical and effluent Chla concentration with time at $(NH_4\text{-}N)_o$=44 mg/Lt. As seen from the figure, 18.7 mg/L Chla was the initial concentration. Although calculated Chla concentration was around 2.3 mg/L at the end of operation, the observed concentration was $(Chla)_e$= 6.5 mg/L. This result indicates that, there was Chla synthesis.

Figure 3.19.d indicates the variation of theoretical biomass concentration and biomass concentration in the system with time. The initial concentration was 740 mg/L and the final concentration was observed as 442 mg/L at the end of 3 days. The calculated biomass concentration was 90 mg/L. Hence, although it seems that there was a decrease in the biomass concentration and there was increase, instead, when compared with the theoretical concentration.

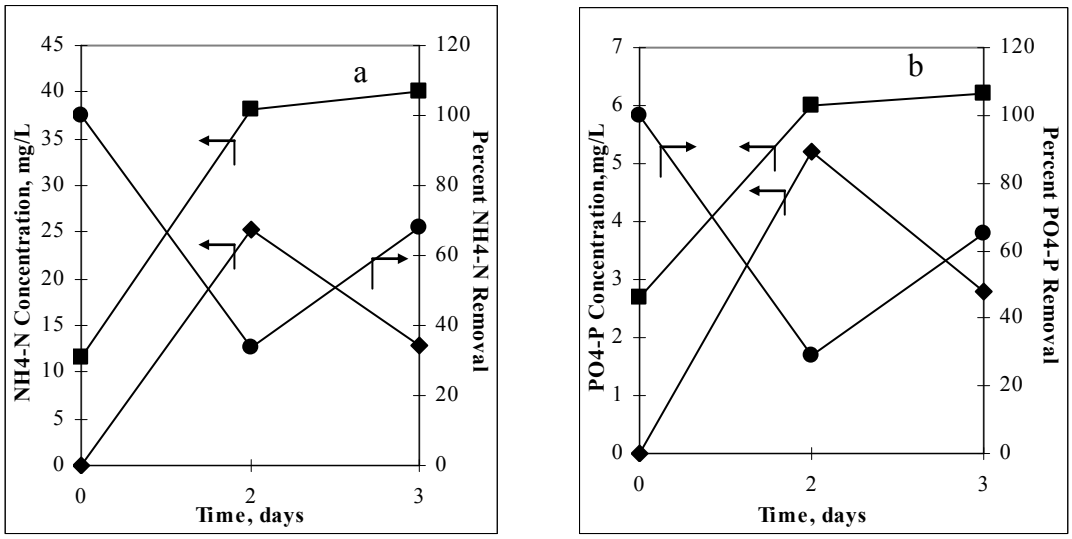

Figure 3.19 (a) Variation of effluent NH_4-N concentration, theoretical NH_4-N concentration and NH_4-N removal efficiency with time at N/P=6.6 (—■— theoretical NH_4-N concentration, —♦— effluent NH_4-N concentration,—●— NH_4-N removal efficiency). (b) Variation of effluent PO_4-P concentration, theoretical PO_4-P concentration and PO_4-P removal efficiency with time at N/P= 6.6 (—■—theoretical PO_4-P concentration, —♦— effluent PO_4-P concentration, —●— PO_4-P removal efficiency)

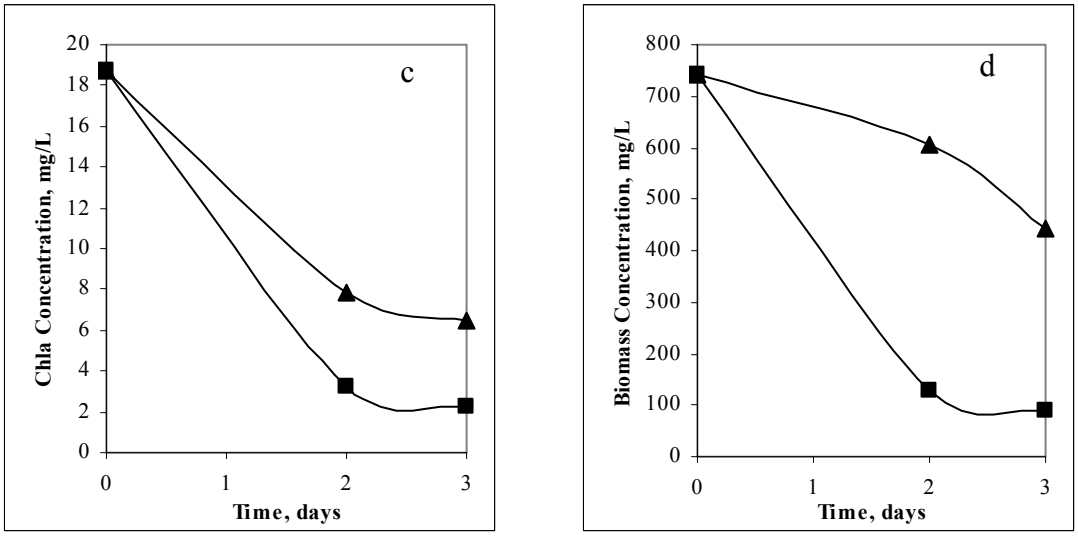

Figure 3.19 (c) Variation of theoretical Chla concentration and effluent Chla concentration with time at N/P= 6.6 (—■— theoretical Chla concentration, —▲ — effluent Chla concentration), (d) Variation of biomass concentration and effluent biomass concentration with time at N/P= 6.6 (—■— theoretical biomass concentration, —▲— effluent biomass concentration).

Figure 3.20.a indicates the variation of effluent NH_4-N concentration and NH_4-N removal efficiency with time at N/P= 7.3. NH_4-N concentration decreased form 40 mg/L to 17.8 mg/L at the end of 3 days of fed- batch operation. This decrease can be evaluated as substantial when compared with the theoretical concentration. As a result 55 % removal efficiency was observed.

Variation of effluent PO_4-P concentration with time at N/P= 7.3 is depicted in Figure 3.20.b. Theoretical PO_4-P concentration was 5.6 mg/L in the system at the end of 3 days. However, the effluent phosphate concentration decreased to $[PO_4\text{-}P]_e$=3.1 mg/L resulting in 60 % removal efficiency .

Figure 3.20.c depicts variation of theoretical and effluent Chla concentration with time at $(NH_4\text{-}N)_o$=40 mg/Lt. As seen from the figure, 11.2 mg/L Chla was the initial concentration. Although calculated Chla concentration was around 2.7 mg/L at the end of operation, the observed concentration was $(Chla)_e$= 5.6 mg/L. This result indicates that, there was Chla synthesis.

Figure 3.20.d indicates the variation of theoretical biomass concentration and biomass concentration in the system with time. The initial concentration was 670 mg/L and the final concentration was observed as 384 mg/L at the end of 3 days. The calculated biomass concentration was 82 mg/L. Hence, although it seems that there was a decrease in the biomass concentration and there was increase, instead, when compared with the theoretical concentration.

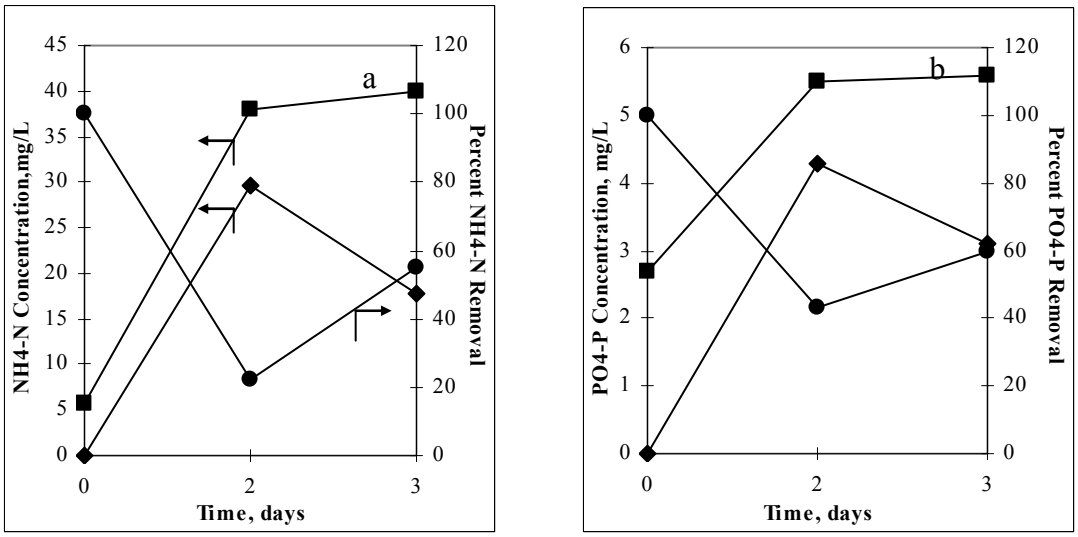

Figure 3.20 (a) Variation of effluent NH_4-N concentration, theoretical NH_4-N concentration and NH_4-N removal efficiency with time at N/P= 7.3 (—■— theoretical NH_4-N concentration, —♦— effluent NH_4-N concentration,—●— NH_4-N removal efficiency).(b) Variation of effluent PO_4-P concentration, theoretical PO_4-P concentration and PO_4-P removal efficiency with time at N/P= 7.3 (—■—theoretical PO_4-P concentration, —♦— effluent PO_4-P concentration, —●— PO_4-P removal efficiency)

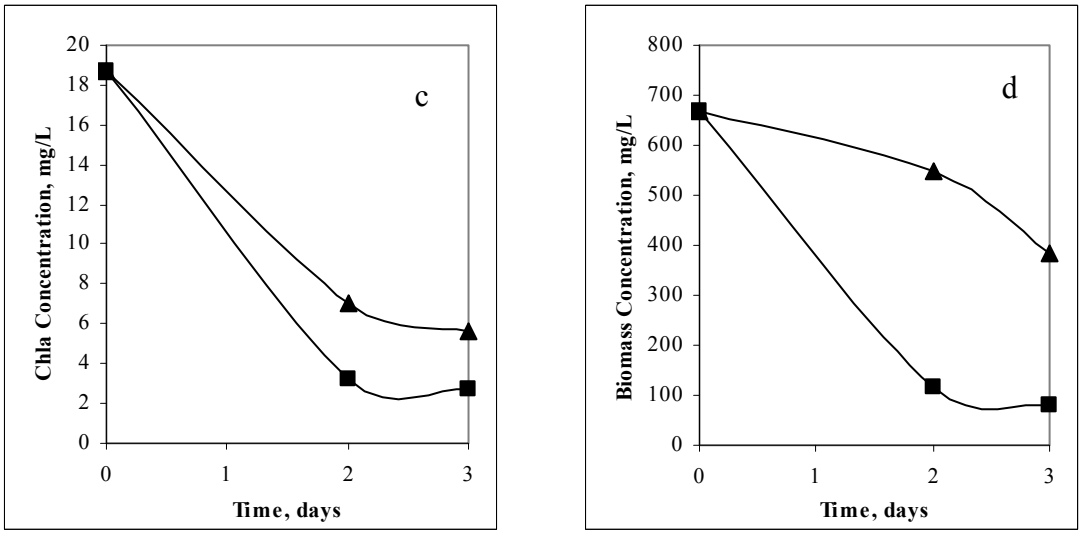

Figure 3.20 (c) Variation of theoretical Chla concentration and effluent Chla concentration with time at N/P= 7.3 (—■— theoretical Chla concentration, —▲— effluent Chla concentration), (d) Variation of biomass concentration and effluent biomass concentration with time at N/P= 7.3 (—■— theoretical biomass concentration, —▲— effluent biomass concentration)

CHAPTER FOUR
CONCLUSIONS

Treatment of synthetic wastewater in fed-batch flasks and continuous immobilized photobioreactor by algal culture *C.vulgaris* was investigated. Immobilized photobioreactor system was operated continuously under different nitrogen/phosphorus ratios and light intensities. Fed-Batch system was operated at different initial nutrient concentrations and nitrogen/phosphorus ratios.

Continuous operation of an immobilized photobioreactor system provided effective nutrient removal. Increasing nitrogen/phosphorus ratio provided better effluent quality in terms of ammonia nitrogen. Effluent NH_4-N concentration decreased from 6.8 mg/L to 0.9 mg/L when the N/P ratio increased from 4/1 to 15/1 for the initial NH4-N concentration of 20 mg/L. As a result over 95%, removal efficiency was obtained at N/P=15/1 ratio.

However, lower N/P ratio (N/P=4/1 days) significantly affect NH_4-N removal performance of the system resulting in decreasing in removal efficiency to around 75% with effluent NH_4-N concentration of 5.1 mg/L. It can be concluded that *Chlorella vulgaris* containing immobilized photobioreactor should be operated with N/P=12/1 and N/P=15/1 ratios in order to obtain higher that 90% removal efficiency.

Low light intensities,1650 lux, at low nitrogen concentrations, 20 mg/L, efficient ammonia nitrogen and phosphorus removal was observed. Increasing light intensity to 3727 lux did not provide significant improvement in removal efficiencies at the same initial nitrogen concentration. However, at high light intensities and higher initial concentrations no efficient removals of these nutrients were observed. The highest phosphorus and nitrogen removal was observed at 1650 lux and 20 mg/L initial NH_4-N with 93% and 89 % removal efficiency. Theses results indicated that, algae immobilized photobioreactor system can tolerate about 20 mg/L initial

ammonia nitrogen concentrations. Light intensity does not help to uptake more nutrients.

The separation of algae from effluent of suspended growth systems as ponds is known as an important problem. Fortunately, no significant algal biomass was observed in the effluent of immobilized growth system. However, the culture immobilized on the sides surfaces of photobioreactor. That caused a kind of self shading in the system. This result can be advantageous for the high natural light intensities during noon time in full scale application to overcome photoinhibition. Nevertheless, the self-shading caused limitation in light availability for the organisms on the surface of support material. As a result, the artificial light requirement increased.

C. vulgaris can remove 12.6 mg/L ammonia nitrogen completely from synthetic wastewater in fed-batch under the optimized experimental conditions. The minimum N/P ratio for almost complete ammonia nitrogen and phosphorus removal was found to be N/P=4.7. The most important factors affecting algal nutrient uptake were determined as initial nitrogen concentration. There was synthesis of Chla and biomass which indicate that, the culture in the system was active and growing by uptaking the nutrients.

When two photobioreactor systems were compared, fed batch operation could provide better nitrogen removing performance compared to immobilized system even at lower hydraulic retention times. The result can be explained as the presence of better homogenous environment in fed batch than that of immobilized system. Light penetration or availability, contact between algae and the substrate were significantly better in fed batch system. In addition, it was possible to keep the high biomass concentration in the system. As a conclusion, fed- batch operation could be a good approach to algal wastewater treatment systems.

The study was designed to evaluate nutrient removal by only algal uptake process. Therefore, the experiments were conducted at neutral pH. Better removal

performance could be observed if pH was not controlled, which will provide elevated pH resulting in stripping of ammonia.

Finally, the experimental results indicated that the algal nutrient removal system is a good approach as a tertiary treatment. So it could be concluded that the algal nutrient removal system can be used for secondary effluent treatment which has insufficient carbon but excess nitrogen and phosphorus content.

CHAPTER FIVE
RECOMMENDATIONS

Photobioreactor system needs further studies to understand the possible full scale application of algal nutrient removal systems. Therefore, the following studies are recommended as further studies;

1. The removal of other nitrogen forms such as NO_3 can be studied by fed-batch experiments,
2. The effect of presence of carbon on nutrient removal by algae can be studied by fed-batch experiments,
3. The effect of light intensity on nutrient removal by algae can be studied by fed-batch experiments,
4. The effect immobilization surface area to liquid volume ratio on nutrient removal in photobioreactor should be investigated,
5. Outdoor experiments at different seasons can be conducted to observe behavior of the system under natural conditions,
9. These systems ,fed-batch and immobilized photobioreactor, can be operated with real wastewater after the operation parameters are optimized.

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APPENDICES

RAW DATA OF EXPERIMENTAL STUDIES

TableA.1 Raw data of continuous experiment at N/P=4/1

Time (days)	Q (L/day)	θ (g/L)	$(NH_4\text{-}N)_o$ (mg/L)	$(NH_4\text{-}N)_e$ (mg/L)	E_N (%)
0	5.6	5.36	18.6	18.6	0
1	5.6	5.36	18.4	8.9	52
2	4.91	6.11	18.3	8	56
3	5.8	5.17	18.3	7.3	60
4	5.8	5.17	17.6	6.3	64
5	5.2	5.77	17.1	5.9	65
6	5.2	5.77	17.2	5.2	70
7	5.3	5.66	17.5	5.1	71
8	5.2	5.77	17.6	4.8	73
9	5.2	5.77	17	4.7	72
10	5.3	5.66	20.7	5.1	75

TableA.2 Raw data of continuous experiment at N/P=6/1

Time (days)	**Q (L/day)**	**θ (g/L)**	**$(NH_4\text{-}N)_o$ (mg/L)**	**$(NH_4\text{-}N)_e$ (mg/L)**	**E_N (%)**
0	5.3	5.66	20	20	0
1	5.4	5.55	19.6	6.9	65
2	5.6	5.36	20.4	6.7	67
3	5.6	5.36	20.4	6.3	69
4	5.6	5.36	20.4	6	71
5	5.5	5.45	19.8	7.1	64
6	5.7	5.26	19.8	7.6	62
7	5.6	5.36	19.8	7.6	62
8	5.3	5.66	19.8	6.3	68
9	5.2	5.77	19.8	4.3	78
10	5.2	5.77	19.8	3.8	81

TableA.3 Raw data of continuous experiment at N/P=9/1

Time (days)	**Q (L/day)**	**θ (g/L)**	**$(NH_4\text{-}N)_o$ (mg/L)**	**$(NH_4\text{-}N)_e$ (mg/L)**	**E_N (%)**
0	5.6	5.36	20.7	20.7	0
1	5.5	5.45	20.7	15.5	25
2	5.5	5.45	20.4	14.3	30
3	5.6	5.36	20.2	14.2	30
4	5.5	5.45	20.2	13.8	32
5	5.7	5.26	20.2	12.8	37
6	5.7	5.26	19.8	11.5	42
7	5.3	5.66	19.8	9.8	51
8	5.2	5.77	19.8	9	55
9	5.2	5.77	19.8	6.3	68
10	5.2	5.77	19.8	5.5	72

TableA.4 Raw data of continuous experiment at N/P=12/1

Time (days)	**Q (L/day)**	**θ (g/L)**	**$(NH_4\text{-}N)_o$ (mg/L)**	**$(NH_4\text{-}N)_e$ (mg/L)**	**E_N (%)**
0	5.3	5.66	20.4	20.4	0
1	5.4	5.55	20.4	7	66
2	5.4	5.55	20.4	6.8	67
3	5.3	5.66	20.3	6.7	67
4	5.5	5.45	19.8	6.8	66
5	5.5	5.45	18.5	6.6	64
6	5.4	5.55	19.9	7.4	63
8	5.3	5.66	19.9	7.6	62
9	5.3	5.66	18.8	7.6	60
10	5.4	5.55	18.8	7.5	60

TableA.5 Raw data of continuous experiment at N/P=15/1

Time (days)	**Q (L/day)**	**θ (g/L)**	**$(NH_4\text{-}N)_o$ (mg/L)**	**$(NH_4\text{-}N)_e$ (mg/L)**	**E_N (%)**
0	5.3	5.66	18.3	18.3	0
1	5.3	5.66	17.8	2.5	86
2	5.4	5.55	17.8	3.2	82
3	5.3	5.66	17.8	2.6	85
4	5.4	5.55	20.4	3.4	83
5	5.5	5.45	20.4	3.1	85
6	5.5	5.45	20.2	3	85
7	5.4	5.55	20.2	0.7	97
8	5.4	5.55	22.6	0.6	97
9	5.4	5.55	23.9	1	96
10	5.3	5.66	23.9	0.9	96

Table A.6 Raw data of continuous experiment at different nitrogen/phosphorus ratios

N/P Ratio	**$(NH_4\text{-}N)_e$ (mg/L)**	**E_P (%)**
4/1	5.1	75
6/1	3.8	81
9/1	5.5	72
12/1	7.5	60
15/1	0.9	96

TableA.7 Raw data of continuous experiment at NH_4-N=20 mg/L and light intensity= 1650 lux

Time (days)	**Q (L/day**	**θ (g/L)**	**$(NH_4\text{-}N)_o$ (mg/L)**	**$(NH_4\text{-}N)_e$ (mg/L)**	**E_N (%)**	**$(PO_4\text{-}P)_o$ (mg/L)**	**$(PO_4\text{-}P)_e$ (mg/L)**	**E_P (%)**
0	5.6	5.36	19.2	19.2	0	1.4	1.4	0
1	5.6	5.36	19.2	7.6	60	1.4	1.2	14
2	5.6	5.36	18.4	7.1	61	1.4	1.2	14
3	4.91	6.11	18.4	5.8	68	1.4	0.6	57
4	5.8	5.17	18.4	1.9	90	1.4	0.3	79
5	5.8	5.17	18.4	1.7	91	1.4	0.3	79
6	5.2	5.77	19.2	1.7	91	1.4	0.1	93
7	5.2	5.77	19.2	0.8	96	1.4	0.1	93
9	5.3	5.66	19.2	0.6	97	1.4	0.1	93
10	5.2	5.77	19.2	2.1	89	1.4	0.1	93

TableA.8 Raw data of continuous experiment at NH_4-N=20 mg/L and light intensity= 3727 lux

Time (days)	**Q (L/day**	**θ (g/L)**	**$(NH_4\text{-}N)_o$ (mg/L)**	**$(NH_4\text{-}N)_e$ (mg/L)**	**E_N (%)**	**$(PO_4\text{-}P)_o$ (mg/L)**	**$(PO_4\text{-}P)_e$ (mg/L)**	**E_P (%)**
0	5.6	5.36	21.3	21.3	0	1.5	1.5	0
1	5.6	5.36	21.3	9.8	7	1.5	1.2	20
2	5.6	5.36	21.3	8.6	21	1.4	1	30
3	4.91	6.11	21.3	8.3	36	1.4	0.8	43
4	5.8	5.17	21.3	6.4	54	1.5	0.8	47
5	5.8	5.17	21.3	5.8	73	1.5	0.6	60
6	5.2	5.77	21.3	4.2	80	1.5	0.4	73
7	5.2	5.77	21.3	2.3	89	1.5	0.4	73
9	5.3	5.66	21.3	4.1	81	1.5	0.3	80
10	5.2	5.77	21.3	3.1	85	1.5	0.3	80

TableA.9 Raw data of continuous experiment at NH_4-N=40 mg/L and light intensity= 3727 lux

Time (days)	**Q (L/day**	**θ (g/L)**	**$(NH_4\text{-}N)_o$ (mg/L)**	**$(NH_4\text{-}N)_e$ (mg/L)**	**E_N (%)**	**$(PO_4\text{-}P)_o$ (mg/L)**	**$(PO_4\text{-}P)_e$ (mg/L)**	**E_P (%)**
0	5.6	5.36	40	40	0	2.9	2.9	0
1	5.6	5.36	40	19.6	51	2.9	1.6	45
2	4.91	6.11	40	22.8	43	2.8	1.5	46
3	5.8	5.17	40	24	40	2.8	1.4	50
4	5.8	5.17	39.2	25.6	35	3	1.3	57
5	5.8	5.17	39.2	24	39	3	1.3	57
6	5.2	5.77	39.2	23.6	40	2.9	1.3	55
7	5.2	5.77	38.8	22	43	2.9	1.4	52
9	5.3	5.66	38.8	22.2	43	2.9	1.5	48
10	5.2	5.77	38.8	19.2	51	2.9	1.2	59

TableA.10 Raw data of continuous experiment at NH_4-N=60 mg/L and light intensity= 3727 lux

Time (days)	**Q (L/day**	**θ (g/L)**	**$(NH_4\text{-}N)_o$ (mg/L)**	**$(NH_4\text{-}N)_e$ (mg/L)**	**E_N (%)**	**$(PO_4\text{-}P)_o$ (mg/L)**	**$(PO_4\text{-}P)_e$ (mg/L)**	**E_P (%)**
0	5.6	5.36	60.8	60.8	0	4.1	4.1	0
1	5.6	5.36	60.8	18.8	69	4.1	1.7	60
2	4.91	6.11	60.8	18.2	70	4.1	1.5	63
3	5.8	5.17	60.8	20	67	4.1	1.4	66
4	5.8	5.17	62.8	26.8	57	4.1	2.5	39
5	5.8	5.17	62.8	30	52	4.3	3.2	26
6	5.2	5.77	62.8	32.4	48	4.3	2.6	40
7	5.2	5.77	60.8	33.6	45	4.3	2.7	37
9	5.3	5.66	60.8	33.2	45	4.3	2.4	44
10	5.2	5.77	60.8	31.2	49	4.3	2.4	44

Table A.11 Raw data of continuous experiment at different light intensities and initial NH_4-N concentrations

Light intensity (Lux)	**$(NH_4\text{-}N)_o$ (mg/L)**	**$(NH_4\text{-}N)_e$ (mg/L)**	**E_N (%)**
1650	20	2.1	89.06
3727	20	3.1	85.44
3727	40	19.2	50.52
3727	60	44.19	48.68

TableA.12 Raw data of fed-batch experiment at initial NH_4-N=12.6 mg/L and initial PO_4-P=3.2 mg/L

Time (days)	**$(NH_4\text{-}N)_{teo}$ (mg/L)**	**$(NH_4\text{-}N)_e$ (mg/L)**	**E_N (%)**	**$(PO_4\text{-}P)_{teo}$ (mg/L)**	**$(PO_4\text{-}P)e$ (mg/L)**	**E_P (%)**
0	0.1	0	100	0.3	0	100
2	10.4	2.3	78	2.8	1.5	46
3	11	1.8	84	2.3	0.4	83

TableA.13 Raw data of fed-batch experiment at initial NH_4-N=12.6 mg/L and initial PO_4-P=3.2 mg/L

Time (days)	**$(Chla)_{teo}$ (mg/L)**	**$(Chla)_e$ (mg/L)**	**$(Biomass)_{teo}$ (mg/L)**	**(Biomass)e (mg/L)**
0	11.2	11.2	770	770
2	1.9	8.8	132	607
3	1.4	10.3	94	459

TableA.14 Raw data of fed-batch experiment at initial NH_4-N=22.4 mg/L and initial PO_4-P=5.6 mg/L

Time (days)	$(NH_4\text{-}N)_{teo}$ (mg/L)	$(NH_4\text{-}N)_e$ (mg/L)	E_N (%)	$(PO_4\text{-}P)_{teo}$ (mg/L)	$(PO_4\text{-}P)e$ (mg/L)	E_P (%)
0	0.5	0	100	0.1	0	100
2	18.6	10.4	44	4.7	1.9	60
3	19.7	8.8	55	4.8	2.1	56

TableA.15 Raw data of fed-batch experiment at initial NH_4-N=12.6 mg/L and initial PO_4-P=3.2 mg/L

Time (days)	$(Chla)_{teo}$ (mg/L)	$(Chla)_e$ (mg/L)	$(Biomass)_{teo}$ (mg/L)	(Biomass)e (mg/L)
0	15.5	15.5	671	671
2	2.7	9.4	115	579
3	1.9	8.5	82	433

TableA.16 Raw data of fed-batch experiment at NH_4-N=32 mg/L and initial PO_4-P=8 mg/L

Time (days)	$(NH_4\text{-}N)_{teo}$ (mg/L)	$(NH_4\text{-}N)_e$ (mg/L)	E_N (%)	$(PO_4\text{-}P)_{teo}$ (mg/L)	$(PO_4\text{-}P)e$ (mg/L)	E_P (%)
0	2.4	0	100	0.5	0	100
2	26.9	20.2	25	6.7	5.3	21
3	28.4	10.6	63	7.1	2.3	68

TableA.16 Raw data of fed-batch experiment at NH_4-N=32 mg/L and initial PO_4-P=8 mg/L

Time (days)	$(Chla)_{teo}$ (mg/L)	$(Chla)_e$ (mg/L)	$(Biomass)_{teo}$ (mg/L)	(Biomass)e (mg/L)
0	13.7	13.7	740	740
2	2.4	6.9	126	475
3	1.6	4.9	90	397

Table A.17 Raw data of fed-batch experiment at different initial nitrogen and phosphorus

$(NH_4\text{-}N)_0$ (mg/L)	$(NH_4\text{-}N)_e$ (mg/L)	E_N (%)	$(PO_4\text{-}P)_0$ (mg/L)	$(PO_4\text{-}P)_e$ (mg/L)	E_P (%)
12.6	1.8	84	3.2	0.4	83
22.4	8.8	55	5.6	2.1	56
32	10.6	63	8	2.3	68

TableA.18 Raw data of fed-batch experiment at N/P=4.6

Time (days)	$(NH_4\text{-}N)_{teo}$ (mg/L)	$(NH_4\text{-}N)_e$ (mg/L)	E_N (%)	$(PO_4\text{-}P)_{teo}$ (mg/L)	$(PO_4\text{-}P)e$ (mg/L)	E_P (%)
0	4	0	100	0.4	0	100
2	33.8	29.2	14	7.3	1.8	75
3	35.6	8.8	75	3.4	1.4	75

TableA.19 Raw data of fed-batch experiment at N/P=4.6

Time (days)	$(Chla)_{teo}$ (mg/L)	$(Chla)_e$ (mg/L)	$(Biomass)_{teo}$ (mg/L)	(Biomass)e (mg/L)
0	18.7	18.7	770	770
2	3.2	7	132	648
3	2.3	8.7	94	484

TableA.20 Raw data of fed-batch experiment at N/P=6.6

Time (days)	$(NH_4\text{-}N)_{teo}$ (mg/L)	$(NH_4\text{-}N)_e$ (mg/L)	E_N (%)	$(PO_4\text{-}P)_{teo}$ (mg/L)	$(PO_4\text{-}P)e$ (mg/L)	E_P (%)
0	11.6	0	100	2.7	0	100
2	38.1	25.2	34	6	5.2	29
3	40	12.8	68	6.2	2.8	65

TableA.21 Raw data of fed-batch experiment at N/P=6.6

Time (days)	$(Chla)_{teo}$ (mg/L)	$(Chla)_e$ (mg/L)	$(Biomass)_{teo}$ (mg/L)	(Biomass)e (mg/L)
0	18.7	18.7	740	740
2	3.2	7.8	127	607
3	2.3	6.5	90	442

TableA.22 Raw data of fed-batch experiment at N/P=7.3

Time (days)	$(NH_4\text{-}N)_{teo}$ (mg/L)	$(NH_4\text{-}N)_e$ (mg/L)	E_N (%)	$(PO_4\text{-}P)_{teo}$ (mg/L)	$(PO_4\text{-}P)e$ (mg/L)	E_P (%)
0	5.6	0	100	2.7	0	100
2	38	29.6	22	5.5	4.3	43
3	40	17.8	55	5.6	3.1	60

TableA.23 Raw data of fed-batch experiment at N/P=7.3

Time (days)	$(Chla)_{teo}$ (mg/L)	$(Chla)_e$ (mg/L)	$(Biomass)_{teo}$ (mg/L)	(Biomass)e (mg/L)
0	18.7	18.7	670	670
2	3.2	7	116	548
3	2.7	6.6	82	384

Table A.24 Raw data of fed-batch experiment at different nitrogen/phosphorus ratios

N/P Ratio	$(NH_4\text{-}N)_0$ (mg/L)	$(NH_4\text{-}N)_e$ (mg/L)	E_N (%)	$(PO_4\text{-}P)_0$ (mg/L)	$(PO_4\text{-}P)_e$ (mg/L)	E_P (%)
4.6	35.6	8.8	75	3.4	1.4	75
6.6	40	12.8	68	6.2	2.8	65
7.3	40	17.8	55	5.6	3.1	60