DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

NITROGEN AND PHOSPHORUS REMOVAL FROM SYNTHETIC WASTEWATER USING ALGAL PHOTOBIOREACTOR SYSTEM

by

Şebnem ASLAN

September, 2005
İZMİR

NITROGEN AND PHOSPHORUS REMOVAL FROM SYNTHETIC WASTEWATER USING ALGAL PHOTOBIOREACTOR SYSTEM

A Thesis Submitted to the
Graduate School of Natural and Applied Sciences of Dokuz Eylül University
In Partial Fulfillment of the Requirement for the Degree of Master of Science in
Environmental Engineering, Environmental Technology Program

by

Şebnem ASLAN

September, 2005
İZMİR

M.Sc THESIS EXAMINATION RESULT FORM

We certify that we have read this thesis and **"NITROGEN AND PHOSPHORUS REMOVAL FROM SYNTHETIC WASTEWATER USING ALGAL PHOTOBIOREACTOR SYSTEM"** completed by **Şebnem ASLAN** under supervision of **Assoc.Prof.Dr. İlgi 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.

Assoc.Prof.Dr. İlgi K. KAPDAN

Supervisor

Prof.Dr. Fikret Kargı

(Committee Member)

Assist. Prof.Dr Vildan Gündoğdu

(Committee Member)

Approved by the

Graduate School of Natural and Applied Sciences

Prof.Dr. Cahit HELVACI
Director

ACKNOWLEDGMENTS

I would like to greatly thank to my supervisor Assoc. Prof. Dr. İlgi Karapınar Kapdan for her valuable advises, incomparable helps, continuous supervision and considerable concern in carrying out the study. It has been a great honor and privilege for me to work with her.

I also would like to thank to technicians Yılmaz Sağer and Orhan Çolak for their help in my laboratory studies.

At the same time I thank to my all friends who helped me in preparing my thesis magnificently.

Furthermore, I greatly acknowledge my family, for being always with me in every single steps of this thesis with their encouragement.

Şebnem ASLAN

NITROGEN AND PHOSPHORUS REMOVAL FROM SYNTHETIC WASTEWATER USING ALGAL PHOTOBIOREACTOR SYSTEM

ABSTRACT

Nitrogen and phosphorus removal from synthetic wastewater by *C. vulgaris* was investigated in this thesis. The study has two parts as 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 NH_4-N and PO_4-P concentrations on nitrogen and phosphorus removal were evaluated and biokinetic coefficients such as k; reaction rate constant, Ks, saturation constant, Y; yield coefficient were determined by using Monod rate expression in the first part of batch experiments. The results indicated that effluent water quality decreases with increasing nutrient concentrations and nitrogen is more effectively removed than that of phosphorus.

In the second part of batch experiments, the effect of light intensity, Nitrogen/ Phosphorus ratio, Light/Dark cycle and initial ChA concentration on nutrient removal was studied. The optimum conditions for maximum NH_4-N removal were determined at maximum light intensity. Phosphate uptake rate by algae was significantly lower than that of nitrogen.

The photobioreactor system was continuously operated at different hydraulic retention times and initial ammonia nitrogen concentration. The maximum removal efficiency was obtained with minimum NH_4-N concentration. Increasing hydraulic retention time provided better effluent quality in terms of ammonia nitrogen.

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

SENTETİK ATIKSUDAN ALG FOTOBİYOREAKTÖR SİSTEMİNDE AZOT VE FOSFOR GİDERİMİ

ÖZ

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

Azot ve fosfor konsantrasyonlarının azot ve fosfor giderimi üzerine etkileri değerlendirilmiştir. Bunun yanında, k; reaksiyon oranı sabiti, K_S; doygunluk sabiti, Y ve ürün katsayısı gibi biyokinetik katsayılar Monod oranı ifadeleri kullanılarak belirlenmiştir. Bulunan sonuçların işaretinde, çıkış suyu kalitesinin artan azot konsantrasyonlarıyla birlikte azaldığı görülmüştür. Ancak azot giderim hızının, fosfor giderim hızına nazaran daha yüksek olduğu belirlenmiştir.

Çalışmanın ikinci bölümünde, ışık şiddeti, azot/fosfor oranı, aydınlık/karanlık döngüsü ve başlangıç klorofil A konsantrasyonu gibi parametrelerin nutrient giderimi üzerine olan etkileri incelenmiştir.

Fotobiyolojik reaktor sistemi sürekli koşullarda farklı hidrolik alıkonma sürelerinde ve farklı giriş azot konsantrasyonlarında işletilmiştir. Çıkış azot derişimi, giriş azot derişimdeki artışa bağlı olarak yükselmiştir. Maksimum azot giderim verimi düşük azot derişimlerinde elde edilmiştir. Artan hidrolik alıkonma süreleri amonyak azotu açısından daha iyi çıkış suyu kalitesi sağlamaktadır.

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

CONTENTS

	Page
THESIS EXAMINATION RESULT FORM …….....	ii
ACKNOWLEDGEMENTS …….....	iii
ABSTRACT …….....	iv
ÖZ…..…….....	vi
CHAPTER ONE –INTRODUCTION……...……...…	**1**
1.1. Literature Review…….	1
1.1.1. Bacterial Nutrient Removal...……..	1
1.1.2. Algal Nutrient Removal.....…….	2
1.1.2.1. Algal Treatment systems……....	3
1.1.2.2. Important Design Parameters for Photobioreactors...……...	5
1.1.2.2.1. Growth Index...…….	5
1.1.2.2.2. Light Inside the Photobioreactor…...……	5
1.1.2.2.3. CO_2/O_2 Balance…….	6
1.1.2.2.4. Temperature...……...	6
1.1.2.2.5. Salinity, Nutrients and pH value……...……	6
1.1.2.2.6. Turbulence……...….	7
1.1.2.2.7. Mixing…...……	7
1.1.2.2.8. Contamination...……	7
1.1.3. Literature on Algal Nutrient Removal……...……...	7
1.2. Objectives and Scope…...…….	17
CHAPTER TWO – MATERIALS AND METHODS…….……	**18**
2.1. Microbial Culture ……..…….…….	18
2.2. Wastewater Composition ……...…….	18
2.3. Experimental Set-up ……...…….	18
2.3.1. Batch Experiments ……...	18
2.3.1.1. Experimental Studies…….	19
2.3.2. Photobioreactor…….	19
2.3.2.1. Experimental Studies……	20

2.4. Analytical Methods …. 20
2.4.1. Sampling …. 20
2.4.2. Chlorophyll A Analysis……..……... 20
2.4.3. Nitrogen and Phosphorus Analysis……..…… 21
2.4.4. Biomass Measurement…….. 21
2.4.5. pH and Light Intensity Measurement…… 21

CHAPTER THREE – RESULTS AND DISCUSSIONS…….…… 23
3.1. Batch Experiments…… 23
3.1.1. Effect of Initial NH_4-N Concentration on Nutrient Removal Performance of the Batch System ……..…. 23
3.1.2. Determination of Batch Kinetic Coefficients…… 39
3.1.3. Effect of Initial Light/dark Cycle on Nutrient Removal Performance of the Chlorella Vulgaris in Batch Operation…….. 45
3.1.4. Effect of Light Intensity on Nutrient Removal Performance of the Batch System…… 51
3.1.5. Effect of N/P Ratio on Nutrient Removal Performance of the Batch System…… 62
3.1.6. Effect of Chlorophyll A Concentration on Nutrient Removal Performance of the Batch System……. 71
3.2. Continuous Experiments in Immobilized Photobioreactor System…….. 79
3.2.1. Effect of NH_4-N Concentration on NH_4-N Removal Performance of Continuous Immobilized Photobioreactor System……... 79
3.2.2. Effect of Hydraulic Retention Time on NH_4-N Removal Performance of Continuous Immobilized Photobioreactor System…….. 85

CHAPTER FOUR - CONCLUSIONS …..……..…….. 91

CHAPTER FIVE – RECOMMENDATIONS…….……..… 95
References…….……...…….……..... 97
Appendices……...…….……..….. 101
Raw Data of Experimental Studies 101

CHAPTER ONE
INTRODUCTION

1.1 Literature Review

Nitrogen and phosphorus are major sources of eutrophication which are released by human activities such as agricultural practices, urbanization, industrialization and other alterations. Eutrophication of aquatic ecosystems can cause algal blooms and hazardous environmental problems, thus it is the most important water quality problem throughout the world. There are some biological nutrient removal systems to prevent eutrophication. These systems can be broadly divided into bacterial nutrient removal and algal nutrient removal.

1.1.1 Bacterial nutrient removal

Several scientific studies on nitrogen and phosphorus removal were reported. Most of these studies are based on biological processes and different combinations of anaerobic, aerobic and anoxic zones (Bardenpho, A_2O, UCT and their modifications), but a combined usage of biological and chemical methods was also used. Sequences of anaerobic, anoxic and aerobic steps are used in bardenpho process for nitrogen and phosphorus removal. While nitrogen is removed by nitrification-denitrification, phosphorus is removed by wasting sludge from system (Metcalf & Eddy, 1991). Furthermore sequencing batch reactor (SBR) operations have a great potential for COD/BOD and phosphate removal. The modifications of sequencing batch operation are used to achieve nitrogen removal by nitrification and denitrification in addition to COD and phosphate removal. The SBR operations contain anaerobic, anoxic and oxic phases in the same reactor (Kargi & Uygur, 2002). Additionally, constructed wetlands and immobilize nitrifiers have been used for nitrogen and phosphorus removal from wastewaters (Picard et al., 2005).

1.1.2 Algal Nutrient Removal

Different algal species have a large biotechnological potential for production of many useful products. For instance; animal feed, food, biofertilizer, soil conditioner, as feed in aquaculture and biological treatment of wastewaters. Recent researches are conducted on the potential use of 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.

Microalgae have an important role in fixing carbon dioxide which causes green house effect. It is expected that, in the future, photosynthetic microorganisms will play a larger role than higher plants in photosynthetic carbon dioxide fixation because they have higher photosynthetic rates per unit biomass.

Algae treat wastewater with two mechanisms. The first one is by producing oxygen that is dissolved in wastewater and used by bacteria to oxidize wastes. Secondly, algae use nutrients in wastewater like ammonia for its own biomass production. Algae are very useful for waste treatment because of fast growth growing which results in production of biomass and consumption of nutrients from the wastes. Algal biomass production requires few simple inputs such as carbon dioxide, water, light and mineral salts or other nutrient sources (N, P). Algae have the potential to reduce dissolved and/or ionize odorous compounds like ammonia, hydrogen sulfide. Microalgae have ecological diversity and can adapt various mediums. Thus, there are multitude solutions for cultivation problems (Ogbonna &Tanaka, 1997; Pulz, 2001; Lee, K. & Lee, C, 2001; Anderson, Kommareddy & Schipull, 2002).

1.1.2.1 Algal Treatment Systems

Algae can be produced in both open and closed systems. Open systems can be broadly divided into natural systems (lakes, lagoons, ponds) and artificial ponds or containers. Closed systems are broadly divided into following groups: (1) vertical or horizontal tubular systems (glass, plastic, and bags), (2) inclined or horizontal flate-plate systems, (3) ultrathin immobilized configuration systems and (4) closed container based systems (tanks, hanging plastic bags).

There are some difficulties in open systems to obtain high productivity. The most important ones are; various temperatures, light intensity throughout the day and the year, significant evaporative loses and diffusion of CO_2 to the atmosphere. Furthermore, open ponds require large surface area and problems with contamination arise. Covering ponds does reduce some of the disadvantages, but enclosed systems will still provide better control of temperature, light intensity, better control of gas transfer and larger surface area-to-volume ratio.

The usage of closed photobioreactors (PBRs) enhance commercial algal biomass production by keeping algae genetics pure and reducing the possibility of parasite infestation. The scale-up of simple container based systems (tanks, hanging plastic bags) as a first generation of closed PBRs as soon faced with serious limitations to supply light efficiently by maximizing the illumination surface-to-volume ratio of the reaction. For this reason, there are several technical approaches to underwater lighting (submersed lambs or light diffusing optical fibers). On the other hand, the vertical arrangements of horizontal running tubes or plates are preferred because of light distribution and appropriate flow (Ogbonna &Tanaka, 1997; Pulz, 2001). Advantages and disadvantages of closed and open systems are listed 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 (PBRs)**
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 restricted 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
Reproducibility of production parameters	Not given, dependent 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

The use of algae for wastewater treatment is depend on many environmental parameters including light intensity, CO_2/O_2 balance, temperature, salinity, nutrients, pH value and turbulence.

1.1.2.2.1 Growth Index: Mathematical modeling of photosynthetic cell growth is one of the most important parameter in designing and optimizing photobioreactors. Generally specific rates during the exponential growth phase are used as growth parameters in growth kinetics and photosynthetic cell growth models. Owing to have a great influence on culture productivity, the cell growth rate during a growth phase would be a good index for process design and optimization. Ogbonna & Tanaka (1997) studied relative significance of the exponential and linear growth rates during light-limited batch cultivation of photosynthetic cells using various types and sizes of photobioreactors as a first step in the photobioreactor design. It is indicated during the cultivation of *Chlorella pyrenoidosa* C-212 and *Spirulina platensis* M-135 that there was not good correlation between the specific growth rates and the linear growth rates or between the specific growth rates and the final cell concentrations. There is also a mathematical model is developed that could explain the existence of the various growth phases during the light-limited batch cultivation. According to this model the linear growth phase is longer than the exponential growth phase under various conditions. Hence, the linear growth rate is better growth index than the specific growth rate.

1.1.2.2.2 Light inside the photobioreactor: Light is the major limiting factor for the photoautotrophic life. The advantage of the illumination intensities is the rate of photosynthesis is directly proportional to light intensity. On the other hand, too high illumination intensities can damage photosynthetic receptor system occurs within a few minutes (photoinhibition). The total light energy supplied per unit volume of photobioreactor (E_t/V), is the most appropriate measure of photobioreactor performance. It has been concluded by Ogbonna & Tanaka (1997) that the linear growth rates decreased with an increase in depth of the photobioreactors. These results indicating that the light distribution inside the reactor must be considered in the rational design and scale-up of photosynthetic processes.

There is more homogenous light distribution in the shallow photobioreactor than deep one. For this reason, surface area per unit volume is used as an important design parameter. An efficient photobioreactor should have a high surface area-to-volume ratio. In cultivation ponds, this is achieved by keeping the pond as shallow as possible. However there are some scale-up problems with such photobioreactors like limiting tube diameters and panel depths.

1.1.2.2.3 CO_2/O_2 Balance: Like other autotrophically growing plant, algae require an inorganic carbon source to perform photosynthesis. Thus, CO_2 must be supplied in algal cultures while increasing O_2 has to be removed before reaching inhibitory concentrations. Oxygen may become a problem in algal cultures of high cell densities not only because of the limitation of the rate of photosynthesis but also upon radiation with appropriate energy, oxygen radicals may develop during the respiratory gas exchange and cause toxic effects on cells due to membrane damage. The natural CO_2 concentration in air only 0.03 % is too low to sustain optimal growth and high productivity. It has been shown in chemical analyses that algal biomass consist of about 50% carbon which means that about 1,8 kg of carbon dioxide are required to produce 1 kg of algae. Thus, some CO_2 entrance should be done. Since pure carbon dioxide is expensive, alternative and cheaper sources of this gas from industrial combustion process, diesel engines, cement plants or fermentation have been tried. Hence, algal ponds should be located near such plants.

1.1.2.2.4 Temperature: Temperature is one of the major parameters which effect growth of microalgae and photosynthetic reactions in algal medium. The influence of temperature is insignificant, when carbon dioxide or light is limiting for photosynthesis. At high temperatures, efficiency of photosynthesis declines. This effect can worsen in suspension cultures by the difference in decrease of CO_2 and O_2 solubility at increased temperatures.

1.1.2.2.5 Salinity, nutrients and pH value: The pH value is the other important parameter in algal cultivation. It has an effect on solubility of carbon dioxide and minerals in the medium, so directly or indirectly influences the metabolism of the algae. There are some factors that influence pH of algal cultures, such as composition and buffering capacity of medium, amount of dissolved CO_2 solubility and metabolic activity of algal cells. Ammonia stripping and precipitation of calcium phosphate can

be occurring in alkaline pH. For optimal photosynthesis sufficient nutrient supply for microalgae is a precondition. The shortage of nutrients will cause disorder in metabolism and disproportionate production of intermediates of photosynthesis. Deviations from the optimum osmotical conditions and salinity will cause physiological reactions and productivity problem.

1.1.2.2.6 Turbulence: Turbulence is important for photosynthetic reactions which have an important effect on light distribution in algal medium.

1.1.2.2.7 Mixing: Mixing is the other important parameter for photobioreactors. It helps to keep cells in suspension, distribute the nutrients, generated heat in within the reactor, improves CO_2 transfer into the reactor, stripping the photosyntheticaly produced O_2, improve mass transfer between cells and the liquid milieu and facilitate the movement of cells in and out of the illuminated part of the reactor. Many photosynthetic cells have no cell and some are mobile or filamentous which makes them fragile and sensitive to shear stress. Thus, it is desirable to keep the hydrodynamic stress as low as possible.

1.1.2.2.8 Contamination: Owing to lack of organic carbon source, the risk of contamination by heterotrophic microorganisms is low. On the other hand, contamination by other photoautotrophs can be a serious problem. Hence, the new photobioreactor should be sterilized.

1.1.3. Literature on Algal Nutrient Removal

Bitch, Yaziz & Baktı (1999) studied that combination of *Chlorella vulgaris* and waterhyacinth (*Eichhornia crassipes*) with high rate algal pond (HRAP) for nitrogen removal of agro-industrial wastewater. During the experiments, the ambient temperature averaging at 32 °C and each run lasted 20 days. The wastewater was included 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. The N removal efficiency in normal HRAP system was about 80%. Integrated system achieved 23% better removal than normal HRAP. The nitrogen removal in combined system could include assimilation by the algae (55%) and waterhyacinth (10%) now accounted for about 65% of the total N removal. The ammonia volatilization amounted about 15% and due to the nitrification-denitrification about

20%. Besides, there is no further requirement for suspended solid removal in integrated treatment system.

The wastewater nutrient removal by marine microalgae grown on a corrugated raceway was investigated by Craggs, McAuley & Smith (1997). Two marine microalgal isolates were taken (SA91B33, *Phaeodactylum tricornutum* and SA91CY1, *Oscillatoria sp*.) from a sewage outfall site in Scotland. Wastewater was diluted 1:1 with sterile seawater and continuously added to raceway algal cultures grown under ambient conditions. The influent nutrient concentrations were about 497,7 mmol NH_4-N m^{-3} and 76,2 mmol PO_4-P m^{-3}. Both species continued to remove 100% of ammonium and orthophosphate. Nutrient removal was probably not just by uptake and assimilation into algal biomass. The pH of raceway effluents was high (>9,5). Hence, ammonium may have been volatilized and orthophosphate precipitated. At the end of the study two isolates were able to remain unialgal culture during summer and autumn conditions. Removal of nutrients was complete and continuous and was not effected by the diurnal cycle. It is indicated that by exploiting the adherent properties of these isolates the system does not require mixing and may enable simple mechanical harvesting of the algal biomass. According to this study it is suggested that the potential for use of marine microalgae for nutrient removal.

Olguin, Galicia, Angulo-Guerrero & Hernandez (2001) studied the effect of low light flux and nitrogen deficiency on the chemical composition of *Spirulina Arthrospira* grown on complex medium containing sea-water supplemented with anaerobic effluents from digested pig waste. The experiments were carried out bench raceways in batch culture conditions. They tried two different light flux of 66(lower) or 144 µmol photon $m^{-2}s^{-1}$ (higher). Growth kinetics was evaluated in complex medium and in modified Zarrouk medium as a control medium during cultivation at two light fluxes. After 12 days biomass concentration was similar in two media when cultivated at 66 µmol photon $m^{-2}s^{-1}$. The initial ammonia concentration of medium was 1 mM, incubated at 32°C and under nun-pH control regime. Thus, it was expected to have large amounts of ammonia stripping and through consumption could become nitrogen deficient after few days. Protein content of the biomass in the

complex medium was significantly lower regardless of the light intensity. However, biomass from the complex medium was enriched in total lipids (28,6 %) at lower light flux. On the other hand, the palmitoleic acid was significantly higher at 144 µmol photon $m^{-2}s^{-1}$ and a high level of gamma linolenic acid in the biomass harvested from the complex medium at lower light intensity. Finally, polysaccharide content was significantly higher at the high light intensity and a very high content of total polysaccharides (28,41 %) was observed in the complex medium.

An evaluation was made of the annual productivity of *Spirulina Arthrospira* and its ability to remove nutrients in outdoor raceways treating anaerobic effluents from pig wastewater under tropical conditions. During four consecutive years, batch or semi-continuous cultures were set up at different seasons. The pH was maintained at 9,5±0,2. The temperature in the range of 29-34 °C and the average light intensity in the range of 476-1784 µmol photon $m^{-2}s^{-1}$. For batch cultures, the productivity was significantly more in 1998 with a pond depth of 0,10 m than 0,065 m. The average productivity in semi-continuous cultures was higher in summer 1999 than in autumn 2000 or winter 2001, when the depth was 0,15 and 0,20 m (14,4 and 15,1 g $m^{-2}d^{-1}$ respectively). The average protein content of the semi-continuous cultures was 48,9% ash-free dry weight. NH_4-N removal was in the range 84-96 % and P removal in the range of 72-87 %, depending on the depth of the culture and the season (Olguin, Galicia, Mercado & Perez, 2003).

Some experimental studies were conducted on nutrient removal from raw sewage and pre-treated cattle-manure by immobilization of microalgae. These experiments were performed by Travieso, Benitez, Weiland, Sanchez, Dupeyron & Dominguez (1996). The immobilization capacities of *Chlorella vulgaris, Chlorella kessleri* and *Scenedesmus quadricauda* were evaluated using internal immobilization with sodium alginate and k-carrageen and external immobilization with using polystyrene and polyurethane foams. The use of sodium alginate pellets of *Chlorella vulgaris* and *Chlorella kessleri* gave good results with raw sewage (Ammonia-N=31±5, O-Phosphate=6±2 mg/lt). Higher efficiencies were obtained with the utilization by *Chlorella vulgaris* in an expanded-bed when the columns operated under natural

light. The maximum orthophosphate removal (72 %) was obtained with *Chlorella vulgaris*, while it was 60 % in the *kessleri* expanded column. According to results natural light increased the nutrient removal compared with the artificial light. Internal immobilization and the use of an upflow pattern failed with pretreated cattle manure (Ammonia-N=237±43, O-Phosphate=34±3 mg/lt, diluted 1:4 with tab water) due to the dark color of this substrate. However, there were good efficiencies obtained at hydraulic loadings in the range of 0,5-2,5 lt/day using downflow columns. Increase of hydraulic loading resulted in a decrease of nutrient removal efficiency. In the case of *Chlorella* column the ammonia nitrogen removal changed from 64 to 48 %. In Scenedesmus column ammonia nitrogen removal decreased from 60 to 42% and orthophosphate from 59 to 44%. According to the results, external immobilization with polyurethane foam is very promising, considering the low cost of this material compared with sodium alginate and k-carrageenan.

Tam & Wong (2000) investigated the effect of immobilized microalgal bead concentrations on simulated settled domestic wastewater nutrient removal. A unicellular green microalga, *Chlorella vulgaris*, was entrapped in calcium alginate beads. A significantly higher nutrient reduction was found in bioreactors containing algal beads than the blank alginate beads. Within 24 h of treatment in bioreactor having optimal bead concentration (12 beads ml^{-1}, equivalent to 1:3 algal beads: wastewater, v/v), complete removal of NH_4^+-N and around 95% PO_4^{3-}-P was achieved. The $NH4^+$-N removal was significantly lower at low (around 4 beads ml^{-1}) and high (>15 beads ml^{-1}) algal bead concentrations. The (NO_2^-+NO_3^-)-N concentrations in all reactors were less than 0,25 mg/lt throughout the study. On the other hand, the effect of bead concentration on phosphate removal was less obvious. In addition to algal cells, the immobilization matrix, the alkaline pH that developed and the aeration of wastewater also contributed to the removal of nutrients from wastewater.

De-Bashan, Moreno, Hernandez & Bashan (2002) studied on nutrient removal from synthetic wastewater by *Chlorella vulgaris* coimmobilized in alginate beads with the microalgae growth-promoting bacterium *Azospirillum brasilense*. The

experiments were carried out under semi-continuous, continuous and batch conditions. Coimmobilized culture under semi-continuous conditions significantly increased the removal of ammonium (100%, 2 days incubation) and soluble phosphorus ions (83%, 2 days incubation) compared to immobilization of the microalgae alone (NH_4^+-N removal=85%, PO_4^{3-}-P removal=33%). In continuous (NH_4^+-N removal=91%) or batch (NH_4^+-N removal=93%) cultures nutrient removal was less efficient than in semi-continuous culture. It is suggested in this study that coimmobilization of a microalgae with microalgae growth-promoting bacteria can serve as a tool in planning novel wastewater treatments.

The characterization of denitrifying photosynthetic bacteria, *Rhodopseudomonas palustris*, isolated from photosynthetic sludge was made by J. K. Kim, Lee, Kim & Moon (1999). The parameters of growth were characterized in anaerobic flask culture. The optimum pH, temperature, and illumination intensity were 5,5, 31 °C and 5000 lux, respectively. The maximum specific growth rate and the rate of gas production by denitrification were 0,095 h^{-1} and 0,2 ml N_2 h^{-1}, respectively. Dissimilatory nitrate reduction to nitrogen gas by the isolate started after the late-log phase and a small amount of nitrite was accumulated at the, and of the culture. The maximum number of 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) studied with *Chlorella kessleri* under batch culture conditions to investigate effect of light/dark cycles on wastewater treatment. It has been indicated that illumination of L/D (12h/12h) cycles gives better efficiency in the removal of organic carbon and phosphorus per increased biomass, while nitrate removal efficiency under the continuous light is slightly higher than that under L/D cycles. After 3 days, nitrate was reduced to 136,5 and 154,1 mg NO_3^-N/lt from 168,1 mg NO_3^-N/lt under continuous illumination and under diurnal cycles, respectively. These results proved that high- density algal cultures using optimized photobioreactors with diurnal cycles will save energy and improve organic carbon sources removal.

Characterization of the photic zone and light penetration depth in cultures with ultrahigh cell densities of the *Spirulina platensis* was investigated by Gitelson, Qiuang & Richmond (1996). In this study, 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), as well as their apparent optical properties (i.e. the reflectance and the vertical attenuation coefficient of downwelling irradiance), were determined by using high-spectral-resolution radiometric measurements. To estimate the depth of the light penetration into a reactor containing an ultrahigh cell density (chlorophyll concentration, up to 300.000 mg/m^3), vertical attenuation coefficient was used. In ultrahigh-cell-density cultures, the photic volume, which is only a small fraction of the reactor volume, greatly differs for the blue and red light compared with the green light. A photosynthetic role for green light under these unique circumstances was suggested.

A laboratory-scale bubble column was tested for hydrocarbon production from secondarily treated piggery wastewater by An, Sim, Lee & Kim (2003). Experiments were conducted on the removal of nitrogen and phosphorus from piggery wastewater during growth of *Botryococcus brauni*, together with measurements of hydrocarbon formation by the alga. The influence was tested of the initial nitrate (10-1020 mg/lt) and phosphorus (14 mg/lt) concentration on the optimum concentration range for a culture in wastewater. A high cell density (>7 g d.wt./lt) was obtained with 510 mg NO_3-N/lt. The growth was nearly independent of the initial phosphate concentration, except under conditions of phosphate deficiency. Dry cell weights of 8,5 mg/lt and hydrocarbon level of 0,95 g/lt were obtained. It was indicated that after 12 days nitrate (788 mg N/lt) removal was 80%.

Fuentes et al (1999) made quantitative analysis of the daily cyclic variation of culture parameters were tested in outdoor continuous culture of *Porpyridium cruentum* in a tubular photobioreactor. This work reported on the daily cyclic variation of oxygen generation rates, carbon consumption rates, photosynthetic activities, growth rates and biochemical composition of the biomass in the system. It was found that there is a linear relationship between the external irradiance and the

average irradiance inside the culture. There was a reduction in photosynthetic activity of the cells at noon and recovery in the afternoon. For this reason, the cells showed a short-term response of parameters such as oxygen generation rate as well as carbon consumption rate with external and average irradiance; a model of photosynthesis rate considering photoinhibition was proposed. This model was a useful tool for determining CO_2 requirements of the system and for the operation and scale up of tubular photobioreactor. The higher the photosynthesis rates, the lower the carbon losses, ranging from 25% at noon to 100% during the night. The daily mean average irradiance inside the culture showed a linear relationship with the growth rate. In the same way, there was a linear relationship among the oxygen generation rate and the growth rate. With respect to the biochemical composition of the biomass, the cells showed a long-term response of metabolic routes to mean daily culture conditions. During the illuminated period, energy was stored carbohydrates were consumed. The fatty acid dry weight content decreased during the daylight period, whereas the fatty acid profile, as total fatty acid, was a function of growth of growth rate. A short-term variation of exopolysaccharides synthesis with solar irradiance was also observed, i.e. the higher the external irradiance the higher the excretion of exopolysaccharides as a protection against adverse culture conditions.

Hu, Westerhoff & Vermaas (2000) investigated the quantitative assessment of factors influencing nutrient removal from ground water by Cyanobacteria. Three cyanobacteria strains were tested in batch mode glass columns under laboratory conditions. *Synechococcus sp*. Strain PCC 7942 displayed the highest nitrate uptake rate, but all species showed rapid removal of nitrate. The nitrate uptake rate increased proportionally with increasing light intensity. An irradiance of 160 µmol of photons $m^{-2}s^{-1}$ was cause nearly maximal growth and nitrate uptake rate. The nitrate uptake rate was affected by 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. Average nitrate uptake rates up to 0,05 mM NO_3-h^{-1} could be achieved at a culture optical density at 730 nm of 0,5 to 1,0 over a 2 day-culture period. In this study it was clear that, after

nitrate and phosphate, one or more trace elements became limiting for long-term growth of *Synechococcus sp.*

A tubular photobioreactor was used for nutrient uptake and growth of the microalgae *Nannochloropsis Sp.* by Cattaneo et al (n.d). The ammonium nitrogen and phosphorus removal from sewage waters by phytoplankton microalgae was studied in a tubular photobioreactor. The system showed, after start up period of about 10 days, an ammonium daily removal yield of 100%, while phosphorus uptake was about 86%. In 6 hours the complete nutrients consumption was obtained with mean rates of 1,5 mg/h and 1 mg/h for $N\text{-}NH_4$ and $P\text{-}PO_4$ respectively. The decreasing of the algal cells number, and consequently of the chlorophyll a concentration, at the end of the experimentation carried to decreasing in the nutrients uptake velocity.

Laliberte, Lessard, Noue & Sylvestre (1997) investigated the effect of phosphorus addition on nutrient removal from wastewater with the cyanobacterium *Phormidium bohneri* in outdoor 24-l triangular bioreactors. Different N/P ratios (1/1, 2/1, 3/1, 6/1) have been tried. For each experiment, after 90h, higher yields and productivities were obtained at the lowest N/P ratios. The addition of phosphates in wastewater increased the biomass production of *P.bohneri* without significantly affecting removal of the inorganic ions ammonium, nitrate and phosphate.

Martinez Sancho, Jimnez Castillo & El Yousfi (1999) studied removal of phosphorus by *Scenedesmus obliguus* in a continuous photobioreactor. The influence of light intensity was analyzed at different rates of dilution, under limited as well as saturating intensities of illumination. According to experimental results, in order to achieve efficient removal of phosphorus from the liquid medium, it is advisable to operate at a high light intensity with the working dilution rate near the critical maximum dilution rate. At all light intensities, conditions of maximum P removal differed from those of maximum biomass productivity; the former being displaced at higher dilution rates. The removal of phosphate from the medium was aided by surface adsorption to the cells and the culture vessel.

Biotreatment of fish farm effluents using the cyanobacterium *Phormidium bohneri* in photobioreactors was investigated by Dumas, Laliberte, Lessard & de la Noue (1998). Three completely mixed 70 lt photobioreactors were used with retention time of 8, 12 and 24 h, respectively. Average ammonia nitrogen removal efficiency from rainbow trout (*Oncorhynchus mykiss*) effluent was 82% and 85% for soluble orthophosphate, over a 1 month period. Rates of ammonia nitrogen removal were independent of the hydraulic loading rate. Thus, the time of retention was not limiting the uptake of ammonia by *P. bohneri*.

Laboratory-scale experiments were performed to develop procedure for biological treatment of recalcitrant anaerobic industrial effluent (from ethanol and citric acid production) by Valderrama, Del Campo, Rodriguez, L.E. de-Bashan & Y. Bashan (2002). Chlorella vulgaris was used as microalgae followed by the macrophyte *Lemna minuscula*. The recalcitrant wastewater was diluted to 10% of the original concentration with wash water. Initially, the ammonium ion concentration in the wastewater was 3-8 mg NH_4-N/lt. After 5 days, the level of ammonium ion removal from the wastewater by *C. vulgaris* ranged from 60% to 78%, with an average of 72%. The initial concentration of phosphorus was 1.5-3.5 mg PO_4^{-3}/lt. the level of phosphorus ion removal from the treated wastewater ranged from 0% to 51%, with an average of 28%. C. vulgaris treatment lowered COD after 5 days of incubation by 61% (from 3100 to 1200 mg/lt). Addition of macrophyte *L. minuscula* to treated wastewater containing a large population of *C. vulgaris* resulted in the dramatic elimination and sedimentation of microalga to the bottom of the reactors.

Sanchez Villasclaras, Martinez Sancho, Espejo Caballero & Delgado Perez studied production of microalgae using olive mill wastewater. The influence of the aeration level and the composition of the culture medium were analyzed in relation to the concentration of alpechin and KNO_3 added in a batch culture of the microalgae *Chlorella pyrenoidosa* and *Scenedesmus obliguus*. The maximum specific growth rate (μ_m) and the biomass productivity (P_{max}) were determined as kinetic parameters. The biomass composition obtained was evaluated for chlorophyll and crude protein

content as well as fatty acid composition corresponding to the lipid fraction. To achieve a balanced biomass composition in terms of proteins and lipids, the most suitable conditions included an alphechin concentration of 10%, without the addition of nitrates and with an aeration level of 1 v/v min. Under these conditions, the μ_m values reached with both microalgae were around 0,03 h-1.

Martinez, Sanocho, Jimnez Castillo and El Yousfi (2000) carried out experimental studies on removal of phosphorus and nitrogen by freshwater alga *Scenedesmus obliguus* under different conditions of stirring and temperature. The working temperatures were chosen, 20°C, 25°C, 30°C, and 35°C. Two levels of mixing were chosen, maximum and minimum allowed by the installation used. That is the maximum was magnetic stirring in addition to air bubbling in the culture medium, which assured complete mixing; the minimum was the absence of magnetic stirring. The cultures were submitted to continuous illumination for the duration of each experiment (8 days), at a mean intensity of 11 334 lux in the interior of the empty reactors. Maximum specific growth rate was obtained in the stirred cultures, the highest μ value being 0,0438 h^{-1} at 30 °C. After exponential growth, stirring increased biomass productivity in the linear growth phase with the optimum appearing at 25 °C. For evaluate the effect of temperature, stirring was not necessary to provide the highest percentage of P elimination ($\%P_{max}$), but did reduce the time needed to reach maximum P removal percentage (t_{max}). The highest $\%P_{max}$ value, 98%, within the shortest time period, t_{max}=94,33 h, was found in the culture with stirring at 25 °C. The highest percentage of ammonium removal ($\%N_{max}$), 100%, resulted at the final culture time (t_f) of 188,33 h, in the stirred culture at 25 °C. N/P ratio of the culture was 12,9.

1.2. Objective and Scope

In the light of the aforementioned studies, it could be clearly stated that algal cultivation is suitable for nutrient removal from wastewaters. Utilization 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 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 batch experiments,
2. to evaluate the effect of light/dark cycle on nutrient removal in batch culture mode,
3. to determine the effect of light intensity on nutrient removal in batch experiments,
4. to determine optimum N/P ratio on nutrient removal in batch experiments,
5. to investigate the nutrient removal performance of *C. vulgaris* at high ChA concentrations.
6. to evaluate the effect of initial nitrogen concentration on nutrient removal in continuously operated immobilized-cell photobioreactor,
7. to determine the minimum hydraulic retention time for effective nutrient removal in 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 13,2-410 mg/lt for NH_4-N and 7,7-199 mg/lt for PO_4-P depending on the experimental conditions.

2.3. Experimental Set-up

2.3.1. Batch Experiments

The batch experiments were conducted by using 1000 ml flasks. At the beginning of each series of experiments, 800 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 daily 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 at least ten days.

2.3.1.1 Experimental Studies

Effect of media composition and environmental conditions on *C. vulgaris* was investigated. NH_4-N concentration was varied between NH_4-N =13,2-410 mg/lt by changing the concentration of NH_4-Cl, which is the main nitrogen source in the synthetic wastewater. The N/P ratio was kept constant at 2/1 in this set of experiment. Light intensity was 4100 lux, initial chlorophyll A concentration was 3 g/lt and L/D cycle was 24/0. Light intensity was gradually increased from 550 to 7250 lux while NH_4-N concentration was kept constant at NH_4-N=40±7 mg/lt in the second set of experiments. L/D cycle, N/P ratio and initial chlorophyll concentration was the same as the first set of experiments. The L/D cycle was varied between 12/12 and 24/0 at light intensity of 7250 lux. N/P ratio was gradually inreased from 2/1 to 10/1. The other conditions were L/D= 20/4, Light intensity=7250 lux, ChA= 3 mg/lt. In the final set of batch experiment, the effect of different initial chlorophyll A concentrations (3 to 6 mg/lt) were tested. Initial NH_4-N concentration, light intensity, L/D cycle and N/P ratio was 40±7 mg/lt, 7250 lux, 20/4 and 3,5/1 ,respectively.

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. Temperature was maintained at 26^{o}C 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 with 7000 lux light intensity, but only 2700 lux light intensity achieved in photobioreactor, N/P =8/1, 20/4 light/dark cycle and 40 mg/lt NH_4-N concentration for 30 days to allow the immobilization of the culture on the support materials. The surface area of support material in the

reactor was around 2,8 m^2. The liquid media was changed every 3 days to provide nutrients.

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 initial NH_4-N and hydraulic retention times. The NH_4-N concentration was varied between 10 mg/lt to 50 mg/lt while light intensity, L/D cycle and N/P ratio was 2700 lux, 20/4 and 8/1 respectively in the first part of the continuous experiments. In the second part, hydraulic retention time of photobioreactor was changed betwwen 1,5 day to 5,5 days at constant NH_4-N concentration of $[NH_4\text{-}N]_o$ =20±3 mg/lt in the second part. The other parameters were adjusted as light intensity=2700 lux, L/D=20/4 and N/P=8/1.

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 after removing algal cells 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 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.

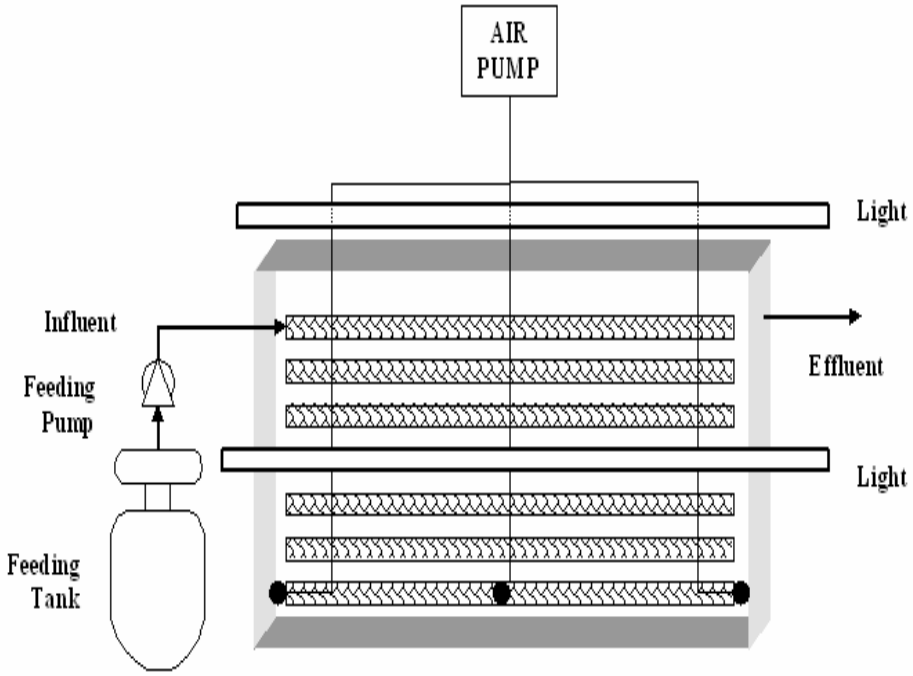


Figure 2.1 Schematic diagram of immobilized photobioreactor

CHAPTER THREE
RESULTS AND DISCUSSIONS

The study contains batch and continuous experiments. In the batch experiment, the effect of media composition and environmental conditions were examined. In continuous experiments, the effects of two operation parameters like hydraulic retention times and initial ammonia nitrogen concentration on nutrient removal performance of *C. vulgaris* in an immobilized photobioreactor system were investigated.

3.1 Batch Experiments

1000 ml flasks were operated batch–wise with synthetic wastewater at different initial NH_4-N, PO_4-P concentrations, light/dark cycle, light intensity, N/P ratio, and ChA concentration in order to investigate the effect of those operating parameters on nutrient removal efficiency. A green alga, *Chlorella vulgaris*, was used as dominant microbial culture in the system.

3.1.1 Effect of Initial NH_4-N Concentration on Nutrient Removal Performance of the Batch System

Initial NH_4-N concentration was gradually increased from around 13,2 mg/lt to 410 mg/lt in the first part of the experiments. Initial light intensity, L/D cycle, N/P ratio and ChA concentration were kept constant as light intensity=4100 lux, L/D cycle=24/0, N/P ratio=2±0,5 and ChA=3,0±0,5 mg/lt, respectively. Biomass concentration was around X_0=0,06 ±0,01 g/ml. NH_4-N, PO_4-P and ChA analyses were carried out on daily samples taken from flasks.

Figure 3.1.a indicates the variation of effluent NH_4-N, PO_4-P and ChA concentration with time at $[NH_4\text{-}N]_o$=13,2 mg/lt, $[PO_4\text{-}P]_o$=7,7 mg/lt initial nutrient concentration and ChA_o=3,2 mg/lt. NH_4-N and PO_4-P concentrations gradually decreased to about $[NH_4\text{-}N]_e$=0,52 mg/lt, $[PO_4\text{-}P]_e$=1,7 mg/lt effluent nutrient concentration, respectively. In addition, the final ChA concentration reached to ChA_e=10,7 mg/lt. As a result of these decreases in the concentrations, 96% for NH_4-N and 77,9% for PO_4-P removal efficiencies were obtained (Figure 3.1.b).

Variation of effluent NH_4-N, PO_4-P concentration and ChA concentration with time at $[NH_4\text{-}N]_o$=21,2 mg/lt, $[PO_4\text{-}P]_o$=15,4 mg/lt initial nutrient and ChA concentration are depicted in Figure 3.2.a. A slow decrease in NH_4-N concentration was observed during the 10 days of batch operation. The effluent ammonia nitrogen concentration decreased to $[NH_4\text{-}N]_e$=5,25 mg/lt resulting in 75 % removal efficiency (Figure 3.2.b). On the other hand, no significant PO_4-P removal was observed and effluent concentration was obtained as 11,4 mg/lt with around 26% removal. The final ChA concentration was ChA_e=8,15 mg/lt at initial ammonia nitrogen concentration of $[NH_4\text{-}N]_o$=21,2 mg/lt.

Figure 3.3.a depicts variation of effluent NH_4-N, PO_4-P and ChA concentration with time at $[NH_4\text{-}N]_o$=41,8 mg/lt, $[PO_4\text{-}P]_o$=21,4 mg/lt and ChA_o=3,06 mg/lt, respectively. As seen from the figure, there was a gradually decrease in NH_4-N ($[NH_4\text{-}N]_e$=17,6 mg/lt) and an increase in ChA concentration (ChA_e=10,75 mg/lt) during the 10 days of batch operation. However, the decrease in PO_4-P was slower ($[PO_4\text{-}P]_e$=10,8 mg/lt) compared to that of NH_4-N concentration. The resulting removal for NH_4-N was 57,9% and 49,5 % for PO_4-P (Figure 3.3.b).

Initial NH_4-N and PO_4-P concentrations were further increased to $[NH_4\text{-}N]_o$=59 mg/lt and $[PO_4\text{-}P]_o$=33 mg/lt. Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at that initial nutrient concentrations is seen in Figure 3.4.a. NH_4-N was slowly removed for the first 3 days. It decreased to 58 mg/lt with 1,7% removal efficiency. After that it slowly reduced to 39,6 at the end of 10 days batch operation. As a result removal efficiency increased to 32,9% (Figure 3.4.b) PO_4-P

was slowly removed for the first 2 days (3,6% removal efficiency). Then it decreased to 26 mg/lt with 21,2% removal efficiency at the end of the 10 days period. The final ChA concentration was obtained as ChA_e=7,15 mg/lt .

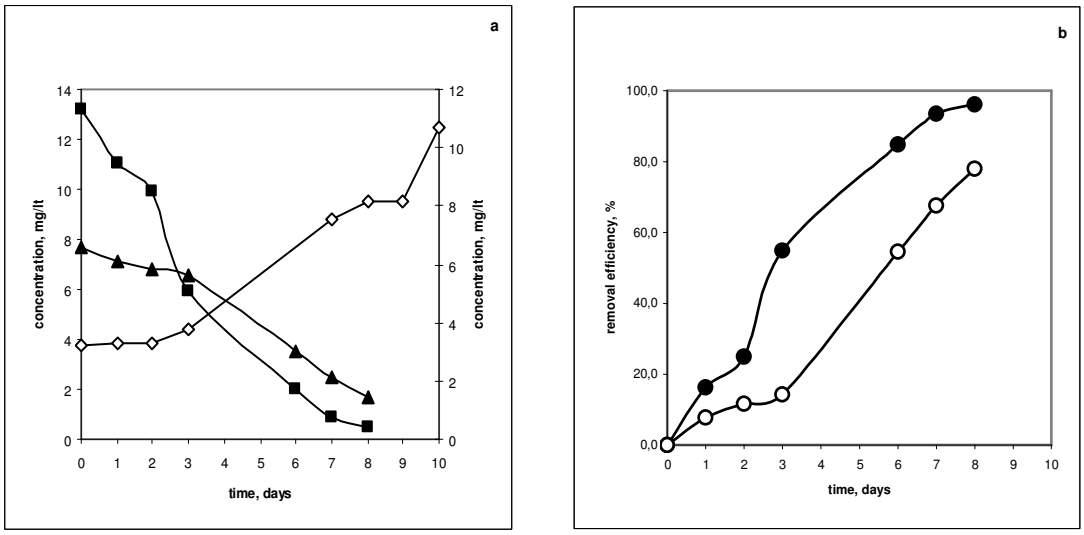


Figure 3.1 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at NH_4-N_o=13,2 mg/lt (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at NH_4-N_o=13,2 mg/lt (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

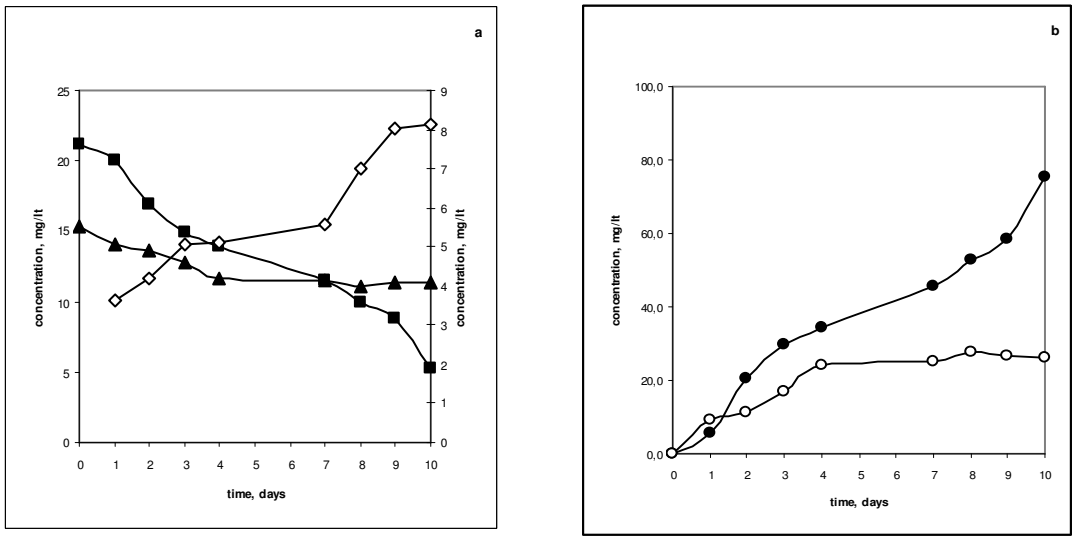


Figure 3.2 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at NH_4-N_o=21,2 mg/lt (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at NH_4-N_o=21,2 mg/lt

(—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

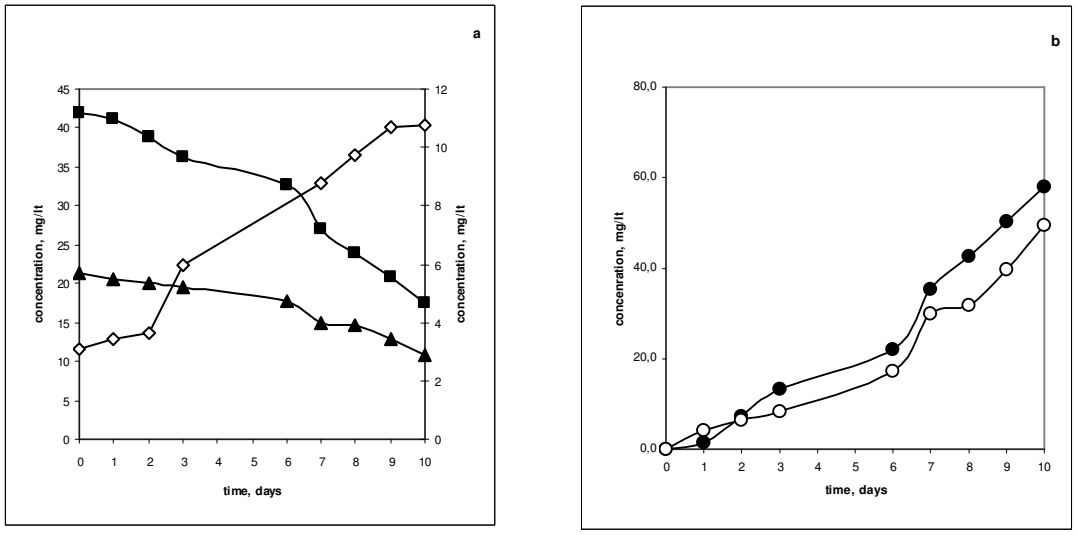


Figure 3.3 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at NH_4-N_o=41,8 mg/lt (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at NH_4-N_o=41,8 mg/lt (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

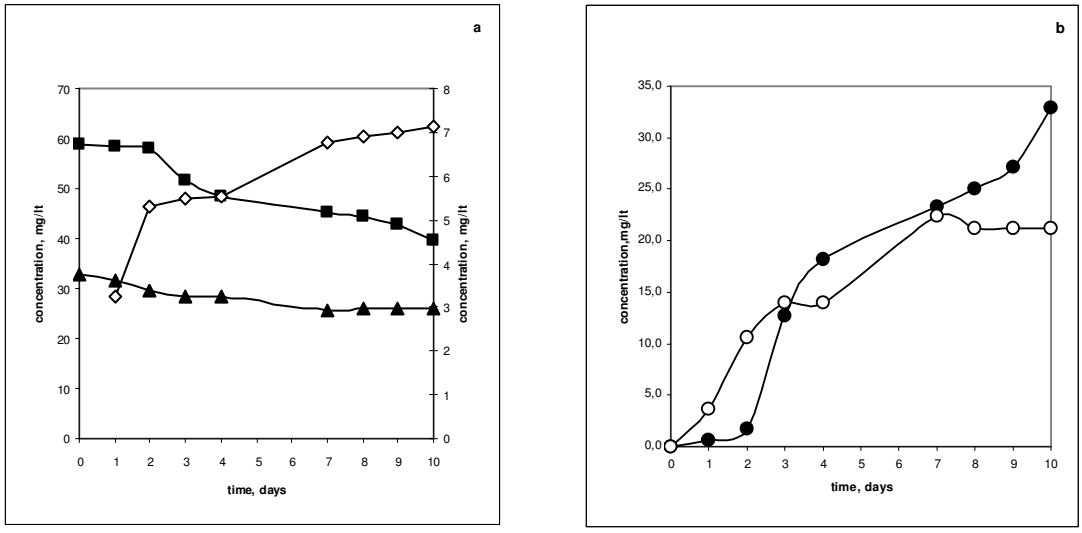


Figure 3.4 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at NH_4-N_o=59 mg/lt (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at NH_4-N_o=59 mg/lt (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

Initial NH_4-N and PO_4-P concentration was adjusted to 74,7 and 41,8 mg/lt, respectively. Figure 3.5.a indicates the variation of effluent NH_4-N, PO_4-P and ChA concentration with time and Figure 3.5.b shows the variation of removal efficiency for NH_4-N and PO_4-P with time. No significant removal in NH_4-N concentration was observed until 3rd days of operation. NH_4-N dropped to about 46 mg/lt with 38,4% NH_4-N removal efficiency between the 3rd and 10th day of operation period. Similarly, there was no significant removal for PO_4-P until 3rd day. After that, PO_4-P decreased slowly to 32,6 mg/lt with 22% removal efficiency. ChA concentration gradually increased to 11,9 mg/lt when operation period was completed.

Figure 3.6.a and Figure 3.6.b indicates the variation of effluent NH_4-N, PO_4-P, ChA concentration and NH_4-N, PO_4-P removal efficiency with time, respectively at $[NH_4\text{-}N]_o$ =92,8 mg/lt. The concentration decreased to $[NH_4\text{-}N]_e$=59,1 mg/lt with 36,3% removal efficiency. There was no significant PO_4-P removal during 10 days operation period. PO_4-P decreased from $[PO_4\text{-}P]_o$=50 mg/lt to $[PO_4\text{-}P]_e$=41,6 mg/lt with 16,8% removal efficiency. The final ChA concentration was ChA_e=11,9 mg/lt .

Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at $[NH_4\text{-}N]_o$=130 mg/lt, $[PO_4\text{-}P]_o$=60,4 mg/lt and ChA_o=3,1 mg/lt initial concentration are depicted in Figure 3.7.a. ChA concentration increased to about ChA_e=12,14 mg/lt. No significant NH_4-N and PO_4-P removal was observed during the operation period. Effluent NH_4-N decreased to $[NH_4\text{-}N]_e$=98,4 mg/lt with 24,1% removal efficiency. Similarly PO_4-P decreased to $[PO_4\text{-}P]_e$=49,2 mg/lt with 18,54% removal efficiency (Figure 3.7.b).

Figure 3.8.a depicts variation of effluent NH_4-N, PO_4-P and ChA concentration with time at $[NH_4\text{-}N]_o$= 149,3 mg/lt, $[PO_4\text{-}P]_o$=71,6 and ChA_o=3,05 mg/lt initial concentration. As seen from the figure there was almost no nutrient removal (NH_4-N=142,8 mg/lt, PO_4-P=68,8 mg/lt) for the first 4 days of batch operation. After 4 days, effluent NH_4-N decreased to $[NH_4\text{-}N]_e$=110,6 mg/lt, PO_4-P decreased from PO_4-P=68,8 to $[PO_4\text{-}P]_e$=56,4 mg/lt. As a result, 25,9% NH_4-N and 21,2% PO_4-P removal efficiencies were achieved at the end of the 10 days period (Figure 3.8.b).

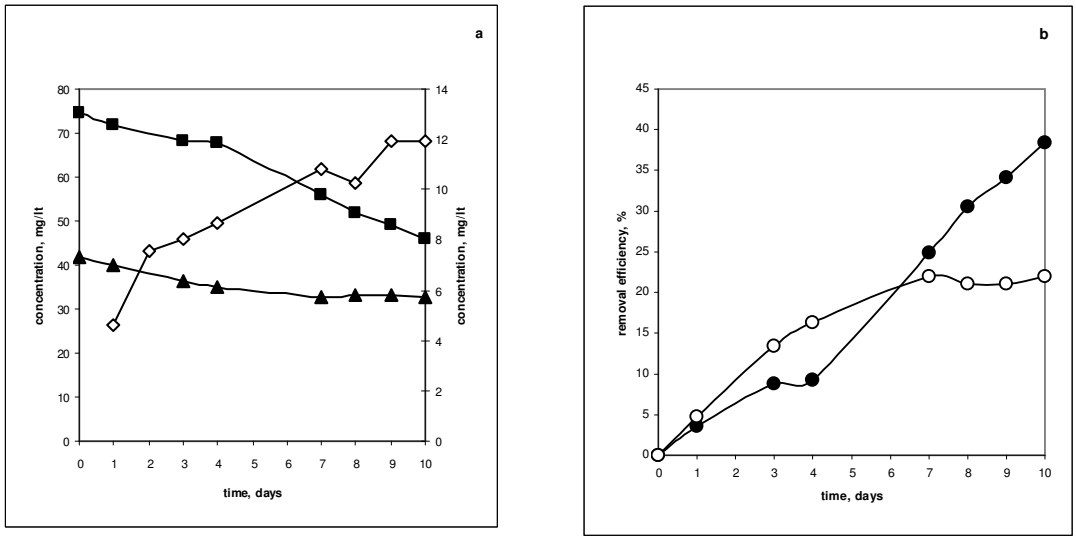


Figure 3.5 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at NH_4-N_o=74,7 mg/lt (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at NH_4-N_o=70 mg/lt (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

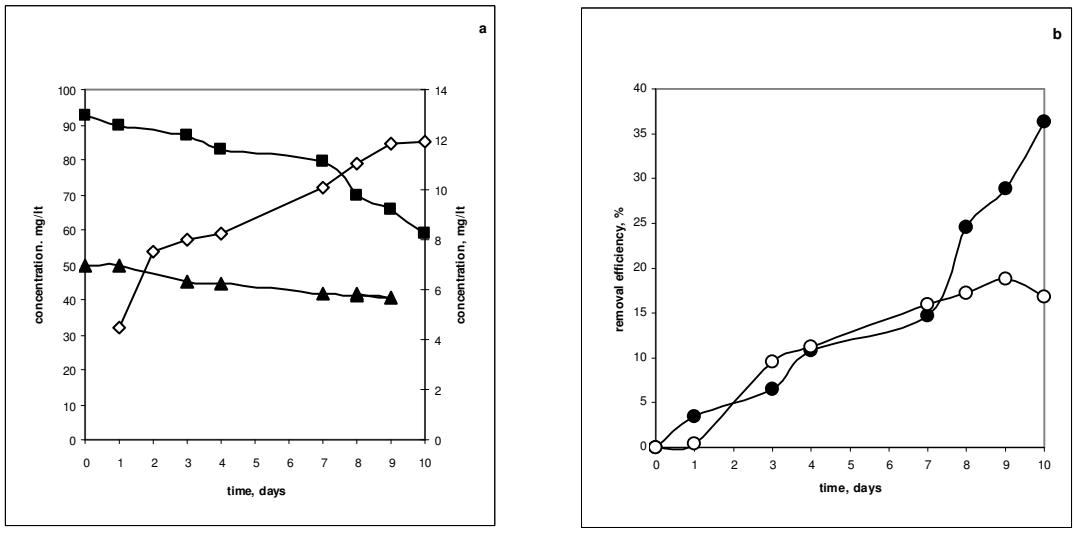


Figure 3.6 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at NH_4-N_o=92,8 mg/lt (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at NH_4-N_o=90 mg/lt (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

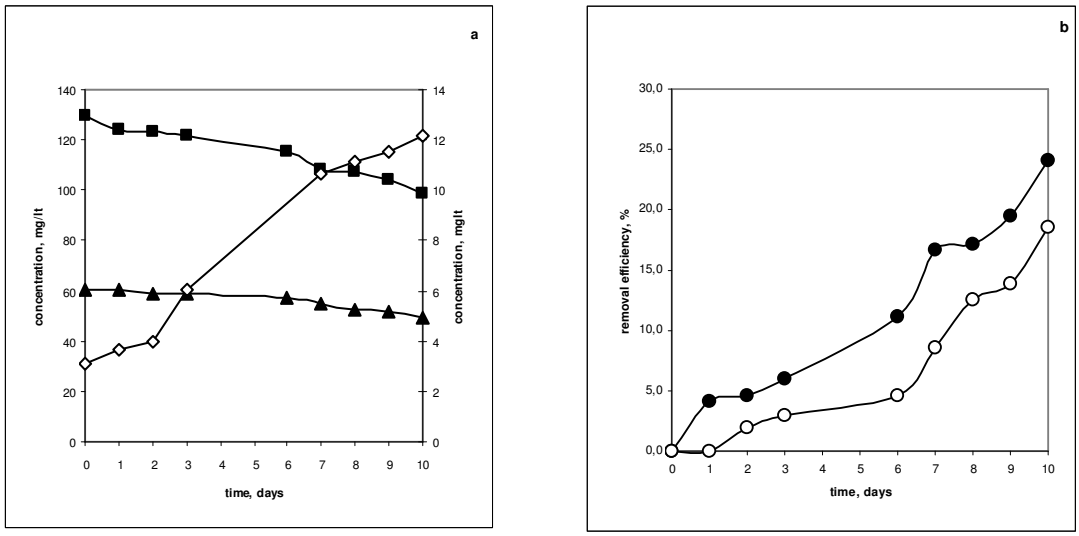


Figure 3.7 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at NH_4-N_o=130 mg/lt (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at NH_4-N_o=130 mg/lt (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

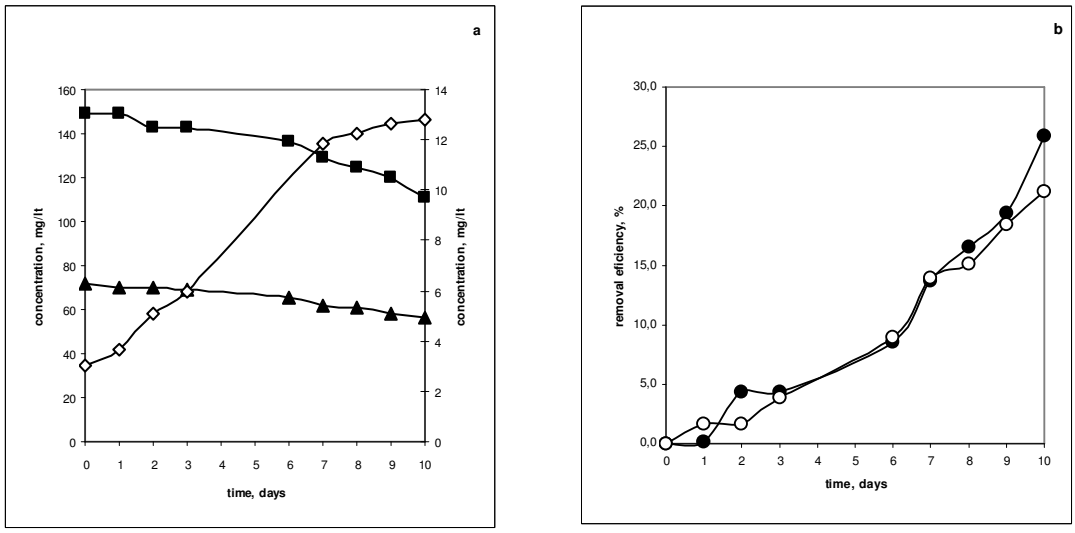


Figure 3.8 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at NH_4-N_o=149,3 mg/lt (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at NH_4-N_o=150 mg/lt (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

Initial NH_4-N and PO_4-P concentration was further increased to $[NH_4\text{-}N]_o$=192 mg/lt, $[PO_4\text{-}P]_o$=93,5 mg/lt, respectively. No significant nutrient removal was observed during the operation period for both nutrients. The final NH_4-N and PO_4-P concentrations were $[NH_4\text{-}N]_e$=152 mg/lt $[PO_4\text{-}P]_e$=79 mg/lt, respectively (Figure 3.9.a). Furthermore, ChA concentration increased from 3,1 mg/lt to ChA_e=12,88 mg/lt. As seen in Figure 3.9.b, NH_4-N, PO_4-P removal efficiency was 20,8% and 15,5% at the end of the 10 days.

In order to investigate the effect of higher initial nutrient concentrations on system performance, initial NH_4-N and PO_4-P concentration was adjusted to $[NH_4\text{-}N]_o$=282 mg/lt, $[PO_4\text{-}P]_o$=139 mg/lt. Figure 3.10.a indicates the variation of effluent NH_4-N, PO_4-P and ChA concentration with time. There was a very slow decrease in NH_4-N ($[NH_4\text{-}N]_e$=196 mg/lt) and PO_4-P ($[PO_4\text{-}P]_e$=118 mg/lt) concentration throughout the operation period. However, ChA concentration significantly increased from ChA_o=4,38 mg/lt to ChA_e=23,08 mg/lt. The resulting removal efficiencies were 30,5% for NH_4-N and 15,1% for PO_4-P removal as shown in Figure 3.10.b.

The batch experiments were conducted with $[NH_4\text{-}N]_o$=316 mg/lt, $[PO_4\text{-}P]_o$=149 mg/lt and ChA_o=3,87 mg/lt. During the operation time, NH_4-N concentration decreased to $[NH_4\text{-}N]_e$=237 mg/lt and final PO_4-P concentration was obtained as $[PO_4\text{-}P]_e$=140 mg/lt (Figure 3.11.a). As a result the removal efficiencies were 25% and 6% for NH_4-N and PO_4-P, respectively (Figure 3.11.b). On the other hand, ChA concentration reached to ChA_e=18,28 mg/lt at the end of 10 days.

Initial NH_4-N and PO_4-P further increased to $[NH_4\text{-}N]_o$=388 mg/lt, $[PO_4\text{-}P]_o$=175 mg/lt. Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at that initial concentrations are seen in Figure 3.12.a. NH_4-N was slowly removed for the first 4 days. It decreased to 330 mg/lt with 14,9% removal efficiency. After 4^{th} day it decreased to $[NH_4\text{-}N]_e$=265 mg/lt. No significant PO_4-P removal ($[PO_4\text{-}P]_e$=161 mg/lt) was observed during the 10 days period. ChA concentration reached to ChA_e=23,29 mg/lt at 10^{th} day of the operation. As it is given in Figure 3.12.b, the

resulting removal efficiency for NH_4-N was 31,7% and 8% for PO_4-P was at the end of the period.

The highest initial NH_4-N and PO_4-P concentration examined in this set of experiment was $[NH_4\text{-}N]_o$=410 mg/lt, $[PO_4\text{-}P]_o$=199 mg/lt, respectively. As seen from Figure 3.13.a, removal performance of the system significantly reduced at high initial nutrient concentration. After 2nd day of batch operation NH_4-N was slowly decreased to $[NH_4\text{-}N]_e$=298 mg/lt which resulted in 27,3% removal efficiency (Figure 3.13 b) At the end of the experiment PO_4-P concentration reduced to $[PO_4\text{-}P]_e$=182 mg/lt with 8,5% removal efficiency.

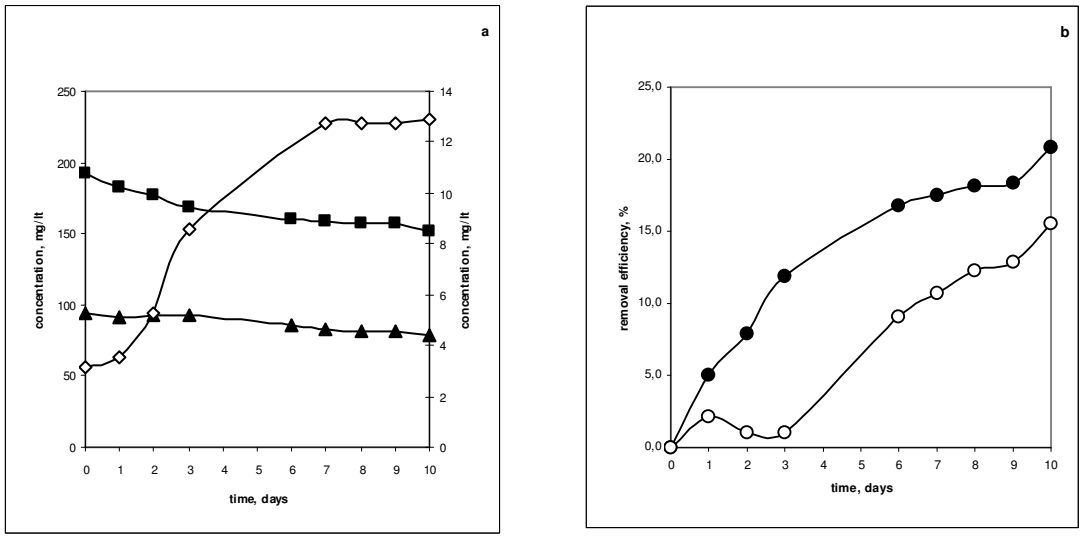


Figure 3.9 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at NH_4-N_o=192 mg/lt (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at NH_4-N_o=190 mg/lt (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

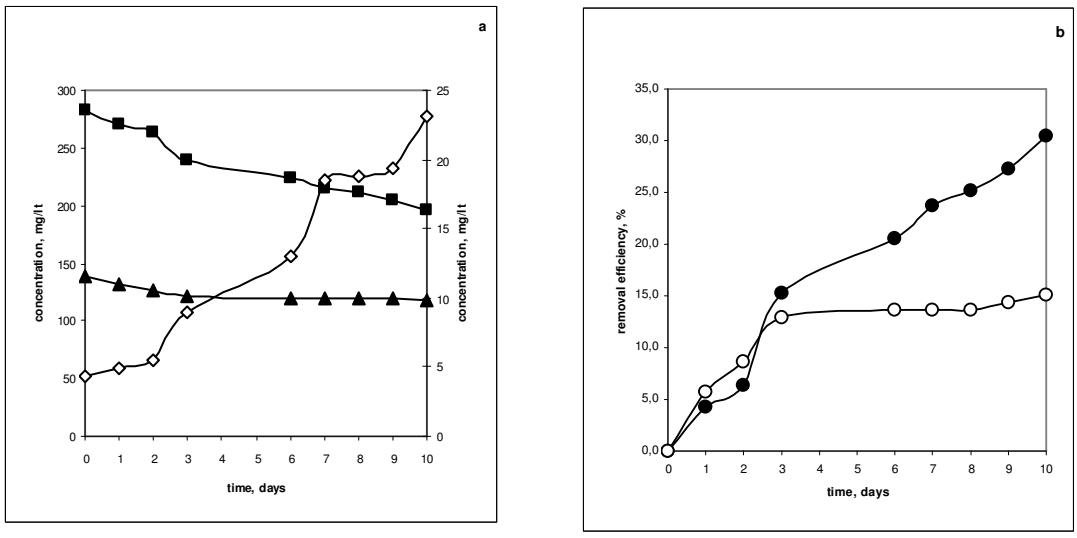


Figure 3.10 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at NH_4-N_o=282 mg/lt (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at NH_4-N_o=280 mg/lt (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

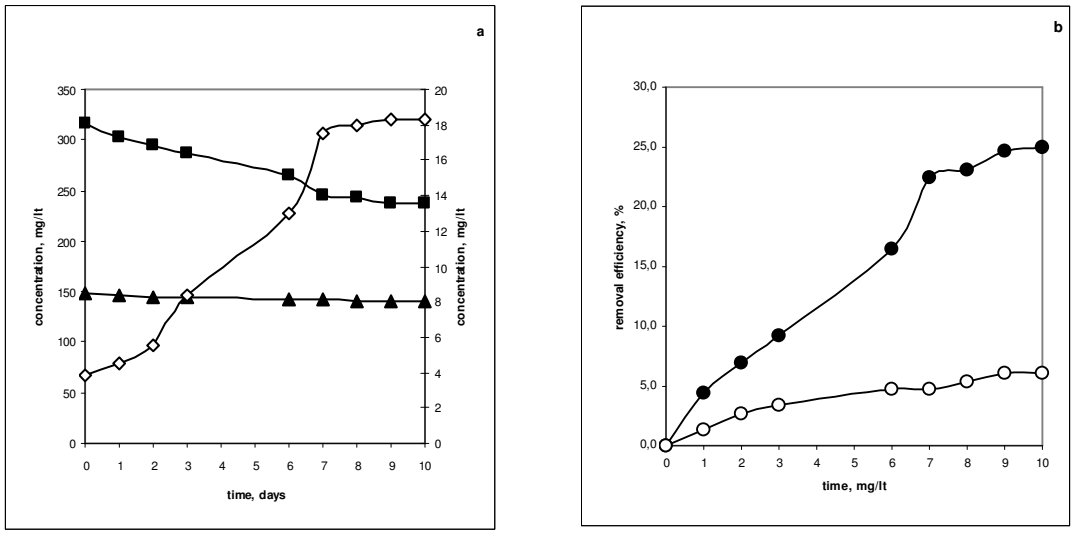


Figure 3.11 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at NH_4-N_o=316 mg/lt (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at NH_4-N_o=316 mg/lt (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

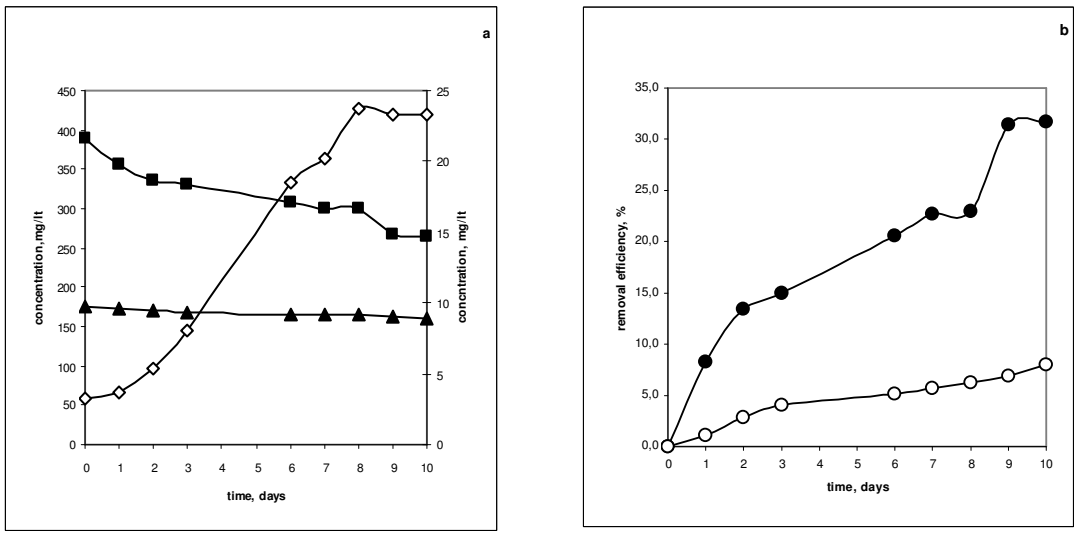


Figure 3.12 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at NH_4-N_o=388 mg/lt (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at NH_4-N_o=388 mg/lt (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

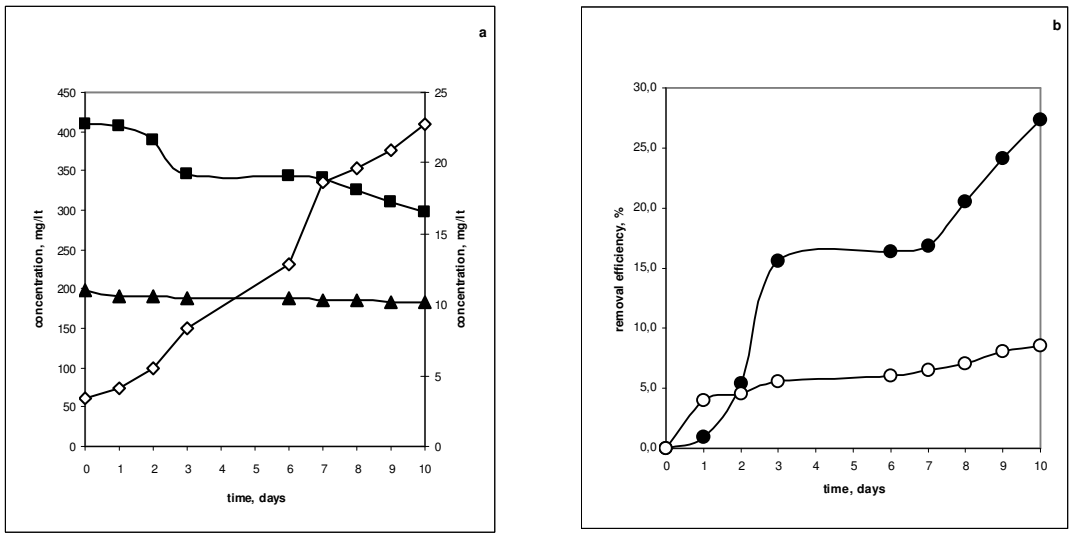


Figure 3.13 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at NH_4-N_o=410 mg/lt (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at NH_4-N_o=410 mg/lt (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

Figure 3.14 summarizes the effect of initial NH_4-N and PO_4-P concentration on effluent NH_4-N and PO_4-P concentration and nutrient removal efficiency. Effluent NH_4-N concentration increases from 0,52 mg/lt to 298 mg/lt with respect to increase in initial NH_4-N concentrations from 13,2 mg/lt to 410 mg/lt as expected. Depending on variation in effluent NH_4-N, removal efficiency decreases from 96,1% to 27,3%, with increasing initial nitrogen concentration. These results indicate that, microalgae *Chlorella vulgaris* can efficiently remove low initial NH_4-N concentrations (NH_4-N≤40 mg/lt) with NH_4-N removal efficiency higher than 57,9% at light intensity=4100 lux, L/D cycle=24/0, N/P ratio=2/1 and initial ChA concentration=3,0±0,5 mg/lt. However, higher concentrations significantly affect NH_4-N removal performance of the batch experiments resulting in decreasing in removal efficiency to around 25%. It can be concluded that *Chlorella vulgaris* containing batch reactor should be operated with ≤22 mg/lt initial NH_4-N concentration in order to obtain higher than ≥75,0% removal efficiency. However, 57,9% removal efficiency around 40 mg/lt initial NH_4-N concentration can be evaluated as high NH_4-N removal performance for the batch conditions. Since, pH was kept constant around pH=7, no significant ammonia removal was observed in control experiments.

Similarly, no efficient phosphate removal was observed by algae. Figure 3.15 indicates the effect of initial PO_4-P concentration on phosphate removal performance of *C. vulgaris*. Effluent phosphate concentration was around 1,7 mg/lt with 78% removal efficiency for $[PO_4\text{-}P]_o$=7,7 mg/lt. However, higher concentrations resulted in mostly less than 30% removal. Although nitrogen and phosphorus uptake by algae did not provide high removal of these nutrients from the synthetic media, final ChA content of the culture significantly increased from 10,67 mg/lt to 27,30 mg/lt with the increase in $[NH_4\text{-}N]_o$= 13,2 mg/lt to $[NH_4\text{-}N]_o$=410 mg/lt or in from $[PO_4\text{-}P]_o$=7,7 mg/lt to 298 mg/lt (Figure 3.16) .

These results indicate that *C .vulgaris* can only be used to remove low concentrations of nutrients such as $[NH_4\text{-}N]_o$<22 mg/lt and $[PO_4\text{-}P]_o$ < 7,7 mg/lt. High concentrations of these nutrients brought about more ChA content which did

not enhance the nutrient removal. Valderrama, Del Campo, Rodriguez, De-Bashan & Bashan, (2002) obtained an average of 72% ammonia nitrogen and 28% phosphorus removal by *C. vulgaris* from ethanol and citric acid production effluent which contained 3-8 mg/lt NH_4-N and 1,5-3,5 mg/lt PO_4-P. Removal of ammonia by immobilized *C. vulgaris* in alginate beads under batch condition provided 86% removal for 3 mg/lt initial concentration; on the contrary no phosphorus removal was obtained (De-Bashan et al., 2002). However, co-immobilization of *C. vulgaris* with growth promoting bacterium *Azospirillum brasilense* enhanced both ammonia and phosphorus removal (De-Bashan et al., 2002, 2004). Phosphorus removal from agroindustrial wastewater with total phosphorus concentration of 111 mg/lt by batch culture of *C.vulgaris* and *Scenedesmus dimorphus* was around 20% after 144 h incubation and increased to 50% after 216 batch operation (Gonzales et al., 1997). Microalgae *Chlorella kessleri* was able to removal only 8-20% phosphate under light/dark cycle for $[PO_4\text{-}P]_o$=10 mg/lt (Lee and Lee, 2001).

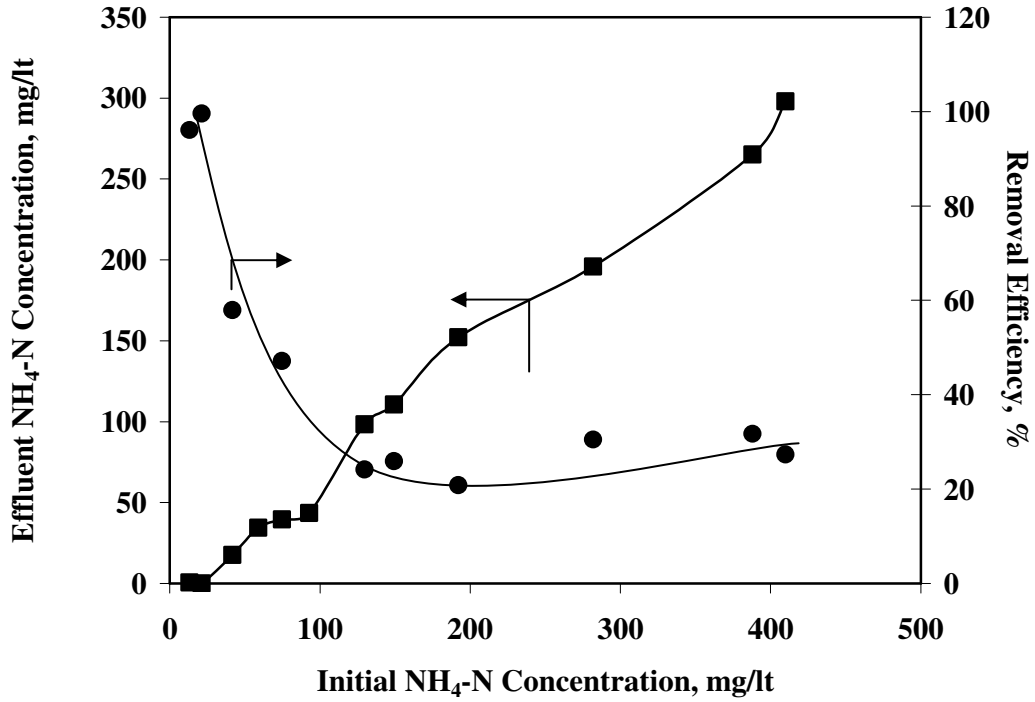


Figure 3.14 Effect of initial NH_4-N concentration on effluent NH_4-N concentration and percent removal (—■— Effluent NH_4-N concentration, —●— NH_4-N removal efficiency)

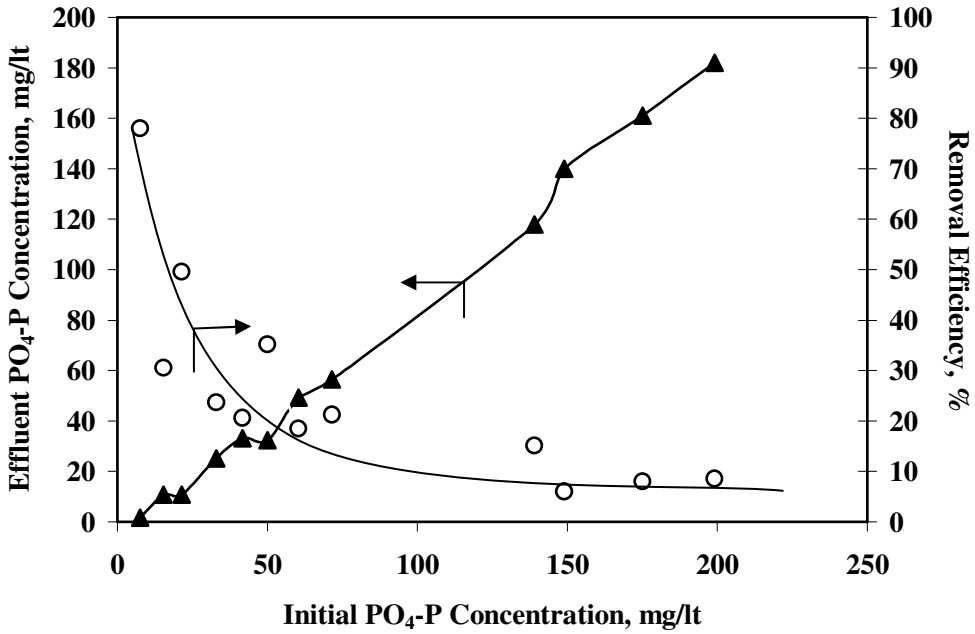


Figure 3.15 Variation of effluent PO_4-P concentration and percent removal with initial PO_4-P
(—▲— Effluent PO_4-Pconcentration, —O— PO_4-P removal efficiency)

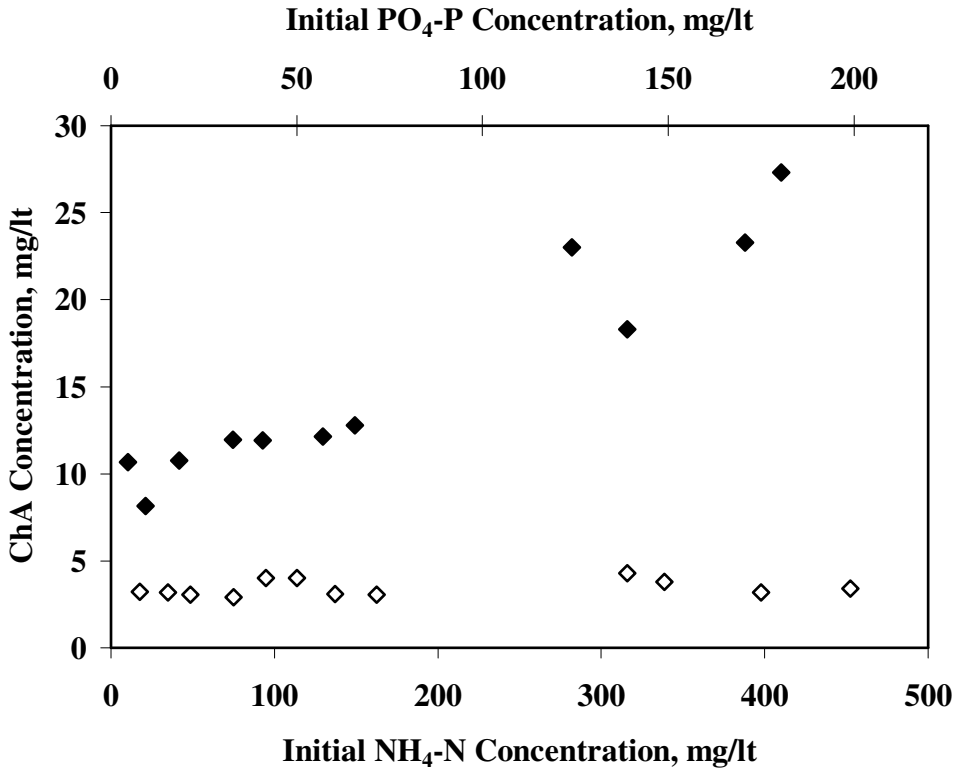


Figure 3.16 Effect of NH_4-N and PO_4-P concentration on chlorophyll A content (—◆— Final ChA concentration, —◊— Initial ChA concentration)

3.1.2. Determination of Batch Kinetic Coefficients

In batch kinetics, initial substrate removal rates, R_i, are used to determine the coefficients. Initial substrate removal rate is calculated as given in equation 1;

$$R_i = - (S_{0+} S_t)/(t_o - t_t) \qquad \text{eq. 1.}$$

where, R_i represents initial rate, S_0 is initial substrate concentrations as NH_4-N or PO_4-P, S_t is the final corresponding substrate concentration at time "t". The first 3 days (t_3) of effluent NH_4-N and PO_4-P concentration were considered in this study to calculate the initial substrate removal rates at different substrate concentrations. The slope of time versus effluent concentration at time "t_3" gives the initial substrate removal rate. Specific rate of substrate removal (R_{xi}) was determined by dividing the initial rates to biomass concentration at the start up of the experiments [$R_{xi}= R_i/[X]_o$]. Biomass concentration was determined from the ChA concentration assuming that ChA content is 1,5% of the biomass (Becker, E. W., 1994).

Initial NH_4-N removal rate for different NH_4-N concentration is depicted in Figure 3.17. The rate was almost constant around 10 mg NH_4-N/ g X d for the initial concentrations between $[NH_4\text{-}N]_o$= 13,2 mg/lt- 129,6 mg/lt. The rate increased to 15,87 mg NH_4-N/ g X d for $[NH_4\text{-}N]_o$= 149 mg/lt. The highest rate was obtained as 90,86 mg NH_4-N/ g X d about 410 mg/lt initial NH_4-N concentrations. Initial rates for PO_4-P removal was calculated by using eq 1 and the results were given in Figure 3.18. The rate increased from 1,88 mg /g X d to around 7,69 mg/g X d for the PO_4-P concentrations between 7,7 mg /lt and 149 mg/lt. At higher concentrations it slightly decreased to around 7,14 mg/g X d.

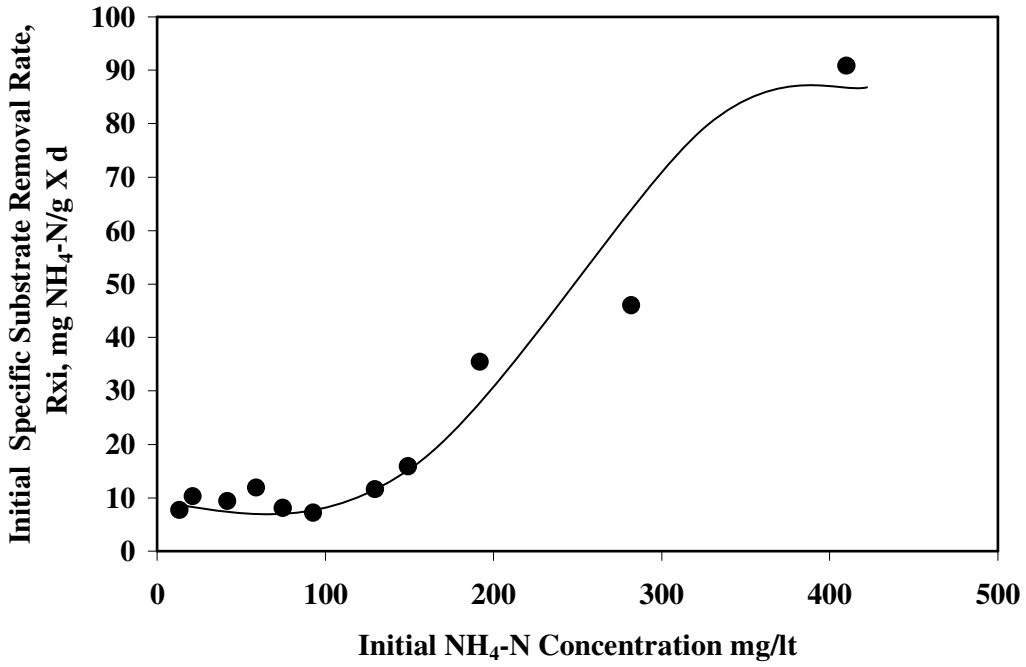


Figure 3.17 Variation of initial specific NH_4-N removal rate with initial NH_4-N concentration

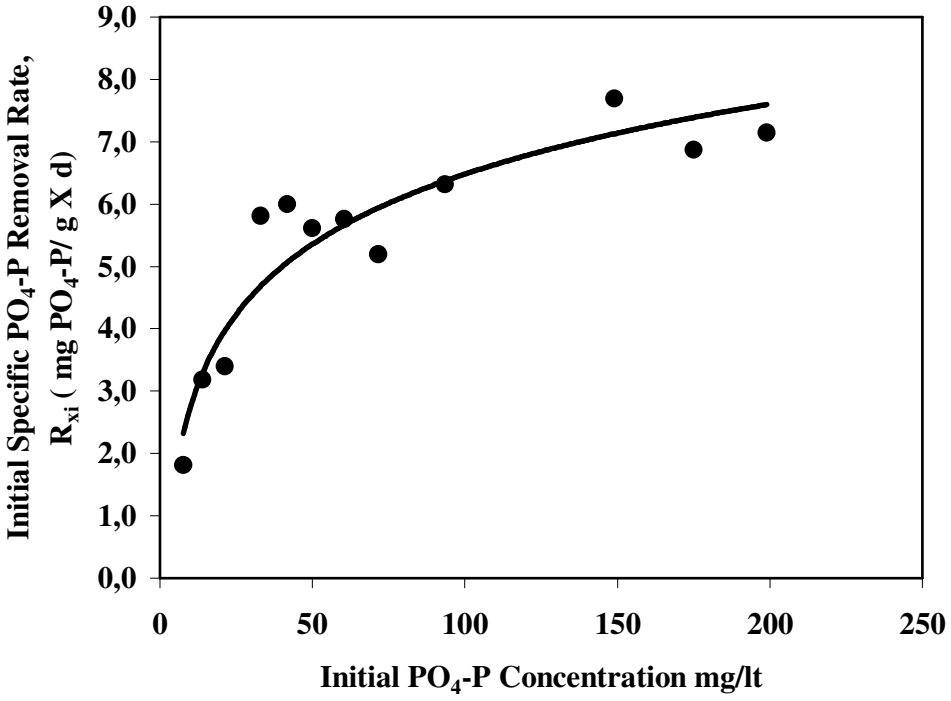


Figure 3.18 Effect of initial PO_4-P concentration on specific PO_4- P removal rate.

Michaelis-Menten kinetic relationship was used in determination of kinetic coefficients, K_m, saturation constant, k, and reaction rate constant. The substrate concentration corresponds to half reaction rate gives the saturation constant. But accurate determination of K_m from this plot is very difficult. Therefore, general Michaelis Menten saturation kinetic equation is used (eq 2)

$$R = \frac{R_{max}\, S}{K_m + S} \qquad \text{eq.2}$$

where, R_{max} is maximum substrate removal rate, S is the effluent substrate concentration. Since initial substrate concentrations and initial substrate removal rates are considered in batch operation, the eq 2 takes the following form;

$$R_{so} = \frac{R_{max}\, S_o}{K_m + S_o} \qquad \text{eq.3}$$

Since $R_{max} = k.X_o$, equation 3 can be written as;

$$R_{so} = \frac{k\, X_o S_o}{K_m + S_o} \qquad \text{eq.4}$$

where, “k” is the reaction rate constant (time $^{-1}$), X_o is the initial biomass concentration or ChA content for algae (~3 mg/lt). Specific rate of substrate removal (R_{xi}) can be calculated by dividing both term of equation 4 to initial biomass concentration as in eq 5.

$$R_{xi} = \frac{R_{so}}{X_o} = \frac{k\, S_o}{K_m + S_o} \qquad \text{eq.5}$$

Equation 5 can be linearized in double reciprocal form as in equation 6 and a plot of $1/R_{xi}$ versus $1/S_o$ yields a linear line with a slope of K_m/k and y-axis intercept of 1/k.

$$\frac{1}{R_{xi}} = \frac{1}{k} + \frac{K_m}{k}\frac{1}{S_o} \qquad \text{eq.6}$$

Experimental data given in Figure 3.17 were plotted in form of $1/R_{xi}$ versus $1/S_o$ as shown in Figure 3.19. From the slope and intercept of best fit line of this plot, kinetic coefficients of ammonia nitrogen removal by *C. vulgaris* were determined as k= 87,5 mg NH_4-N/g X d and K_m= 367 mg/lt. Similarly, best fit line of $1/R_{xi}$ versus $1/S_o$ plot for PO_4-P is given in Figure 3.20 and kinetic coefficients for PO_4-P removal by *C. vulgaris* were found as k= 8,6 mg PO_4-P/g X d and K_m= 28 mg/lt from intercept and the slope best fit line of this plot, respectively.

Yield coefficient for NH_4-N and PO_4-P removal was calculated by using equation 7 and 8 respectively;

$$(X)_f-(X)_i= Y_N\,[(NH_4\text{-}N)_o - (NH_4\text{-}N)_e] \qquad \text{eq. 7}$$

or

$$(X)_f-(X)_i= Y_P\,[(PO_4\text{-}P)_o - (PO_4\text{-}P)_e] \qquad \text{eq. 8}$$

where $(X)_f$ is the final biomass concentration (g/lt) after 10 days of batch operation, $(X)_i$ is the initial biomass concentration (g/lt) at the beginning of the experiments, $[NH_4\text{-}N]_o$ and $[NH_4\text{-}N]_e$ are initial and final NH_4-N concentrations (g/lt), respectively. Slop of $[(X)_f-(X)_i]$ versus $[(NH_4\text{-}N)_o - (NH_4\text{-}N)_e]$ plot gives Y_N (g X/g NH_4-N) which is yield coefficient for NH_4-N and it was determined as Y_N =13,7 (g X/g NH_4-N) by using eq 7 as given in Figure 3.21. The same calculation (eq 8) to determine yield coefficient for PO_4-P removal resulted in Y_P= 52,3 g X/g PO_4-P. (Figure.3.22)

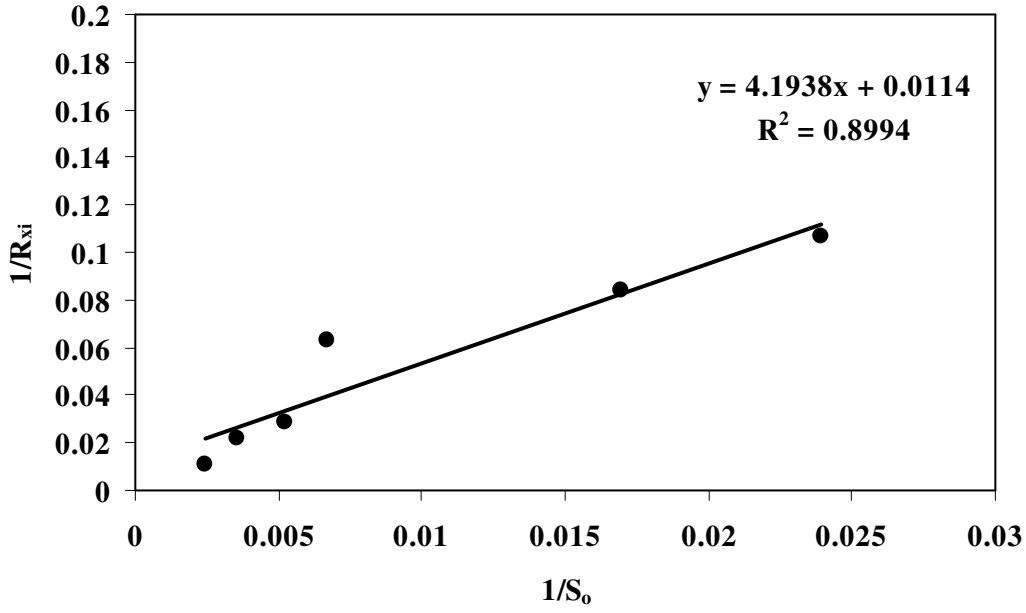


Figure 3.19 Determination of kinetic coefficients, Km and k, for NH_4-N removal

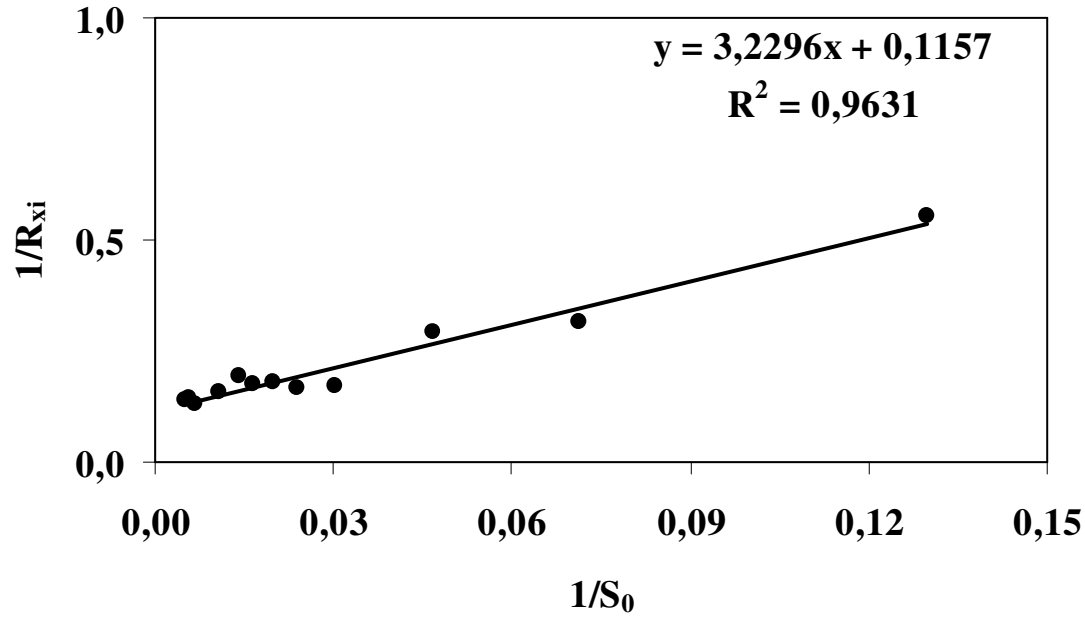


Figure 3.20 Double reciprocal plot of initial specific phosphate removal rate versus phosphate concentration to determine of kinetic coefficients for PO_4-P removal by *C. vulgaris*.

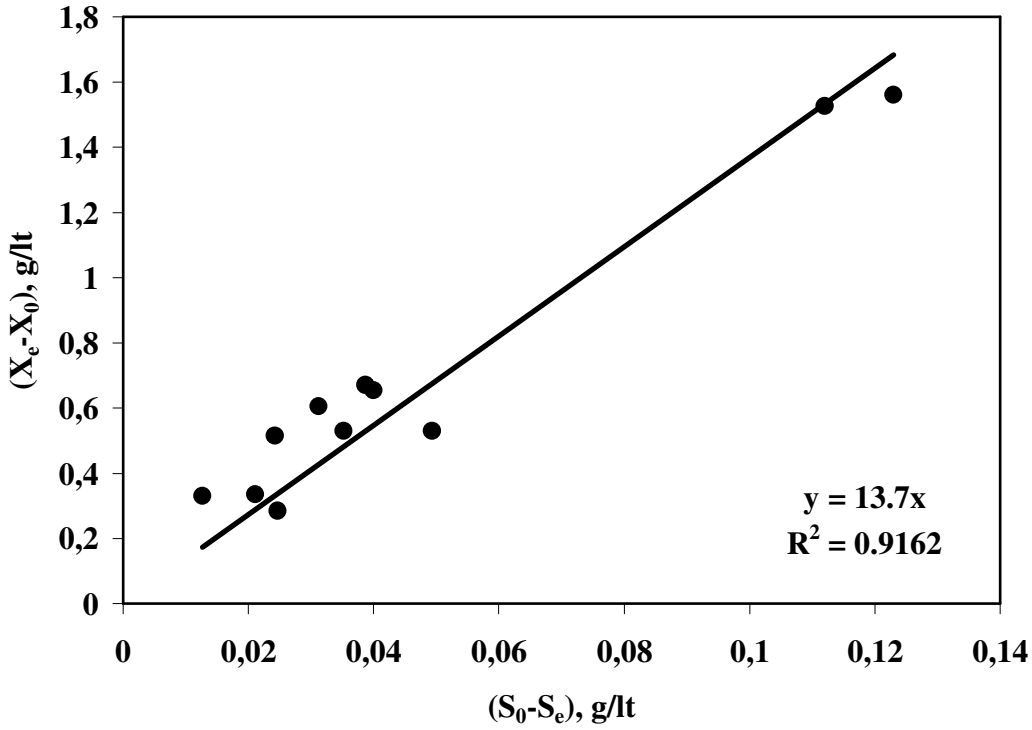


Figure 3.21 Determination of yield coefficient for NH_4-N removal by *C. vulgaris*

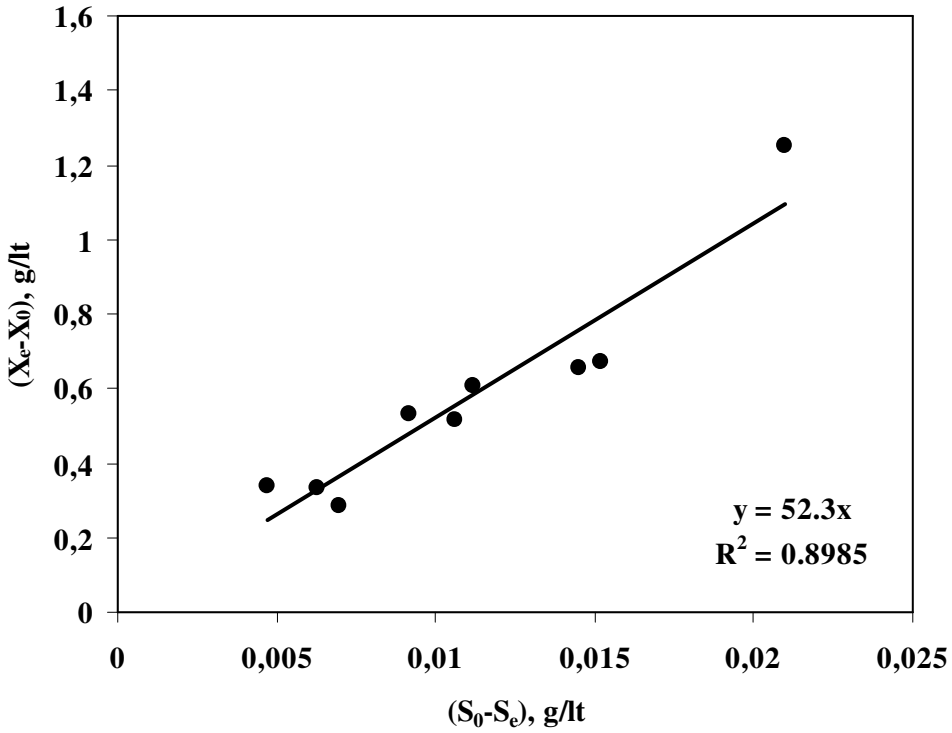


Figure 3.22 Determination of yield coefficient for PO_4-P removal by *C. vulgaris*

3.1.3. Effect of Light/Dark Cycle on Nutrient Removal Performance of the *Chlorella Vulgaris* in Batch Operation

Continuous illumination of algae for nutrient removal from wastewater is not economically feasible and naturally possible to apply in full scale. Therefore, minimum light (illumination) or dark period (no illumination) requirement of *C. vulgaris* for effective nitrogen and phosphorus removal were investigated in this set of experiment. The total cycle was 24 h with increasing light period from 12 h to 24 h (L/D=12 h/ 12h-24 h/0 h). Biomass concentration was X_o= 0,14±0,02 mg/lt, N/P ratio was adjusted to 2/1 and initial NH_4-N concentration was 41 ±2 mg/lt, light intensity and ChA concentration was kept constant at 4100 lux and 3±0,5 mg/lt, respectively.

Variation of effluent NH_4-N, PO_4-P, ChA and NH_4-N, PO_4-P removal efficiencies with time at 12h/12h L/D cycle are indicated in Figure 3.23.a and Figure 3.23.b, respectively. Initial NH_4-N, PO_4-P and ChA were $[NH_4\text{-}N]_o$=39,4 mg/lt, $[PO_4\text{-}P]_o$=19,4 mg/lt and ChA_o=2,74 mg/lt, respectively. ChA concentration reached to 10,33 mg/lt on the 10^{th} day of batch operation. For the first 3 days, no significant NH_4-N removal efficiency was observed. After 3 days NH_4-N decreased gradually to 18,2 mg/lt with 53,8% removal efficiency. PO_4-P decreased from 19,4 mg/lt to 12,8 mg/lt with 34,02% removal efficiency at the end of batch experiment.

Figure 3.24.a, b depicts variation of effluent NH_4-N, PO_4-P, ChA concentrations and NH_4-N, PO_4-P removal efficiency with time at 18h/6h L/D cycle, respectively. Initial nutrient concentrations were $[NH_4\text{-}N]_o$=43,5 mg/lt, and $[PO_4\text{-}P]_o$=19,4 mg/lt. ChA concentration was ChA_o=2,57 mg/lt at the beginning of the experiment, which then reached to ChA_e=9,63 mg/lt at the end of experiment. Removal of NH_4-N became stable at about 40% removal efficiency between 6^{th}-8^{th} days of operation, however on the last day the concentration reduced to 19,1 mg/lt with 56% removal efficiency. PO_4-P removal was 34% with $[PO_4\text{-}P]_e$=12,8 mg/lt.

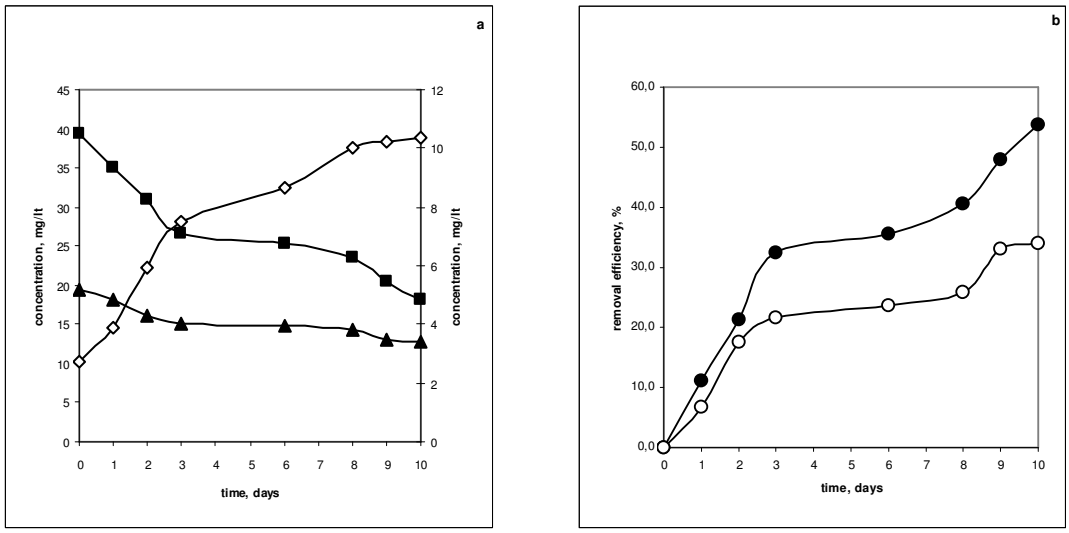


Figure 3.23 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at light /dark cycle=12h/12h (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at light /dark cycle=12h/12h (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

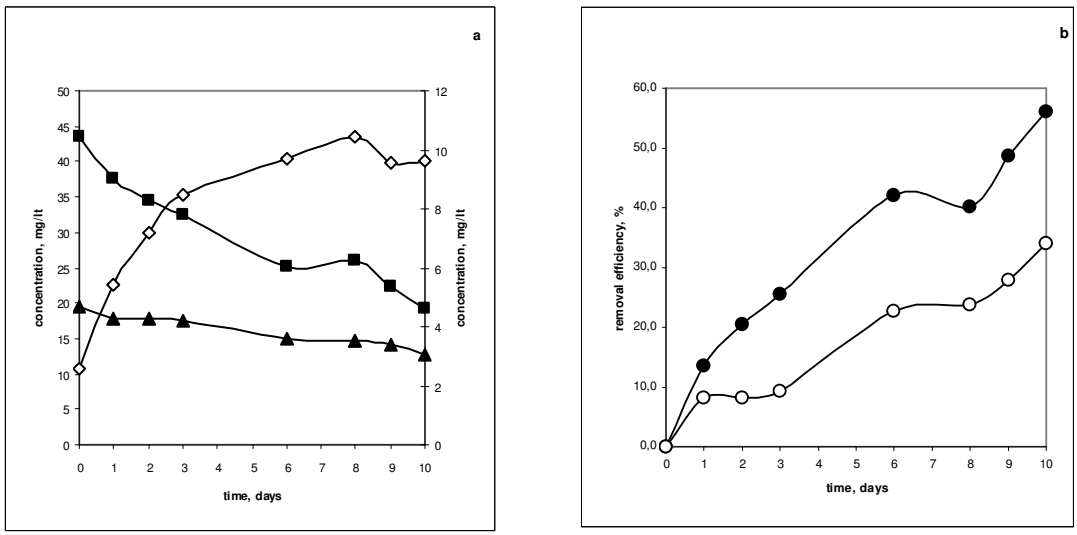


Figure 3.24 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at light /dark cycle=18h/6h (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at light /dark cycle=18h/6h. (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

The L/D cycle was adjusted to L/D=20h/4 h. The variation of effluent nutrient and ChA are depicted in Figure 3.17.a. No significant increase in ChA concentration was obtained at 20 hours of light exposure period. Final ChA concentration was ChA_e=12,17 mg/lt which was almost the same as other L/D cycles. As seen in Figure 3.25.b, there was only 28,4% nitrogen removal efficiency for the first 3 batch operation days. However, effluent NH_4-N decreased from 33,8 mg/lt to 10 mg/lt with 78,8% removal efficiency at the end of the 10^{th} day of the experiment. Effluent PO_4-P decreased gradually from 22 mg/lt to 8,6 mg/lt resulting in 60,9% which was the highest percent removal in phosphate by *C. vulgaris*.

Finally, exposure period to light was increased to 24h without any dark period. In other words, continues illumination was provided. Figure 3.26.a, b indicates the variation of effluent NH_4-N, PO_4-P, ChA concentrations and nutrient removal efficiencies with time at L/D=24 h/0h, respectively. After 10 days of experiment, ChA increased to 10,75 mg/lt, NH_4-N decreased gradually from 41,8 mg/lt to 17,6 mg/lt with 58% removal efficiency during the batch operation. No significant improvement was observed in PO_4-P removal under continuous illumination. The percent removal was only 49,5% with $[PO_4\text{-}P]_e$=10,8 mg/lt.

In summary, L/D=12h/12 gave around 53% removal with 33,8 mg/lt effluent NH_4-N concentration. Further increase in light exposure period to 18 hours resulted in almost the same NH_4-N removal. However, the light cycle of 20 h provided better nutrient removal in terms of ammonia- nitrogen and gave around 79% removal efficiency. It was expected that continuous illumination could provide higher removal performance but the results indicated that only 58% efficiency can be obtained. The L/D cycle had a slight effect on phosphorus uptake by algae. The percent removal increased from 34% to around 49% with the increase in light period from 12 h to 24 h. Similar to ammonia nitrogen removal, maximum phosphorus uptake was observed at 20h/4h L/D cycle with 61% efficiency. The measurement of biomass concentration and ChA content at the end of 10 days batch operation also indicated that it does not vary with the length of light period, and remains around 11 mg ChA/lt. These results indicated that algae need a minimum dark period for the

metabolic activity (Figure.3.27). Craggs, McAuley and Smith, (1996) obtained %100 removal of ammonium and orthophosphate by marine microalgae grown on a corrugated raceway at diurnal L/D cycle. Influent nutrient concentrations remained uniform about 497,7±60,7 mmol N/m^3 and 76,2±4,9 mmol P/m^3 in the wastewater. According to K. Lee & Lee (2001) illumination of L/D (12h/12h) cycles gives better efficiency in the removal of organic carbon and phosphorus per increased biomass, while nitrate removal efficiency under the continuous light is slightly higher than that under L/D cycles for *Chlorella kessleri* at batch culture conditions.

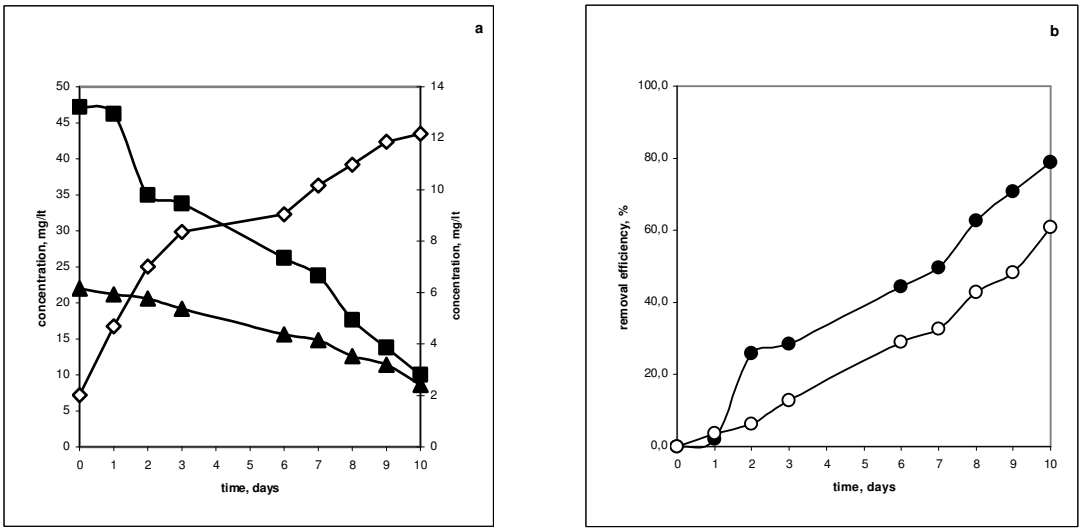


Figure 3.25 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at light /dark cycle=20h/4h (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at light /dark cycle=20h/4h (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

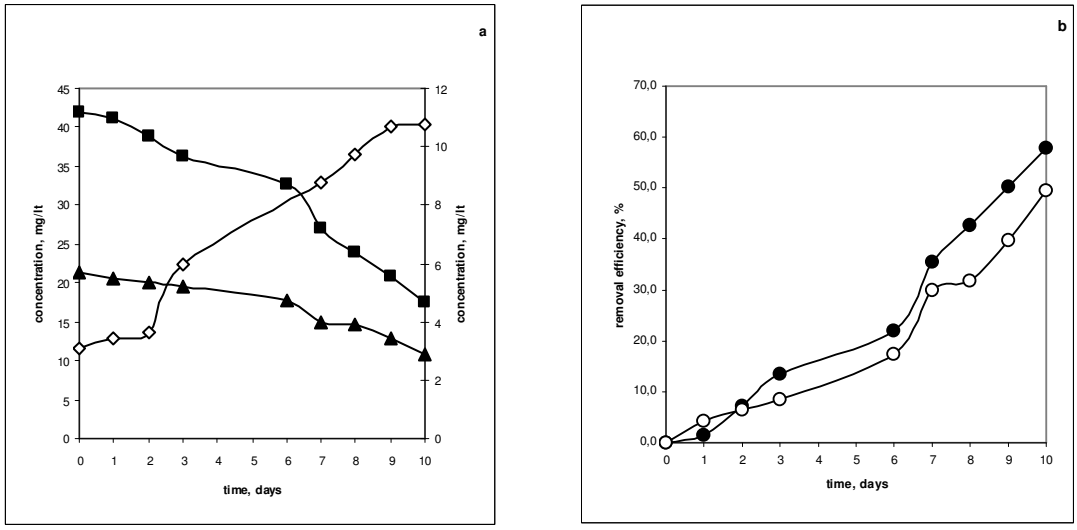


Figure 3.26 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at light /dark cycle=24h/0h (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at light /dark cycle=24h/0h (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

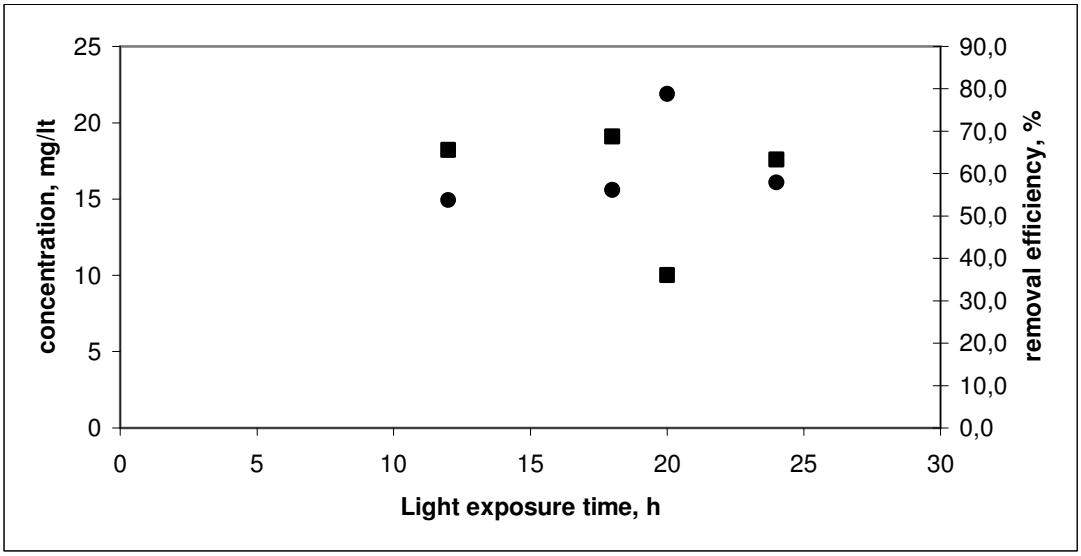


Figure 3.27 Variation of final NH_4-N concentrations and removal efficiencies with light exposure period (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

Table 3.1 Variation of NH_4-N and PO_4-P removal efficiency with different L/D cycles.

Light/Dark cycle	% NH_4-N Removal	% PO_4-P Removal
24/0	57,9	49,5
20/4	79	61
18/6	56	34
12/12	54	34

3.1.4. Effect of Light Intensity on Nutrient Removal Performance of the Batch System

In order to examine the effect of light intensity on nutrient removal efficiency in batch experiments with microalgae *Chlorella vulgaris*, light intensity was gradually increased from 550 lux to 7250 lux. Although maximum NH_4-N removal was obtained at around $[NH_4\text{-}N]_o$=10 mg/lt, the initial NH_4-N concentration was adjusted to $[NH_4\text{-}N]_o$=48±2 mg/lt to evaluate the system performance at high NH_4-N concentrations. The L/D cycle and N/P ratio were kept constant at 20/4 and 2, respectively. In addition, the initial ChA concentration was around ChA_o=3± 0,5 mg/lt. The variations of nutrient removal with time at different light intensities were given in Figures 3.27 to 3.34 and the results were summarized in Figure 3.35.

At the beginning of this part of experiment, the performance of *Chlorella vulgaris* at 550 lux initial light intensity was examined. Variation of effluent NH_4-N, PO_4-P and ChA concentrations at 550 lux light intensity are shown in Figure 3.20.a. Initial nutrient concentrations were $[NH_4\text{-}N]_o$=48 mg/lt and $[PO_4\text{-}P]_o$=22,4 mg/lt. NH_4-N and PO_4-P decreased gradually to $[NH_4\text{-}N]_e$=35,8 mg/lt and $[PO_4\text{-}P]_e$=19,8 mg/lt within 10 days. On the other hand, ChA increased from ChA_o=2,19 mg/lt to ChA_e=11,02 mg/lt at the end of the operation period. As seen in Figure 3.27.b, removal efficiency of NH_4-N and PO_4-P were 26% and 11,6%, respectively on 10^{th} day.

Figure 3.28.a depicts variation of effluent NH_4-N, PO_4-P and ChA concentrations at 740 lux light intensity. Initial NH_4-N, PO_4-P and ChA were adjusted as 48 mg/lt, 22,2 mg/lt and 2,02 mg/lt, respectively. After 10 days of operation ChA concentration increased to 11,35 mg/lt. NH_4-N, PO_4-P concentrations decreased to about $[NH_4\text{-}N]_e$=32,2 mg/lt and $[PO_4\text{-}P]_e$=19,6 mg/lt. As a result final removal efficiencies were obtained as 32,9% for NH_4-N and 11,75% for PO_4-P (Figure 3.28.b).

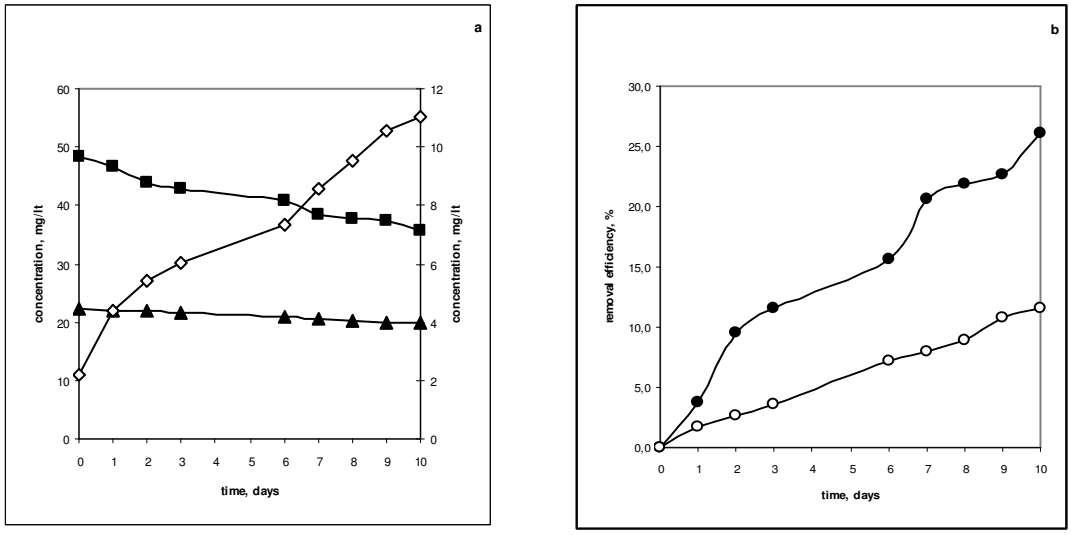


Figure 3.27 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at light intensity=550 lux (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at light intensity=550 lux (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

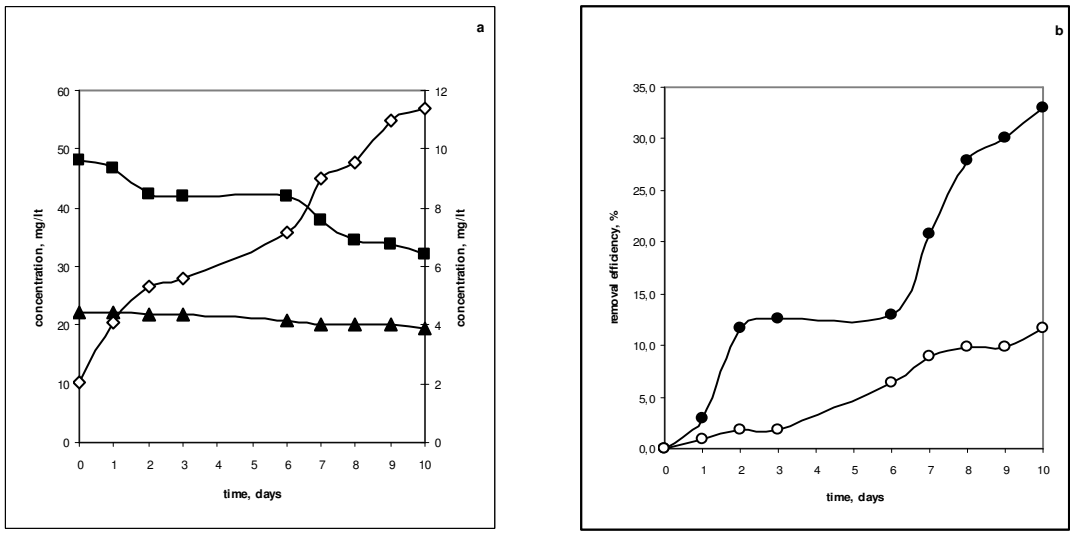


Figure 3.28 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at light intensity=740 lux (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at light intensity=740 lux (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at 1030 lux light intensity are indicated in Figure 3.29.a. At the end of the operation period NH_4-N and PO_4-P decreased from $[NH_4\text{-}N]_o$=48,6 mg/lt, $[PO_4\text{-}P]_o$=22,8 mg/lt to $[NH_4\text{-}N]_e$=27,6 mg/lt, $[PO_4\text{-}P]_e$=19,6 mg/lt, respectively. Furthermore, ChA increased from ChA_o=2,1 mg/lt to ChA_e=12,25 mg/lt on 10^{th} day of the experiment. As it is given in Figure 3.29.b, NH_4-N removal efficiency was 43,2% while PO_4-P removal was only 14%.

Light intensity was increased to 1610 lux and the results are given in Figure 3.30.a, b. ChA concentration reached to 12,2 mg/lt on 10^{th} day of the period. NH_4-N concentration dropped to 31,2 mg/lt for the first 8 days with 35,5% removal efficiency and final NH_4-N concentration was obtained as 24 mg/lt. Depending on this decrease in concentration, NH_4-N removal efficiency increased from 35,5% to 50,4%. PO_4-P removal was slower than NH_4-N removal. Hence, PO_4-P concentration decreased slowly from 22,4 mg/lt to 17,6 mg/lt with 21,4% removal efficiency at the end of 10 days period.

Figure 3.31.a, b depicts variation of effluent NH_4-N, PO_4-P, ChA concentrations and NH_4-N, PO_4-P removal efficiency with time at 2600 lux light intensity. Final ChA concentration was ChA=12,34 mg/lt after 10 days. No significant NH_4-N removal was observed for the first 3 days of batch operation. There was only 17,5% removal efficiency with effluent concentration of 39,6 mg/lt. However, effluent NH_4-N gradually decreased from 39,6 mg/lt to 23 mg/lt with 52,1% removal efficiency at the end of the experiment. Besides, PO_4-P removal efficiency was 31,86% with effluent PO_4-P concentration of $[PO_4\text{-}P]_e$=15,4 mg/lt.

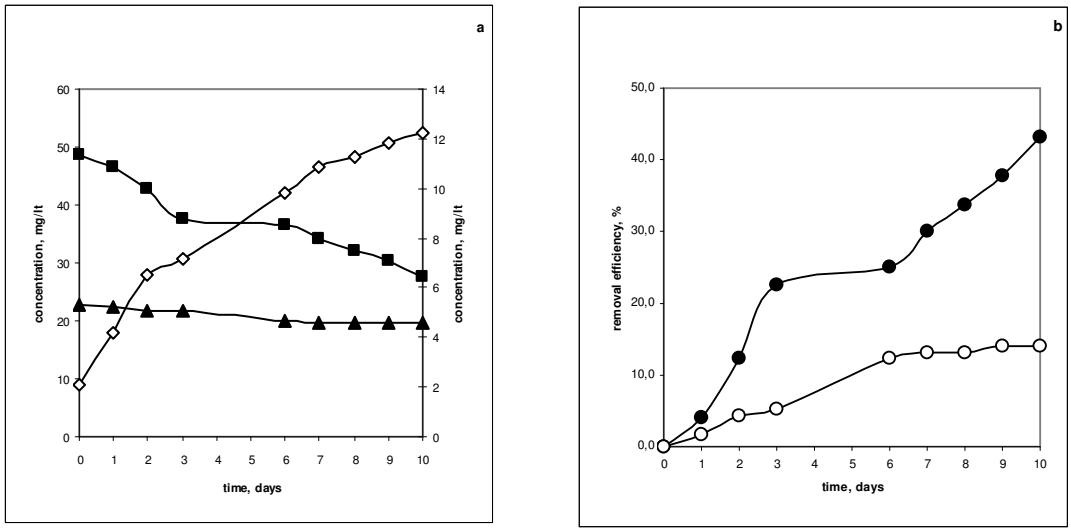


Figure 3.29 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at light intensity=1030 lux (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at light intensity=1030 lux (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

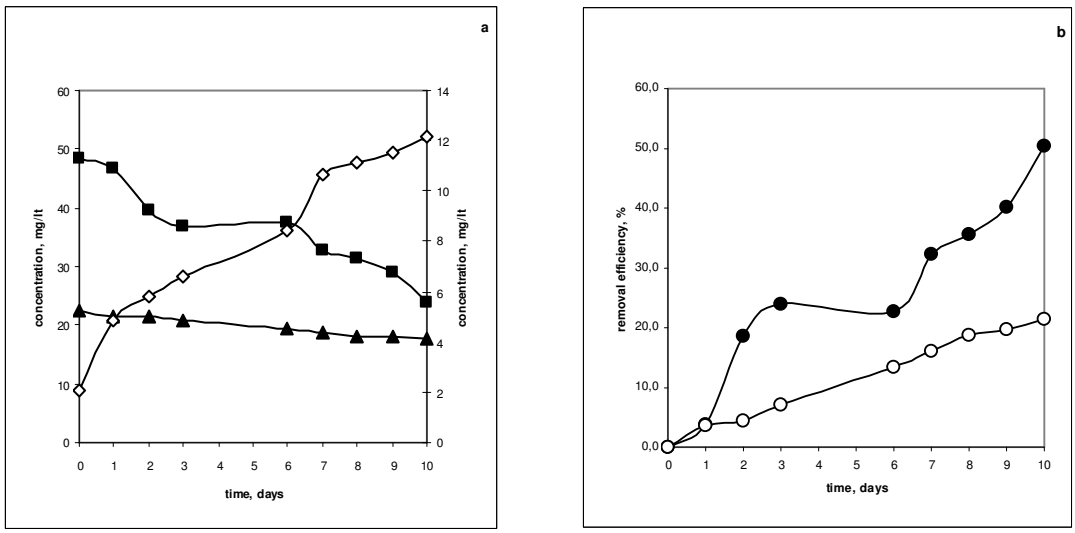


Figure 3.30 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at light intensity=1610 lux (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at light intensity=1610 lux (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

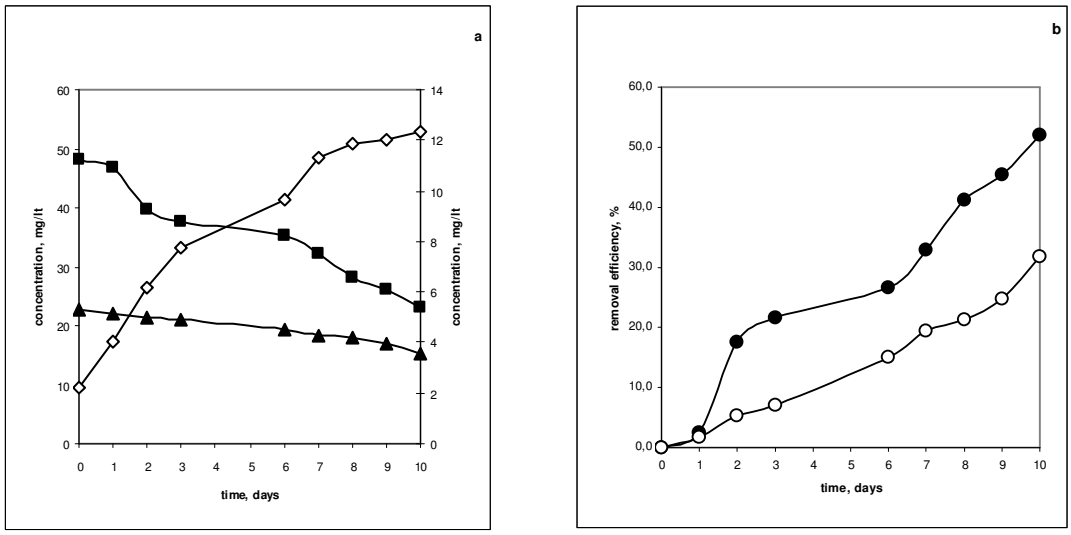


Figure 3.31 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at light intensity=2600 lux (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at light intensity=2600 lux (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

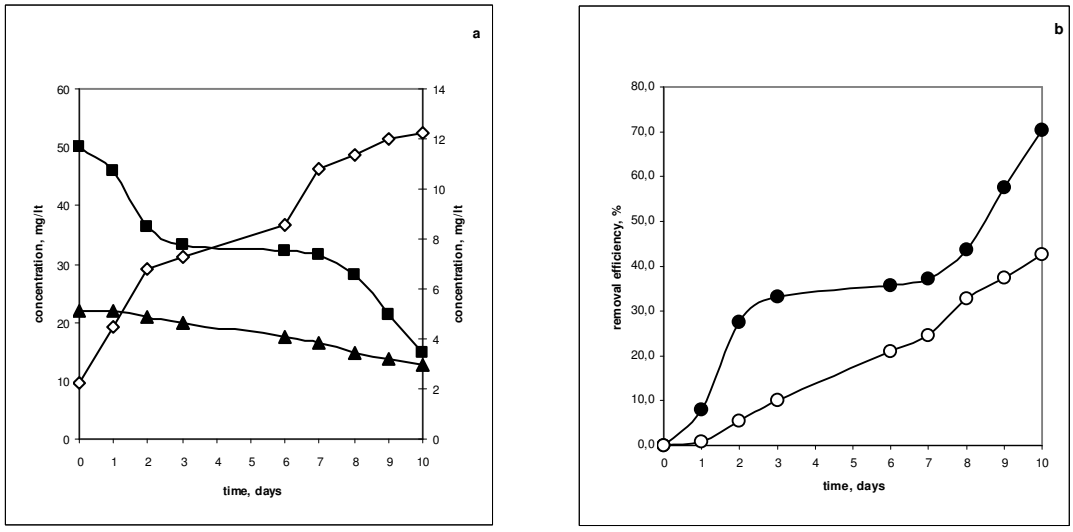


Figure 3.32 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at light intensity=3380 lux (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at light intensity=3380 lux (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

Variation of effluent NH_4-N, PO_4-P, ChA concentration and NH_4-N, PO_4-P removal efficiencies at 3380 lux light intensity are shown in Figure 3.32.a, b, respectively. 12,25 mg/lt ChA concentration was achieved at the end of the 10 days of batch operation. Significant NH_4-N removal, E=70,4%, with effluent concentration of 14,8 mg/lt was observed at 10^{th} days. PO_4-P decreased gradually from $[PO_4\text{-}P]_o$= 22 mg/lt to $[PO_4\text{-}P]_e$=12,6 with 42,7% removal efficiency.

Light intensity was further increased to 4100 lux and the results are given in Figure 3.33.a and Figure 3.33.b. No significant increase in ChA concentration was obtained at high light intensity of 4100 lux compared to other low light intensities. Final ChA concentration was ChA_e=12,17 mg/lt which was almost the same as lower light intensities. As seen in Figure, there was only 28,4% nitrogen removal efficiency was observed for the first 3 days of batch operation. However, effluent NH_4-N decreased from 33,8 mg/lt to 10 mg/lt with 78,8% removal efficiency at the end of the 10^{th} day of the experiment. Effluent PO_4-P decreased gradually from 22 mg/lt to 8,6 mg/lt resulting in 60,9% which was the highest percent removal in phosphate by *C. vulgaris*.

The highest light intensity examined in this set of experiment was 7250 lux. Results of this experiment are indicated in Figure 3.34.a, b. Initial NH_4-N, PO_4-P and ChA concentrations were $[NH_4\text{-}N]_o$=42,6 mg/lt, $[PO_4\text{-}P]_o$=18,6 mg/lt and ChA_o=2,02 mg/lt, respectively. Effluent ChA reached to ChA_e= 12,54 mg/lt at the end of the experiment. PO_4-P decreased gradually from 18,6 mg/lt to 8,2 mg/lt with 56,0% removal efficiency during the batch operation time. *Chlorella vulgaris* significantly removed NH_4-N from 40,6 mg/lt to 2,6 mg/lt with 93,9% removal efficiency which is the highest ammonia nitrogen removal obtained among the examined light intensities.

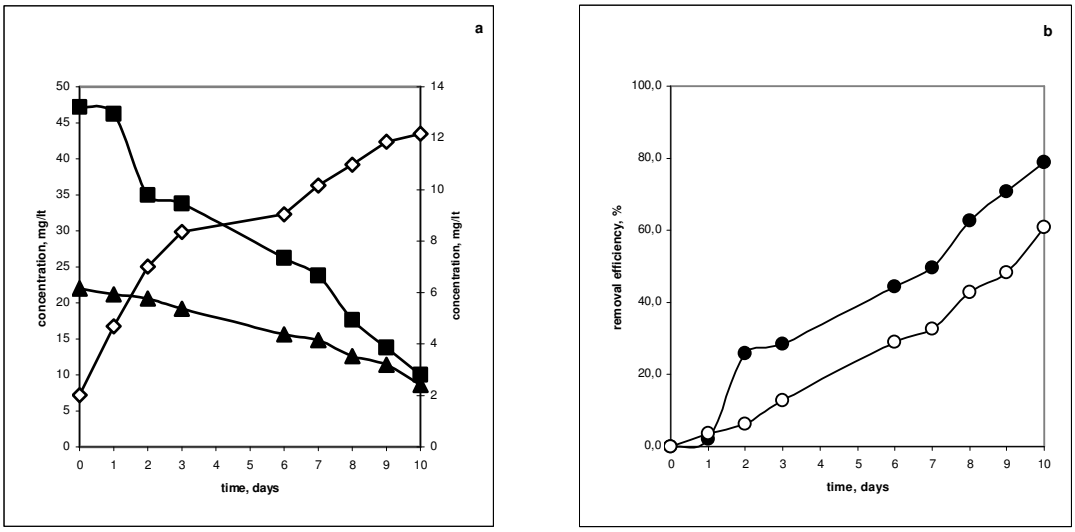


Figure 3.33 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at light intensity=4100 lux (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at light intensity=4100 lux (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

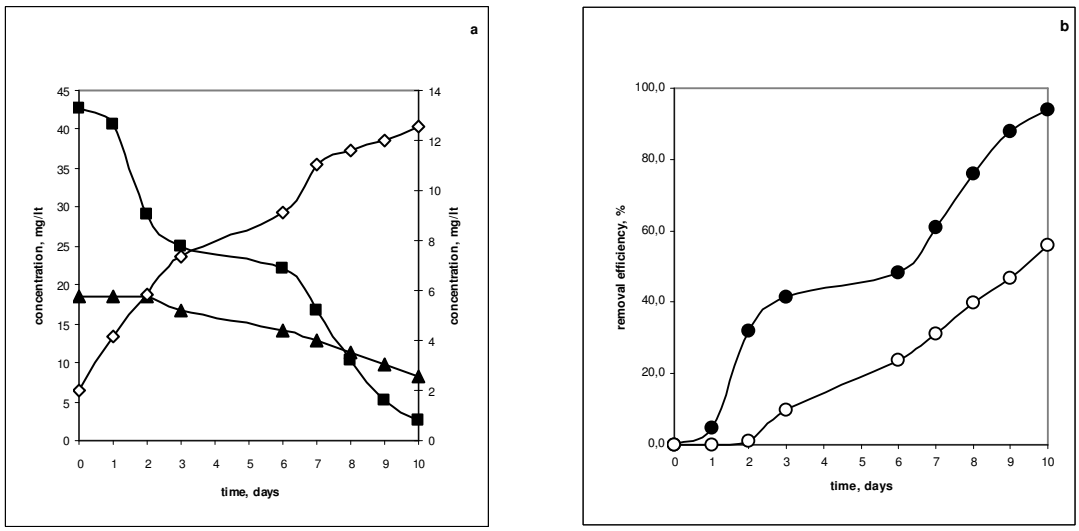


Figure 3.34 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at light intensity=7250 lux (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at light intensity=7250 lux (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

The effect of light intensity on the NH_4-N removal is summarized in Figure 3.35. Effluent NH_4-N concentration was decreased from around 48 mg/lt to 33 mg/lt for the light intensity between 550 lux and 740 lux. The resulting NH_4-N removal efficiency was about 30% for these light intensities. Effluent NH_4-N concentration decreased to around 25 mg/lt for 1030 lux-2600 lux light intensity with 50% NH_4-N removal efficiency. Higher light intensities significantly affected batch operation performance. Efficiency increased to 70,4%, 78,8% at 3380 lux and 4100 lux light intensities, respectively. Furthermore, NH_4-N removal efficiency increased to 93,9% with 7250 lux light intensity. These results indicate that, for the maximum NH_4-N removal efficiency at 48±2 mg/lt NH_4-N concentration with *Chlorella vulgaris*, the light intensity must be 7250 lux. In conclusion, light is the limiting factor for the nutrient removal by microalgae *C.vulgaris*.

Similar results were observed for PO_4-P removal. Increasing light intensity enhanced the phosphorus removal by *C. vulgaris*. However, the effect of light intensity on phosphorus uptake was not as strong as that is in ammonia nitrogen. No significant phosphorus uptake was observed up to 1030 lux and the removal efficiency was around 13±2%. Percent removal increased from 31% to 41 % with the decrease in effluent concentrations of 15,4 mg /lt and 12,6 mg/lt when light intensity was increased from 2600 lux to 3380 lux, respectively. The highest removal was observed at 4100 lux with 61% removal (Figure 3.36).

Table 3.2 indicates effect of light intensity on ChA content, substrate removal rates (R_{so}) and specific substrate removal rates (R_{xi}) Initial substrate removal rate is calculated as in equation 1;

$$R_{so} = -(S_o + S_t)/(t_o - t_t) \qquad \text{eq. 1.}$$

where, R_{so} represents initial rate, S_o is initial substrate concentrations as NH_4-N or PO_4-P, S_t is the final corresponding substrate concentration at time “t”. The first 3 days (t_3) of effluent NH_4-N and PO_4-P concentration were considered in this study to calculate the initial substrate removal rates. The slope of time versus effluent

concentration at time "t_3" gives the initial substrate removal rate. Specific rate of substrate removal (R_{xi}) was determined by dividing the initial rates to biomass concentration at the start up of the experiments [$R_{xi}= R_i/ (X_o)$].

Initial substrate removal rate increased from 1,96 mg/lt d to 6,44 mg/lt d for ammonia nitrogen removal with the increase in light intensity from 550 lux to 7250 lux. As expected, the maximum specific substrate utilization rate was 47,56 mg/ g X d which was obtained at 7250 lux. As seen from the table, both rates for phosphorus removal are lower than that of nitrogen removal. In addition, increasing light intensity did not significantly affect the final ChA concentration which was around 12 mg/lt. Martinez, Castillo & El Yousfi (1999) investigated the effect of light intensity (869 lux -15067 lux) on phosphorus removal by *Scenedesmus* in a chemostat. It was reported that light intensity does not directly affect phosphorus removal but internal P content declined with higher incident light intensity. This result was interpreted as cellular response to avoid accumulating ATP when sufficient light energy was available. Hu, Westerhoff & Vermaas (2000) found that maximal photoautotrophic growth rate of *Synechococcus sp* increased with light intensity (6-180µmole photon/ m^2 s). However, prolonged exposure of low density to 180 µmole photon/ m^2 s caused photobleaching. In addition, J. K. Kim, Lee, Kim & Moon (1999) cultivated the phototrophic denitrifier *Rhodopseudomonas palustris* in anaerobic flask culture at various illumination light intensities between 1000-6000 lux. The optimum light intensity was determined as 5000 lux.

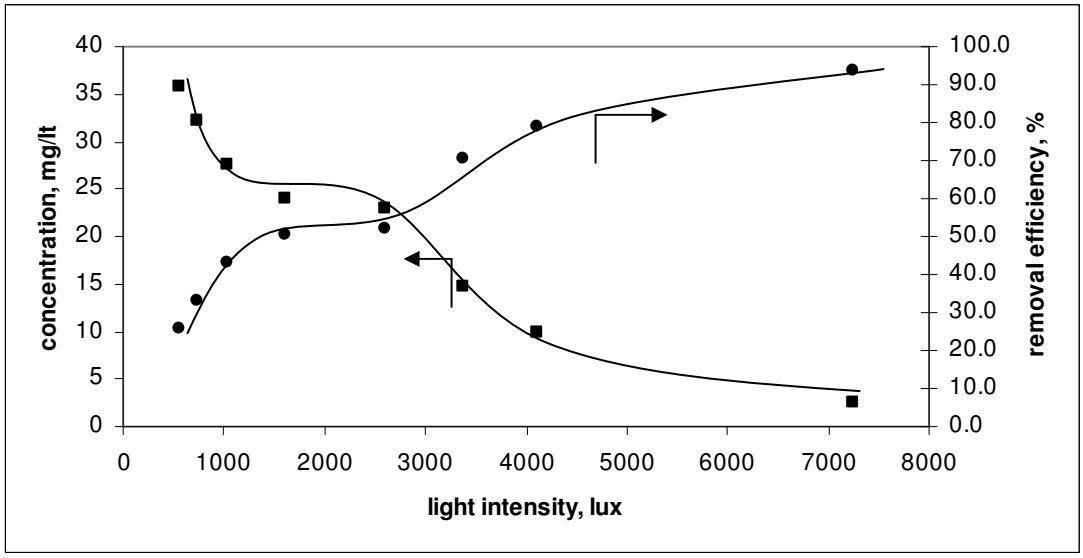


Figure 3.35 Variation of final NH_4-N concentrations and removal efficiencies with light intensity (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

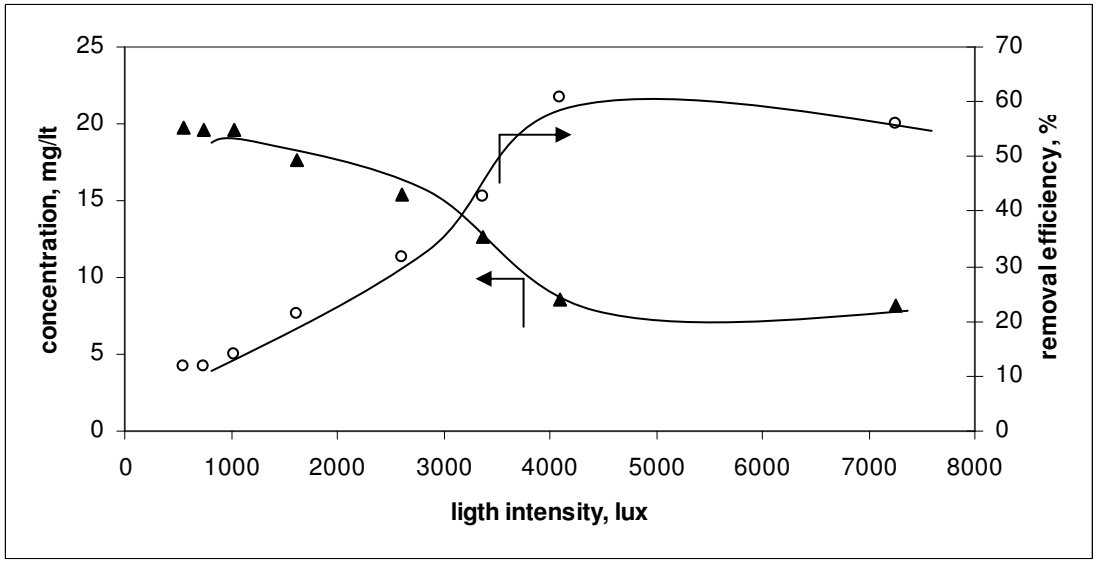


Figure 3.36 Variation of final PO_4-P concentrations and removal efficiencies with light intensity (—▲— effluent PO_4-P concentration, —○— PO_4-P removal efficiency).

Table 3.2 Effect of light intensity on ChA content, substrate utilization rates (R_{so}) and specific substrate utilization rates (R_{xi})

	NH_4-N		PO_4-P		ChA	
Light Intensity	**R_{xi}**	**R_{so}**	**R_{xi}**	**R_{so}**	**ChA_o**	**ChA_e**
Lux	**mg/g X d**	**mg/lt d**	**mg/g X d**	**mg/lt d**	**mg/lt**	**mg/lt**
550	13,35	1,96	1,77	0,26	2,19	11,026
740	16,34	2,22	1,03	0,14	2,03	11,35
1030	21,37	3	2,99	0,42	2,09	12,25
1650	30,18	4,2	3,59	0,5	2,07	12,2
2600	25,48	3,84	3,71	0,56	2,24	12,34
3380	39,55	5,96	5,04	0,76	2,2	12,25
4100	45,07	6,1	6,65	0,9	1,78	12
7250	47,56	6,44	4,13	0,56	2,02	12,54

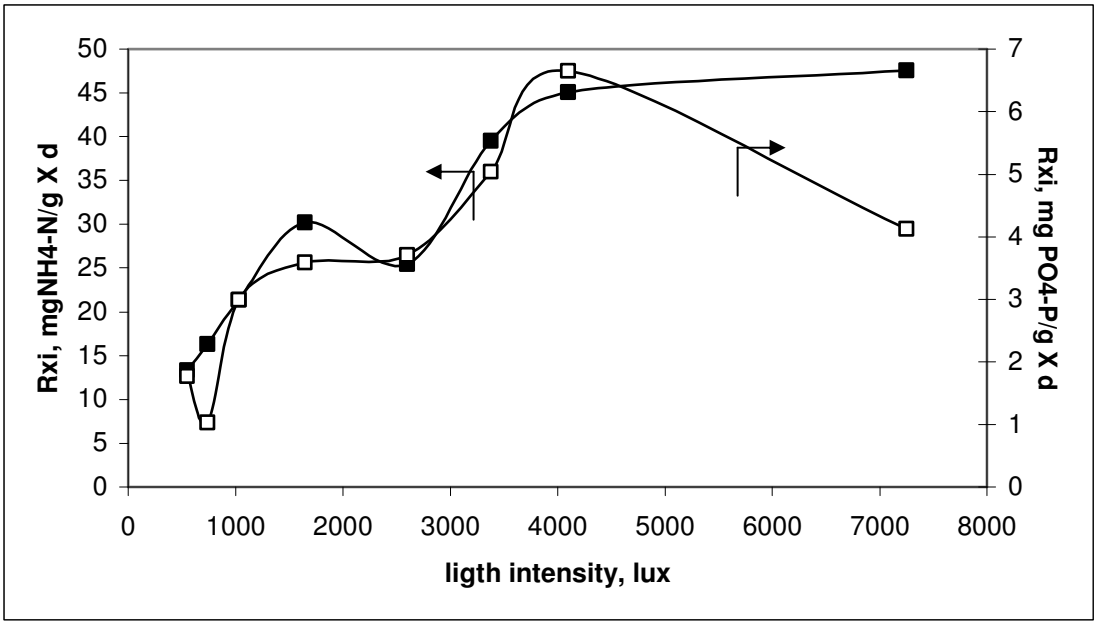


Figure 3.37 Effect of light intensity on specific substrate utilization rates (R_{xi}) for NH_4-N and PO_4-P (—■—NH_4-N specific substrate utilization rate, —□— PO_4-P specific substrate utilization rate).

3.1.5. Effect of N/P Ratio on Nutrient Removal Performance of the 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, batch operation was conducted at different N/P ratio which was varied between 2/1- 10/1. In other words, the aim of this experiment was to enhance phosphorus removal. NH_4-N concentration was kept constant around 40±1 mg/lt while phosphate concentration was gradually decreased from 20±1 mg/lt to 4±0,1 mg/lt. The other environmental conditions were adjusted as light intensity= 7250 lux, ChA_o= 3±0,5 mg/lt and biomass concentration was X_o= 0,07±0,01 g/lt. The variation of effluent NH_4-N, PO_4-P, ChA concentrations and nutrient removal efficiencies with time at different N/P ratios were given in through Figures 3.38-43, and results are summarized in Figure 3.44.

As the first part of this parameter, nutrient removal performance of *Chlorella vulgaris* at N/P=2 was examined. Variation of effluent NH_4-N, PO_4-P and ChA concentrations with time at N/P=2 is shown in Figure 3.38.a. Initial NH_4-N, PO_4-P and ChA was $[NH_4\text{-}N]_o$=42,6 mg/lt, $[PO_4\text{-}P]_o$=18,6 mg/lt and ChA_o= 2,02 mg/lt, respectively. Final ChA concentration reached to ChA_e= 12,54 mg/lt at the end of the 10 days batch period. The removal efficiencies of NH_4-N and PO_4-P were given in Figure 3.38.b. As seen in these Figures, around 76% of NH_4-N was removed within 8 days of operation. Effluent NH_4-N decreased from 42,6 mg/lt to 10,2 mg/lt and PO_4-P decreased from 18,6 mg/lt to 11,2 mg/lt with 40% removal efficiency. Gradual decrease in NH_4-N and PO_4-P removal was observed for the rest of operating period. Final concentrations were obtained as $[NH_4\text{-}N]_e$=2,6 mg/lt, $[PO_4\text{-}P]_e$= 8,2 mg/lt resulting in 94% and 56% removal efficiencies, respectively.

Figure 3.39.a, b depicts variation of effluent NH_4-N, PO_4-P, ChA concentration and NH_4-N, PO_4-P removal efficiency with time at N/P=3,5, respectively. Initial nutrient concentration was adjusted as $[NH_4\text{-}N]_o$= 41,4 mg/lt $[PO_4\text{-}P]_o$=12,1 mg/lt.

ChA concentration increased from ChA_o=2,88 mg/lt to 22,9 mg/lt at the end of the batch experiment. After first 3 days of operation, significant nutrient removal was achieved. NH_4-N concentration was decreased to 0,29 mg/lt with 99% removal efficiency. However, PO_4-P concentration decreased to 6,5 mg/lt with 46,3% removal efficiency.

Variation of effluent NH_4-N, PO_4-P, ChA concentrations and NH_4-N, PO_4-P removal efficiencies are indicated in Figure 3.40.a and Figure 3.40.b with time at N/P=5,7, respectively. Initial NH_4-N, PO_4-P and ChA concentrations were $[NH_4\text{-}N]_o$=41,6 mg/lt, $[PO_4\text{-}P]_o$=7,3 mg/lt and ChA_o=3,05 mg/lt. At the end of 10 days period ChA concentration was ChA_e=23,01 mg/lt. After first 3 days of experiment, significant nutrient removal was observed and then NH_4-N decreased from 34 mg/lt to 0,1 mg/lt with 99,8% removal efficiency. PO_4-P decreased from 5,9 mg/lt to 1,5 mg/lt with 79,5% removal efficiency at the end of 10 days. Almost complete removal of NH_4-N was achieved within 7 days.

N/P ratio was further increased to N/P=6,8 and the results are given in Figure 3.41.a and Figure 3.41.b. Initial ChA concentration increased to 23,16 mg/lt. Initial NH_4-N concentration was 41,6 mg/lt and effluent NH_4-N concentration dropped to 33,6 mg/lt for the first 3 days with 19,2% removal efficiency. And then, effluent NH_4-N concentration decreased linearly from 33,6 mg/lt to 0,1 mg/lt. Depending on this decrease in concentration, NH_4-N removal efficiency increased from 19,2% to 99,8% at N/P= 6,8/1 ratio. Moreover, PO_4-P decreased gradually from 6,1 mg/lt to 1,5 mg/lt with 75,4% removal efficiency.

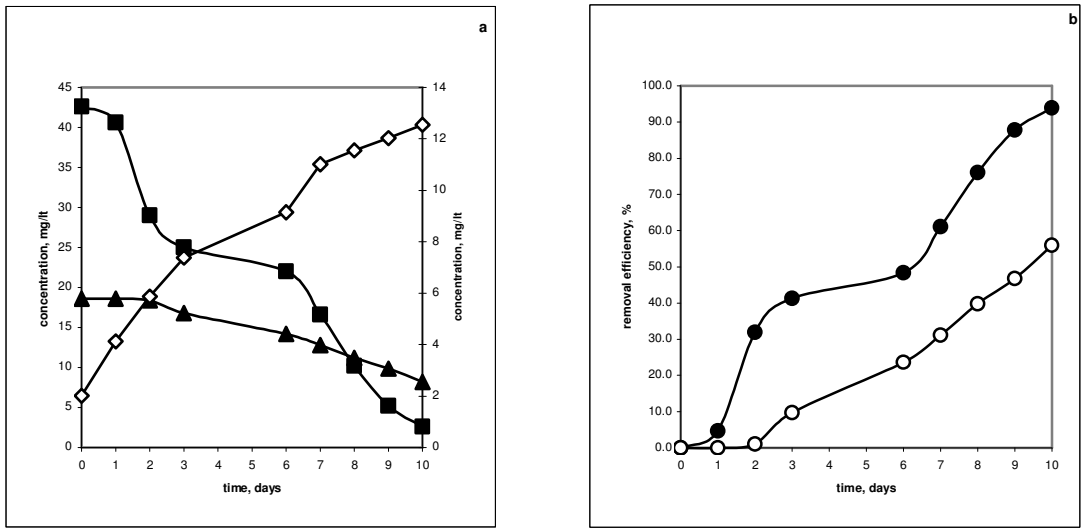


Figure 3.38 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at N/P ratio=2,3 (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at N/P ratio=2 (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

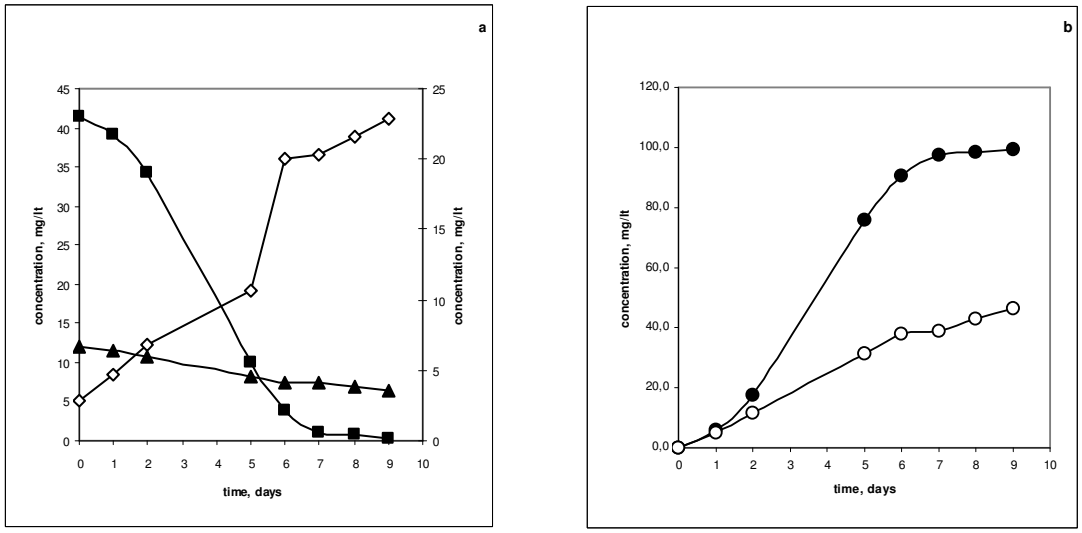


Figure 3.39 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at N/P ratio=3,5 (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at N/P ratio=3,5 (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

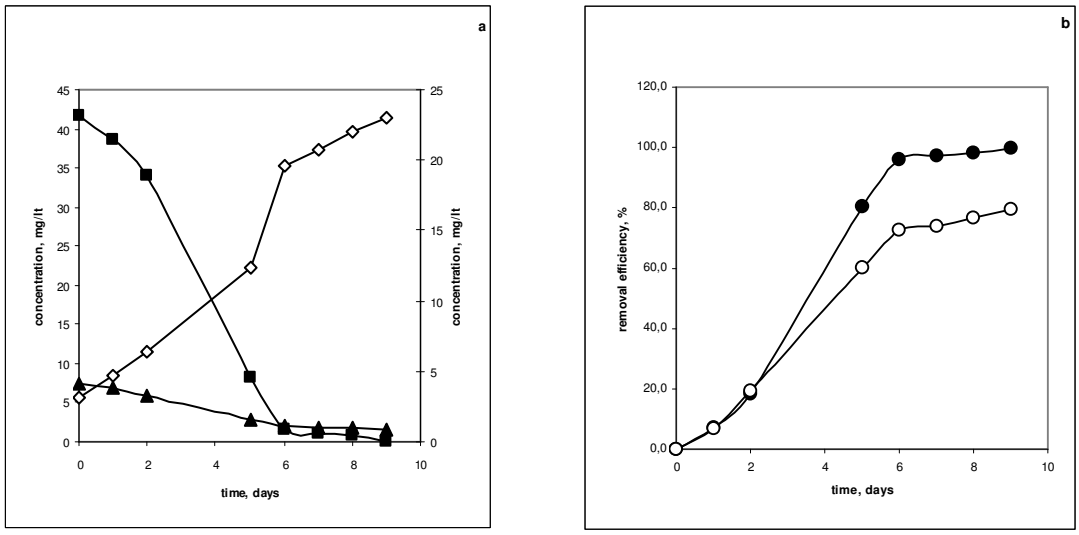


Figure 3.40 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at N/P ratio=5,7 (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at N/P ratio=5,7 (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

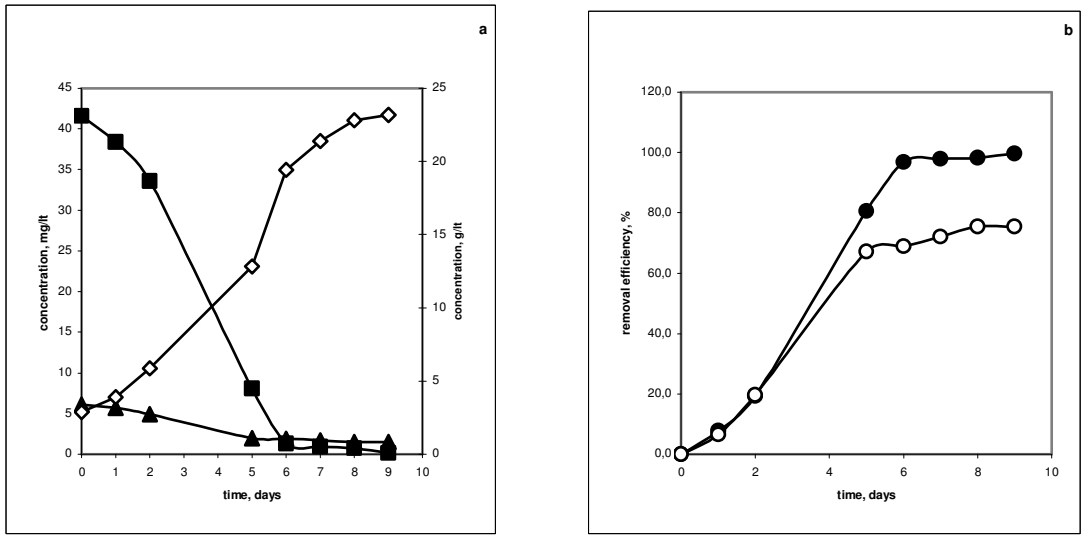


Figure 3.41 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at N/P ratio=6,8 (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at N/P ratio=6,8 (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

Figure 3.42.a and Figure 3.42.b depict variation of effluent NH_4-N, PO_4-P, ChA concentrations and nutrient removal efficiencies with time at N/P=8. Initial ChA concentration ChA_o=2,73 mg/lt reached to 22,7 mg/lt at the end of batch operation period. Initial NH_4-N and PO_4-P were adjusted as $[NH_4\text{-}N]_o$=41,6 mg/lt, $[PO_4\text{-}P]_o$= 5,2 mg/lt, respectively. There was a linear removal of NH_4-N between 0^{th}-5^{th} days of batch operation as seen in Figure3.42.a. NH_4-N decreased from 41,6 mg/lt to 5,7 mg/lt with 86,3% removal efficiency. Then removal efficiency became stable at about 99%. Besides, PO_4-P decreased slowly from 5,2 mg/lt to 1,2 mg/lt with 77% removal efficiency.

Finally N/P ratio was increased to 10 and initial NH_4-N, PO_4-P and ChA were adjusted to 41,8 mg/lt, 4,1 mg/lt and 2,80 g/lt, respectively. Figure 3.43.a and Figure 3.43.b points out variation of effluent NH_4-N, PO_4-P, ChA concentrations and nutrient removal efficiency with time. A rapid increase from 2,8 mg/lt to 21,33 mg/lt was observed in ChA concentration. NH_4-N decreased from 41,8 mg/lt to 0,7 mg/lt within 6 days of experiment with 98% removal efficiency. In last 4 days of operation, NH_4-N removal was almost completed. As seen in Figure, PO_4-P removal was not successful as NH_4-N removal. The percent removal was around 66%.

Variation of NH_4-N and PO_4-P concentration with N/P ratio is summarized in Figure 3.44. Significant ammonia nitrogen removal was observed all N/P ratios except for N/P=2. With N/P=2 ratio nutrient removal efficiency was only about 60%. On the other hand, other N/P ratios resulted in high nitrogen removal efficiencies as about 99%. Similarly, phosphorus removal was also enhanced by increasing N/P ratio to 5,7. The PO_4-P concentration was reduced from 21,4 mg/lt to 10,8 mg/lt with around 50% removal efficiency at N/P=2 but effluent concentration reduced to 1,3 mg/lt (E=79%) at N/P = 5,7. Although ammonia nitrogen was not affected by increasing ratio, phosphorus removal decreased gradually to 66% for N/P=10.

ChA concentration and specific substrate utilization rates were also influenced by the N/P ratio (Table 3.3). Final ChA concentration was around 12,54 mg/lt for N/P=2 but increased to 22±1 at higher ratios. An increase in specific substrate utilization

rate for ammonia nitrogen was observed with increasing in N/P ratio. Maximum rate was obtained as 47,56 mg NH_4-N /g X d at N/P= 2,3. However, the rate slightly decreased with increasing ratio. This result could be explained as insufficient phosphorus concentration for the uptake of ammonia at high N/P ratios. Specific phosphate removal rate was 4,13 mg PO_4-P/ g X d for N/P = 2,3, but increased to 4,43 mg PO_4-P/ g X d at N/P=5,7. Then a significant decrease in the rate to 2.43 mg PO_4-P /g X d was observed for N/P=10. It could be because of phosphorus limitation in the growth media. In summary, N/P=5,7 was determined as the optimum ratio to obtain almost complete nitrogen and around 80% phosphorus removal. Higher yields and productivities were obtained with cyanobacterium *Phormidium bohneri* at the 6/1 N/P ratio. The addition of phosphates in wastewater increased the biomass production of *P.bohneri* without significantly affecting removal of the inorganic ions ammonium, nitrate and phosphate (Laliberte, Lessard, Noue & Sylvestre, 1997). An, Sim, Lee & Kim; (2003) determined that, the growth of *Botryococcus braunii* was nearly independent of the initial phosphate concentration, except under conditions of phosphate deficiency.

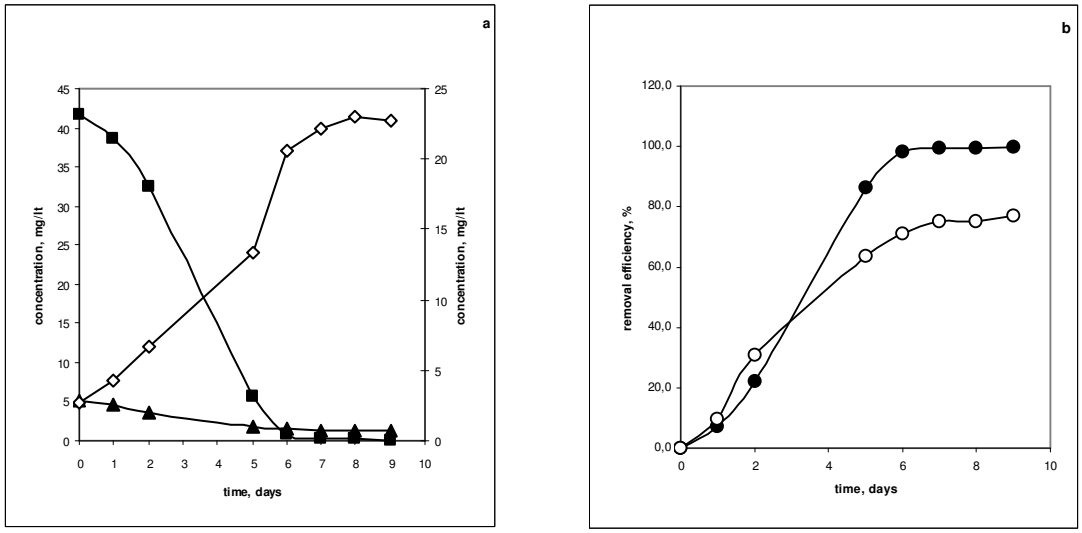


Figure 3.42 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at N/P ratio=8 (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at N/P ratio=8 (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

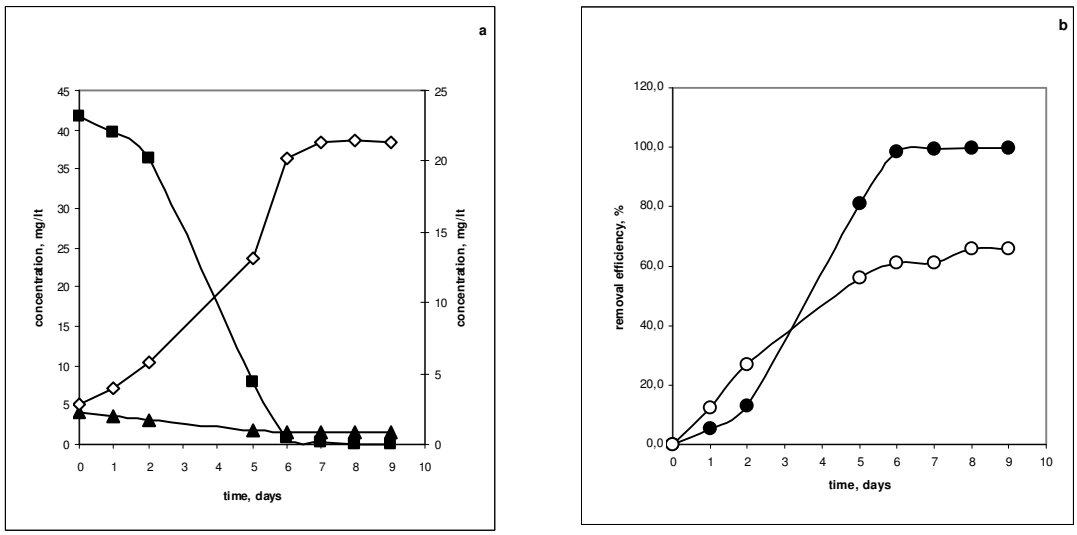


Figure 3.43 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at N/P ratio=10 (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at N/P ratio=10 (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

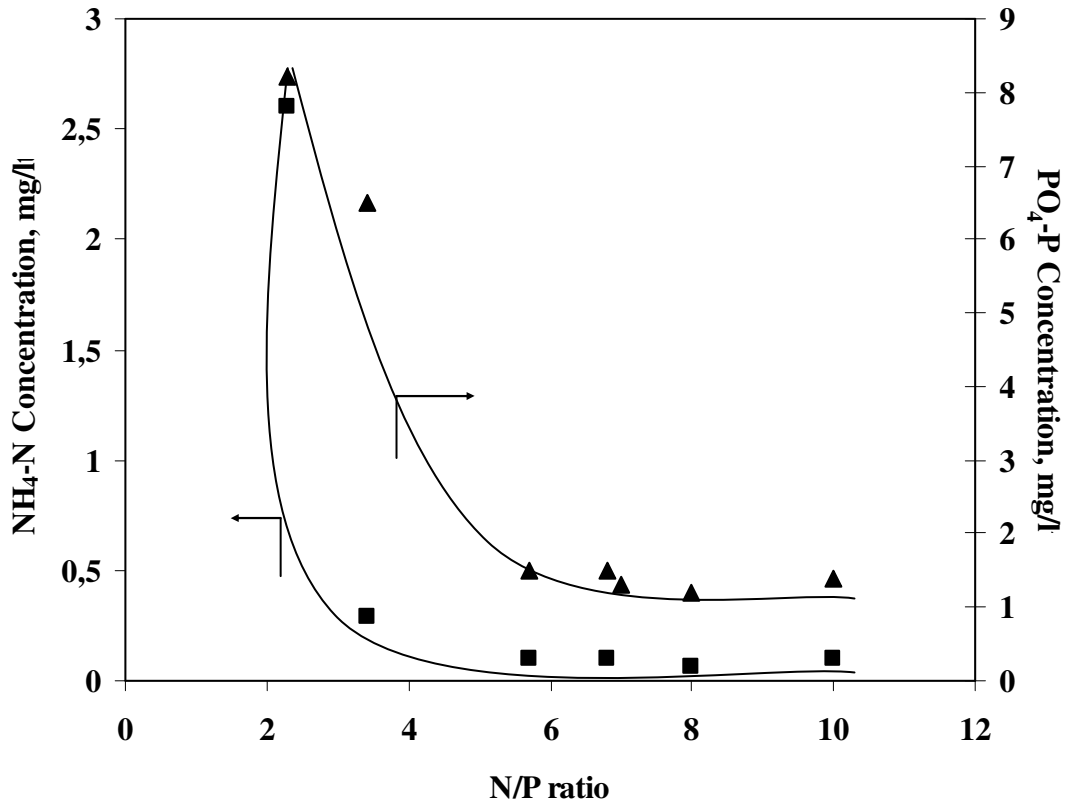


Figure 3.44 Variation of final NH_4-N and PO_4-P concentrations with different N/P ratios (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration).

Table 3.3 Effect of N/P ratio on chlorophyll A content, substrate utilization rates (R_{so}) and specific substrate utilization rates(R_{xi})

	NH_4-N		PO_4-P		ChA	
N/P	**R_{xi} mg/g X d**	**R_{so} mg/lt d**	**R_{xi} mg/g X d**	**R_{so} mg/lt d**	**ChA_o g/lt**	**ChA_e g/lt**
2,3	47,56	6,44	4,13	0,56	2,02	12,54
3,5	33,97	6,56	4,00	0.77	2,88	22,92
5,7	33,91	6,94	4,43	0,91	3,06	23,02
6,8	36,78	7,11	4,40	0,85	2,88	23,17
8,0	40,81	7,48	3,66	0,67	2,73	22,70
10,0	37,86	7.11	2,43	0,46	2,80	21,33

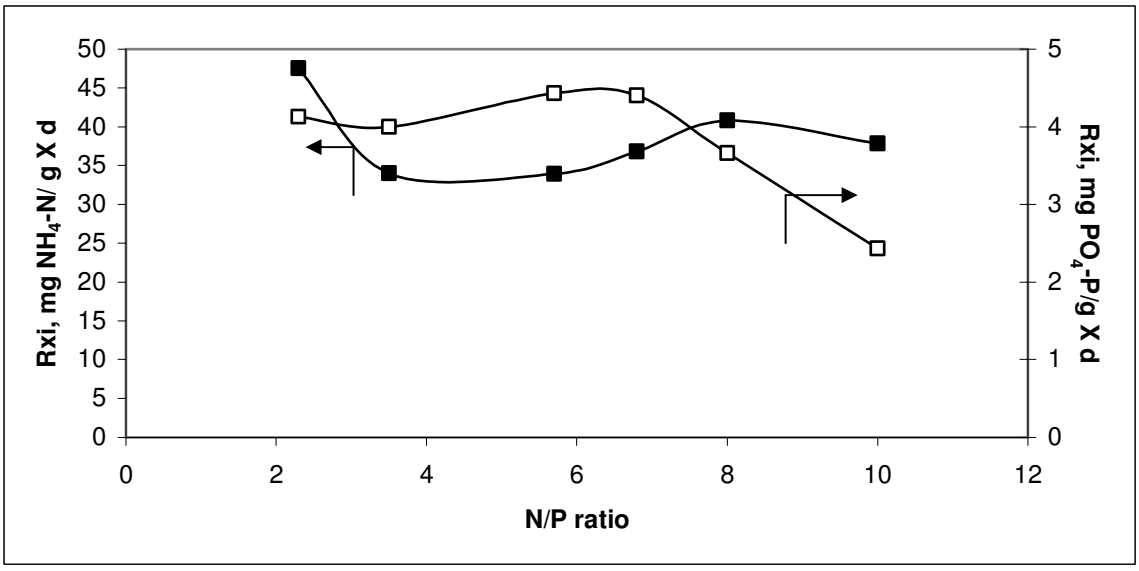


Figure 3.45 Effect of N/P on specific substrate utilization rates (R_{xi}) for NH_4-N and PO_4-P (—■—specific NH_4-N utilization rate, —□— specific PO_4-P utilization rate).

3.1.6. Effect of Chlorophyll A concentration on Nutrient Removal Performance of the Batch System

In order to improve phosphorus removal at 3,8±0,1/1 N/P ratio, the ChA concentration was increased from 2,9 mg/lt to 6,0 mg/lt. NH_4-N concentration, L/D cycle and light intensity were kept constant at 41±2 mg/lt, 20h/4h and 7250 lux , respectively, as determined in previous experiments. Results of this set of experiment were given through Figure 3.46-3.51.

Figure 3.46.a and Figure 3.46.b depict variation of effluent NH_4-N, PO_4-P, ChA concentration and NH_4-N, PO_4-P removal efficiency with time at N/P=3,5, respectively. Initial nutrient concentration was adjusted as $[NH_4\text{-}N]_o$= 41,4 mg/lt $[PO_4\text{-}P]_o$=12,1 mg/lt. ChA concentration increased from ChA_o=2,88 mg/lt to 22,9 mg/lt at the end of the batch experiment. After first 3 days of operation, significant nutrient removal was achieved. NH_4-N concentration was decreased to 0,29 mg/lt with 99% removal efficiency. However, PO_4-P concentration decreased to 6,5 mg/lt with 46,3% removal efficiency.

Figure 3.47.a and Figure 3.47.b depict variation of effluent NH_4-N, PO_4-P, ChA concentration and NH_4-N, PO_4-P removal efficiency with time at ChA_o=4 mg/lt, respectively. Final ChA concentration reached to 20,74 mg/lt after 10 days of batch operation period. Linear NH_4-N removal was observed between 0-8^{th} day the concentration NH_4-N decreased from 42 mg/lt to 0,56 mg/lt with 98,7% removal efficiency. For the last 3 days of experiment, the NH_4-N removal rate was stable at about 99%. On the other hand, PO_4-P decreased slowly during the operation period from 10,7 mg/lt to 6,3 mg/lt with 41,2% removal efficiency.

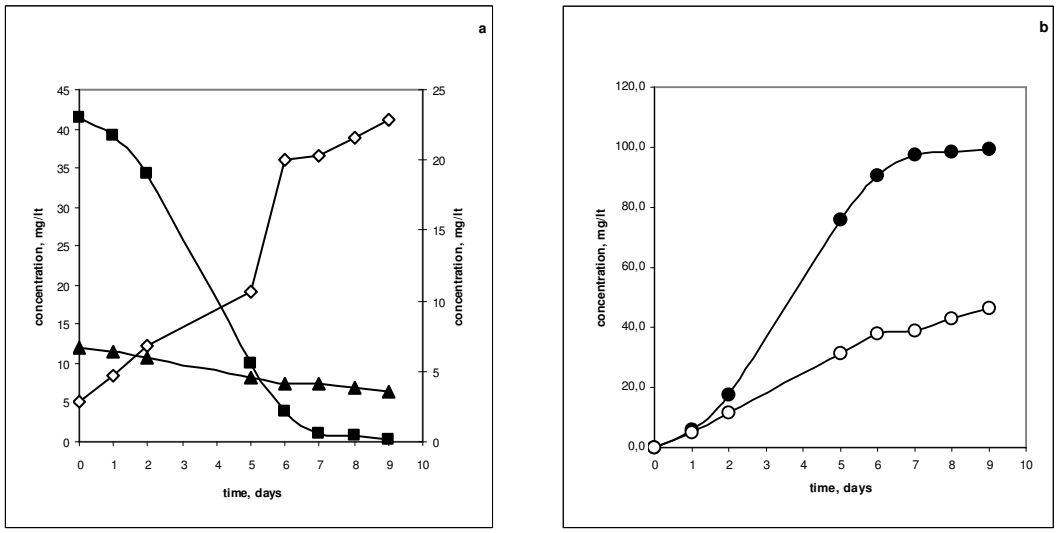


Figure 3.46 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at ChA_0=3,5 mg/lt (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at ChA_0=3,5 mg/lt (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

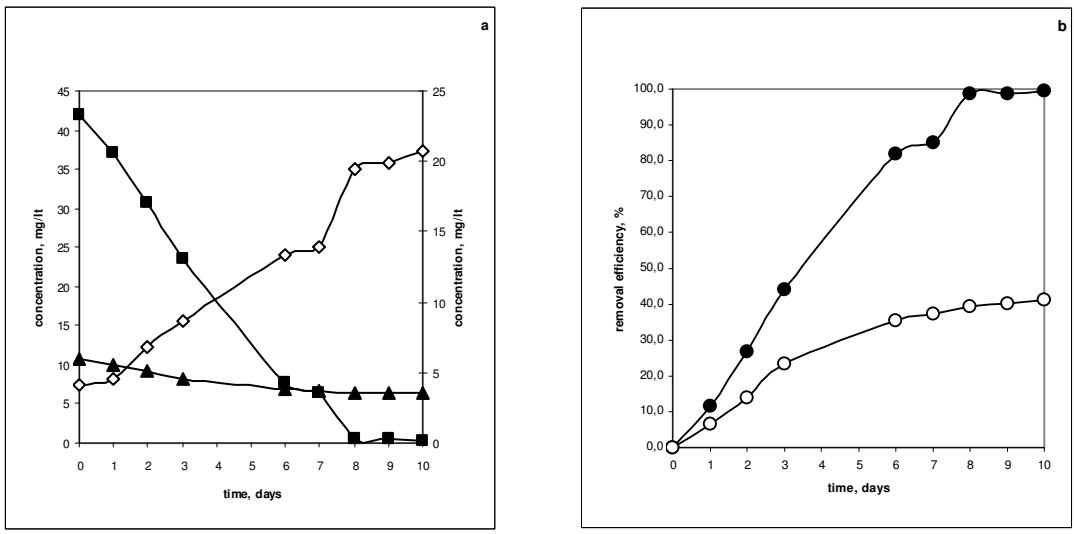


Figure 3.47 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at initial ChA_0=4,0 mg/lt (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at initial ChA_0=4 mg/lt (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

Initial ChA concentration was increased to 4,6 mg/lt and initial NH_4-N and PO_4-P concentration was 41,8 mg/lt, 10,8 mg/lt, respectively. ChA concentration increased rapidly to 21,31 mg/lt through 10 day. As seen in Figure 3.48.a and Figure 3.48.b, NH_4-N decreased linearly from 41,8 mg/lt to 0,65 mg/lt resulting in 98,4% removal efficiency. PO_4-P removal was slower than NH_4-N removal. The concentration decreased from 10,8 mg/lt to 6,9 mg/lt with 36% removal.

Initial ChA was adjusted to ChA_o=5 mg/lt and initial NH_4-N, PO_4-P were $[NH_4\text{-}N]_o$=41,8 mg/lt, $[PO_4\text{-}P]_o$=10,7 mg/lt, respectively. At the end of batch experiment with *Chlorella vulgaris*, 24,56 mg/lt ChA concentration was achieved at 9th day. During the first 6 days of operation, NH_4-N removed rapidly from 41,8 mg/lt to 0,9 mg/lt with 97,8% removal efficiency. Almost complete nitrogen removal was achieved at the end of batch operation. Besides, PO_4-P decreased from 10,7 mg/lt to 6,2 mg/lt with 42% removal efficiency (Figure 3.49.a and Figure 3.49.b).

In order to investigate the effect of higher initial ChA concentration on batch experiment performance, system was operated with ChA_o=5,4 mg/lt. Results are given in Figure 3.50.a and Figure 3.50.b. The final ChA concentration was 28,86 mg/lt at 9th day of operation. Initial NH_4-N and PO_4-P concentrations was 42,2 mg/lt, 11,1 mg/lt, respectively. Significant NH_4-N removal was observed during the batch operation period. NH_4-N was almost completely removed in 8 days resulting in 0,24 mg/lt effluent concentration. No significant removal of PO_4-P was observed as it was in lower initial ChA concentrations. The final PO_4-P concentration was 5,8 mg/lt which provided only 47,7% removal efficiency.

The highest initial ChA concentration examined in this set of experiment was 6 mg/lt. Variation of effluent NH_4-N, PO_4-P concentrations and nutrient removal efficiencies with time, are seen in Figure 3.48.a and Figure 3.51.b. At the end of the batch operation, ChA concentration reached to 40,5 mg/lt. Initial NH_4-N and PO_4-P was 42,2 mg/lt, 11,2 mg/lt, respectively. NH_4-N decreased linearly from 40,5 mg/lt to 0,18 mg/lt within 7 days. The effluent PO_4-P concentration was 6,3 mg/lt after 7 days.

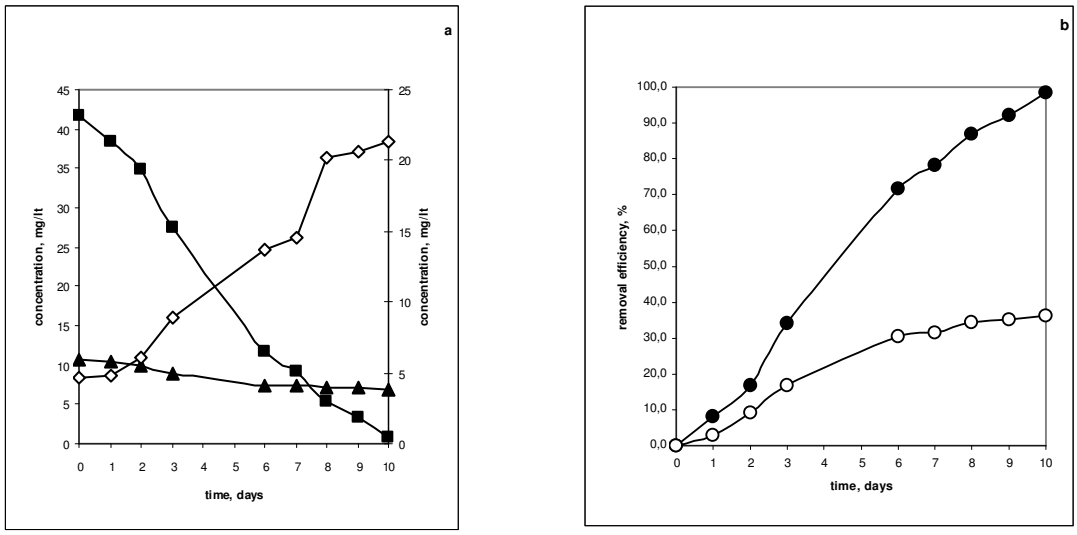


Figure 3.48 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at ChA_0=4,6 mg/lt (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at ChA_0=4,6 mg/lt (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

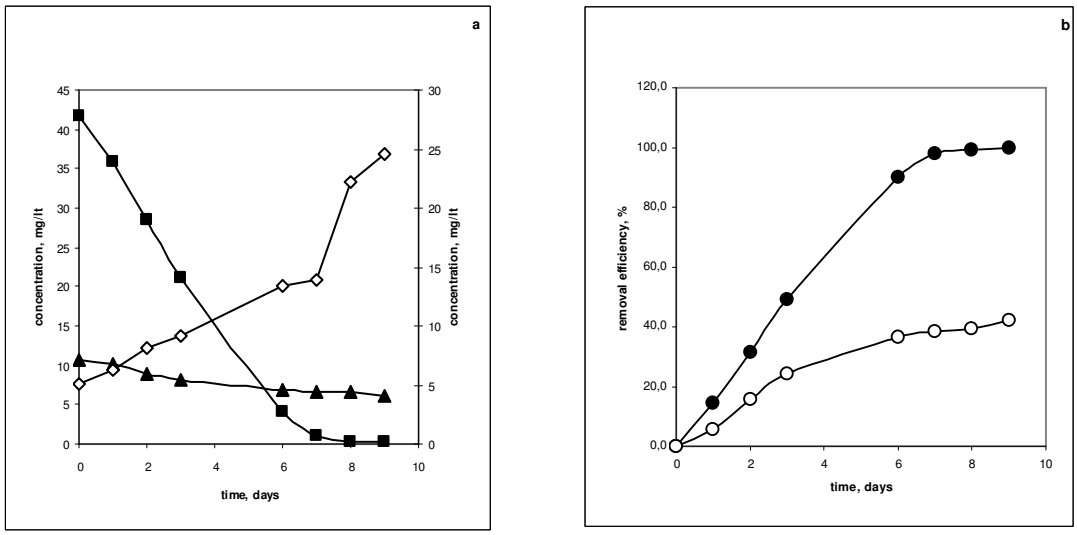


Figure 3.49 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at ChA_0=5 mg/lt (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at ChA_0=5 mg/lt (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

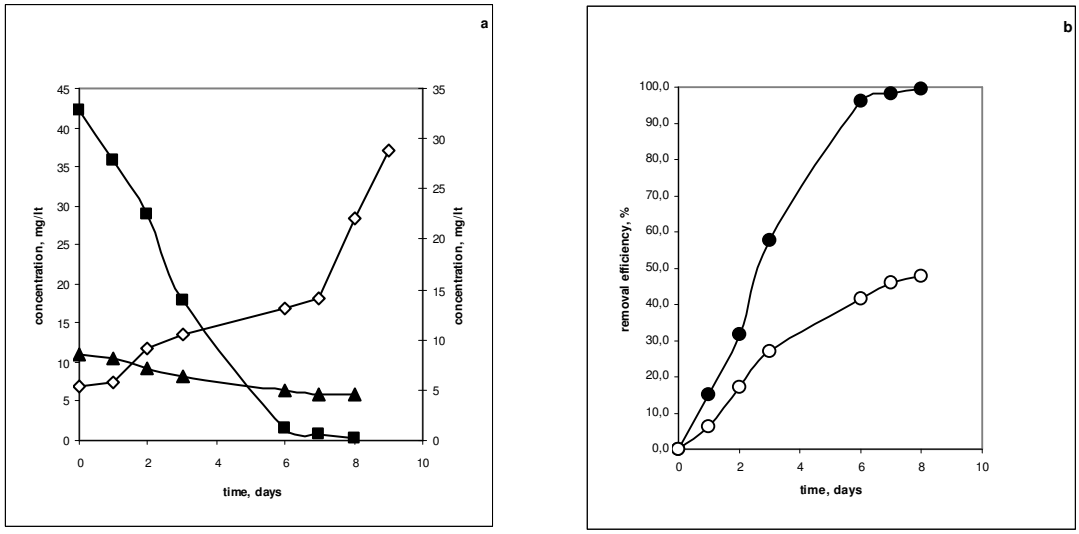


Figure 3.50 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at ChA_0=5,4 mg/lt (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at ChA_0=5,4 mg/lt (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

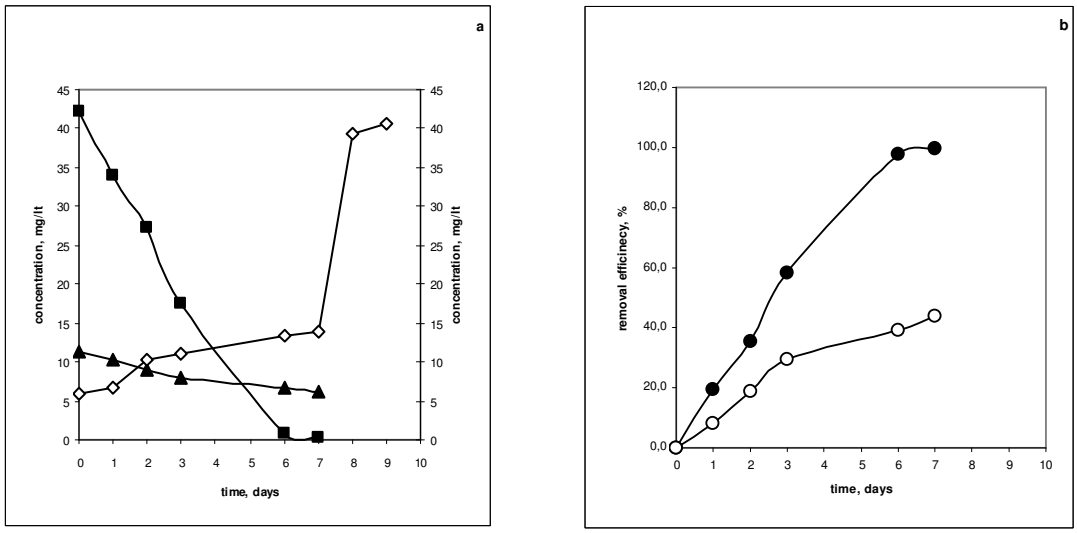


Figure 3.51 (a) Variation of effluent NH_4-N, PO_4-P and ChA concentration with time at ChA_0=6 mg/lt (—■— effluent NH_4-N concentration, —▲— effluent PO_4-P concentration, —◊— ChA concentration).

(b) Variation of NH_4-N and PO_4-P removal efficiency with time at ChA_0=6 mg/lt (—●— NH_4-N removal efficiency, —○— PO_4-P removal efficiency).

In all ChA concentrations, effluent ammonia nitrogen concentration was less than 0,5 mg/lt which resulted in more than 98% removal efficiency (Table 3.4). However, phosphate removal was not significantly affected by ChA concentration. Effluent phosphate concentration varied between 6,9 mg/lt -5,3 mg/lt for all ChA concentrations. As a result, no more than 48% removal efficiency was obtained (Table 3.4). Specific substrate removal rates varied with initial ChA. The highest rates were observed as 23,1 mg NH_4-N/ g X d and 3,10 mg PO_4-P/ g X d at 4 mg/lt ChA concentration (Table 3.5). In addition, final ChA concentration increased up to 40 mg/lt at the maximum starting concentration of 6 mg/lt.

Table 3.4 Variation of NH_4-N and PO_4-P removal efficiency with different initial ChA concentrations.

ChA concentration (mg/lt)	**% NH_4-N Removal**	**% PO_4-P Removal**
2,9	99,3	46
4	99,5	41
4,6	98,4	36
5	99,7	42
5,4	99,4	48
6	99,6	48

Table 3.5 Variation of chlorophyll a content, substrate utilization rates (R_{so}) and specific substrate utilization rates (R_{xi}) with ChA concentration.

NH_4-N		**PO_4-P**		**ChA**	
R_{xi} mg/g X d	**R_{so} mg/lt d**	**R_{xi} mg/g X d**	**R_{so} mg/lt d**	**ChA_o mg/lt**	**ChA_f mg/lt**
19,84	4,71	2,70	0,64	3,5	17,58
23,10	6,19	3,10	0,83	4,0	20,74
16,70	5,14	1,98	0,61	4,6	21,31
20,00	6,90	2,58	0,89	5,1	25,00
21,95	7,99	2,80	1,02	5,4	28,86
20,05	8,06	2,76	1,11	6,0	40,53

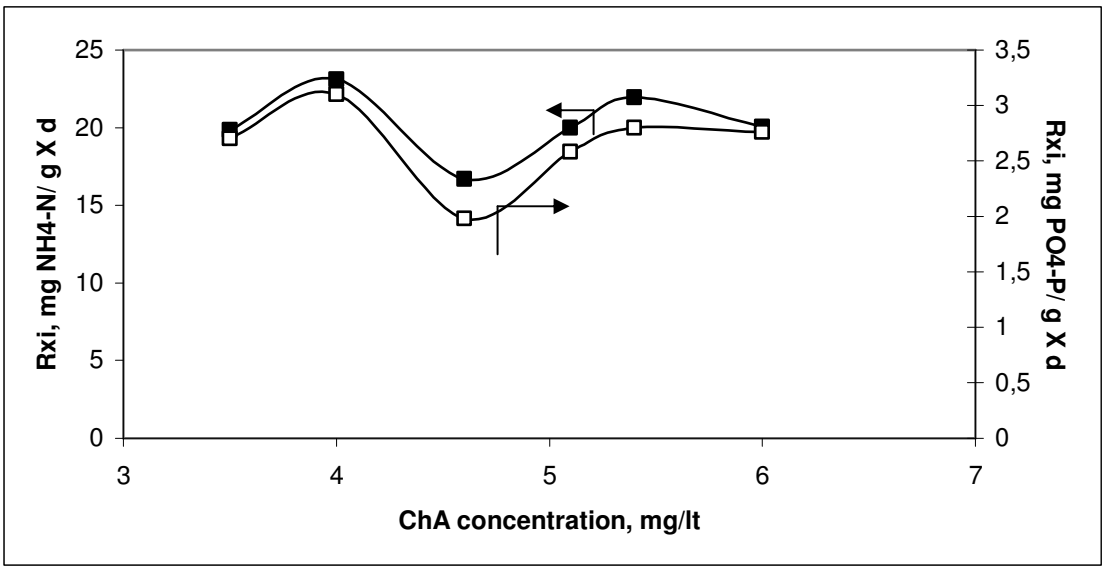


Figure 3.52 Effect of ChA on specific substrate utilization rates (R_{xi}) for NH_4-N and PO_4-P (—■—specific NH_4-N utilization rate, —□— specific PO_4-P utilization rate).

3.2 Continuous Experiments in Immobilized Photobioreactor System

In the second part of study, the nutrient removing performance of continuously operated immobilized photobioreactor system with the same organism was investigated. The system was operated at different initial NH_4-N concentrations (10-50 mg/lt) and hydraulic retention times (θ_H=1,5 days-5,5 days).

3.2.1. Effect of NH_4-N concentration on NH_4-N Removal Performance of Continuous Immobilized Photobioreactor System

In order to examine the effect of initial NH_4-N concentration on the nutrient removal performance of immobilized photobioreactor, five different initial NH_4-N concentrations were tested between 10 mg/lt-50 mg/lt. A microalgae *Chlorella vulgaris* was used as dominant microalgal culture. Light intensity, L/D cycle, N/P ratio and retention time was kept constant at about 2700 lux, 20h/4h, 8 and 2,5 days, respectively. NH_4-N analyses were carried out daily samples taken from influent and effluent wastewater of the system.

Figure 3.52 depicts variation of NH_4-N concentration and NH_4-N removal efficiency with time at 10 mg/lt NH_4-N concentration. As seen in Figure, NH_4-N removal was stable at about 65% removal efficiency between 3-6th days. After 6th day NH_4-N decreased gradually from 3,6 mg/lt to 1,7 mg/lt. As a result of this decrease in the concentration, the efficiency increased from 65% to 83%.

Variation of effluent NH_4-N concentration and removal efficiency with time at 20 mg/lt NH_4-N concentration are given in Figure 3.53. As seen in the Figure, NH_4-N decreased gradually from 20 mg/lt to 5 mg/lt with 75% removal efficiency. In the last 3 days of operation, effluent NH_4-N concentration was almost stable at about 5 mg/lt.

Initial NH_4-N concentration was adjusted to 30 mg/lt. Figure 3.54 indicates the variation of effluent NH_4-Nconcentration and NH_4-N removal efficiency with time. The effluent NH_4-N concentration decreased from 17 mg/lt to 6,5 mg/lt during 6 days of continuous operation. However, then a slight increase to around 9 mg/lt in

the effluent concentration was observed. As a result the efficiency decreased from 79% to 68%.

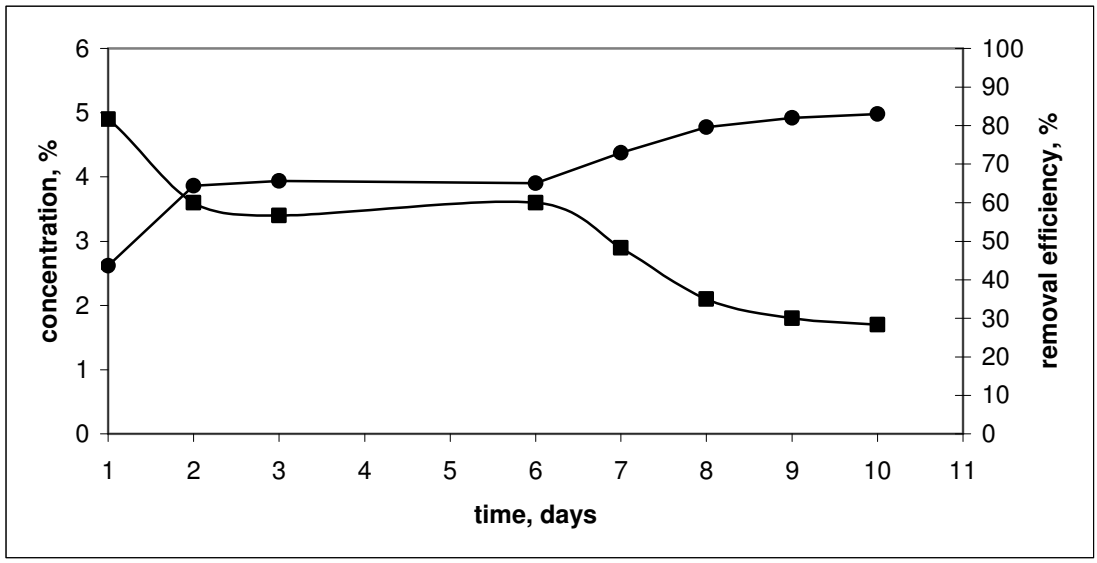


Figure 3.53 Variation of effluent NH_4-N concentration and removal efficiency with time at initial NH_4-N=10±1 mg/lt, θ_H =2,5 days (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

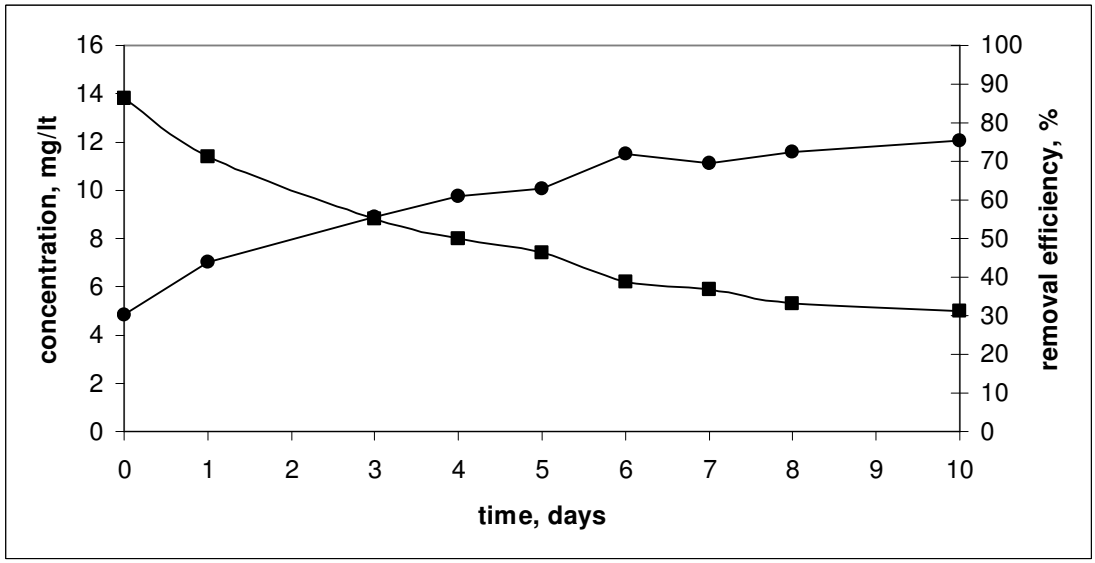


Figure 3.54 Variation of effluent NH_4-N concentration and removal efficiency with time at initial NH_4-N=20±1 mg/lt, θ_H =2,5 days (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

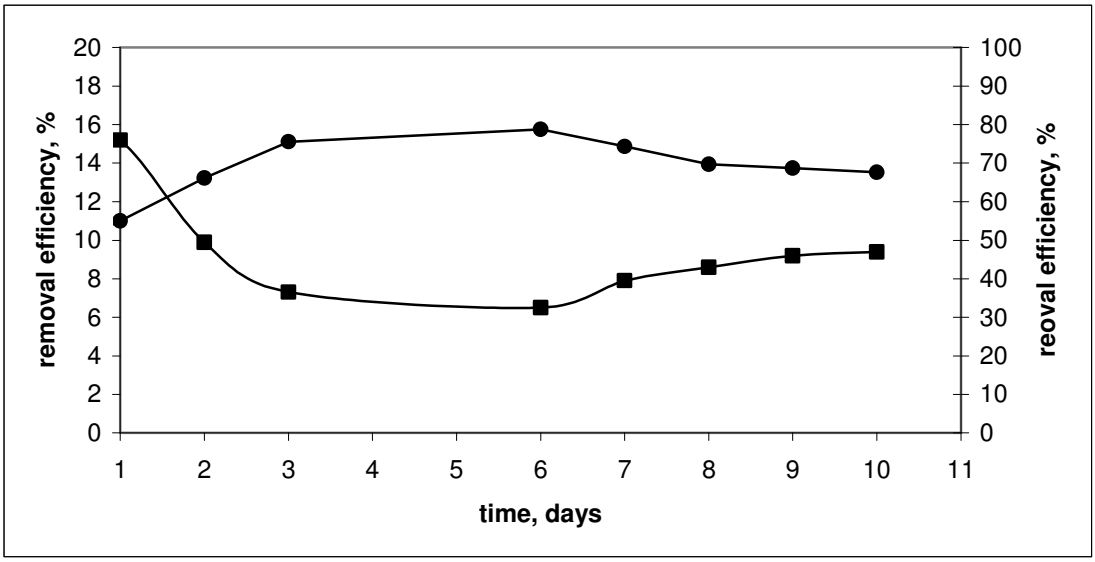


Figure 3.55 Variation of effluent NH_4-N concentration and removal efficiency with time at initial NH_4-N=30±1 mg/lt, θ_H =2,5 days (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

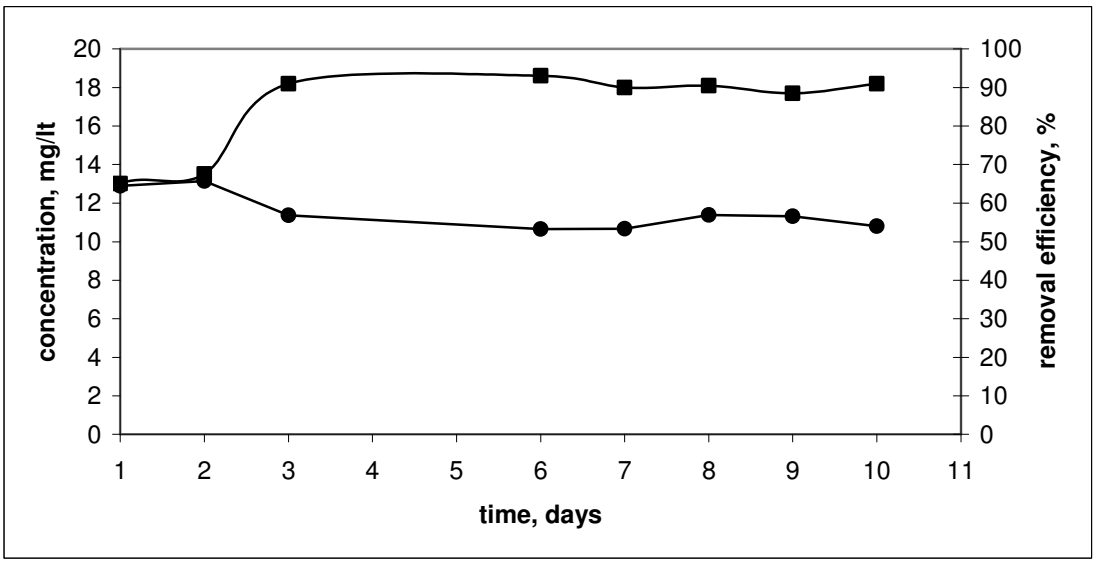


Figure 3.56 Variation of effluent NH_4-N concentration and removal efficiency with time at initial NH_4-N=40±1 mg/lt, θ_H =2,5 days(—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

Initial NH_4-N concentration was further increased to around 40 mg/lt. Variation of effluent NH_4-N concentration and NH_4-N removal efficiency with time at that initial NH_4-N concentration is seen in Figure 3.55. The effluent concentration was around 13 mg/lt because of the previous experiment. Then effect of high initial concentration was observed and the effluent concentration increased to 18 mg/lt. No significant change was observed for the rest of operation. As a result the removal efficiency was around 54%.

Finally, initial NH_4-N concentration was increased to 50±1 mg/lt. The performance of the system significantly reduced. Effluent NH_4-N concentration varied around 32 mg/lt for the first 5 days of continuous operation. It slightly decreased to around 26±1 mg/lt for the rest of operation. So, final efficiency was 49% (Figure 3.56).

Figure 3.57 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 increases from 1,7 mg/lt to 25 mg/lt with respect to increase in initial NH_4-N concentrations from 10 mg/lt to 49 mg/lt as expected. Depending on that variation in effluent NH_4-N, removal efficiency decreases from 83% to 48,6%, respectively. These results indicate that, *Chlorella vulgaris* microalgal culture can efficiently remove low initial NH_4-N concentrations ($[NH_4\text{-}N]_o \leq$ 20 mg/lt). Higher concentrations ($[NH_4\text{-}N]_o$=50 mg/lt) significantly affect NH_4-N removal performance of the system resulting in decreasing in removal efficiency around 48,6% with effluent NH_4-N concentration of 25 mg/lt. It can be concluded that *Chlorella vulgaris* containing immobilized photobioreactor should be operated with 10 mg/lt initial NH_4-N concentration in order to obtain higher that 83% removal efficiency. However, 75% removal efficiency around 20 mg/lt initial NH_4-N concentrations can be evaluated as high NH_4-N removal performance for the *C.vulgaris* immobilized photobioreactor.

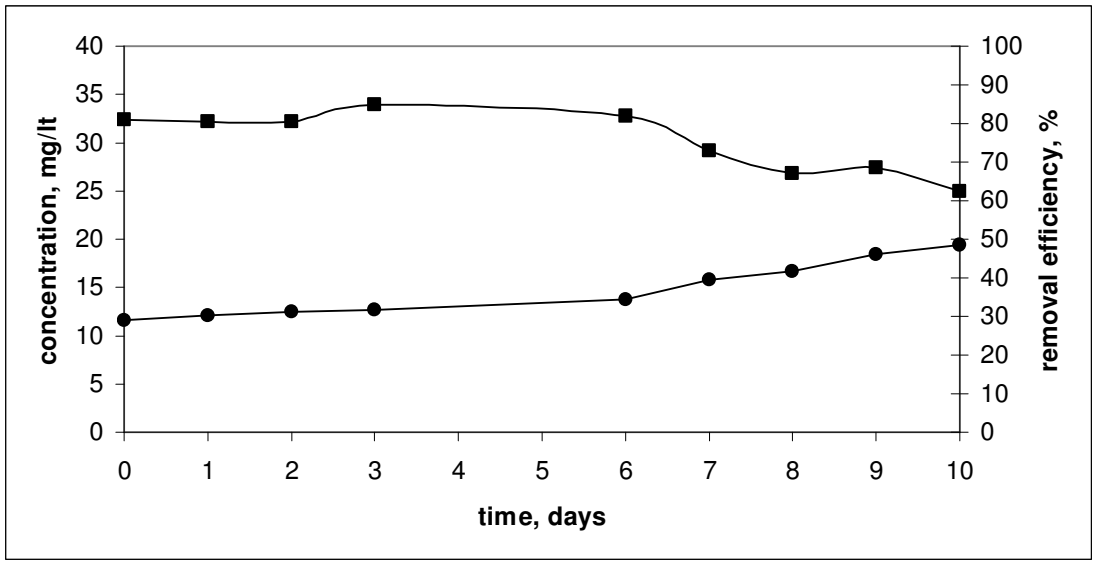


Figure 3.57 Variation of effluent NH_4-N concentration and removal efficiency with time at initial NH_4-N=49±1 mg/lt, θ_H =2,5 days (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

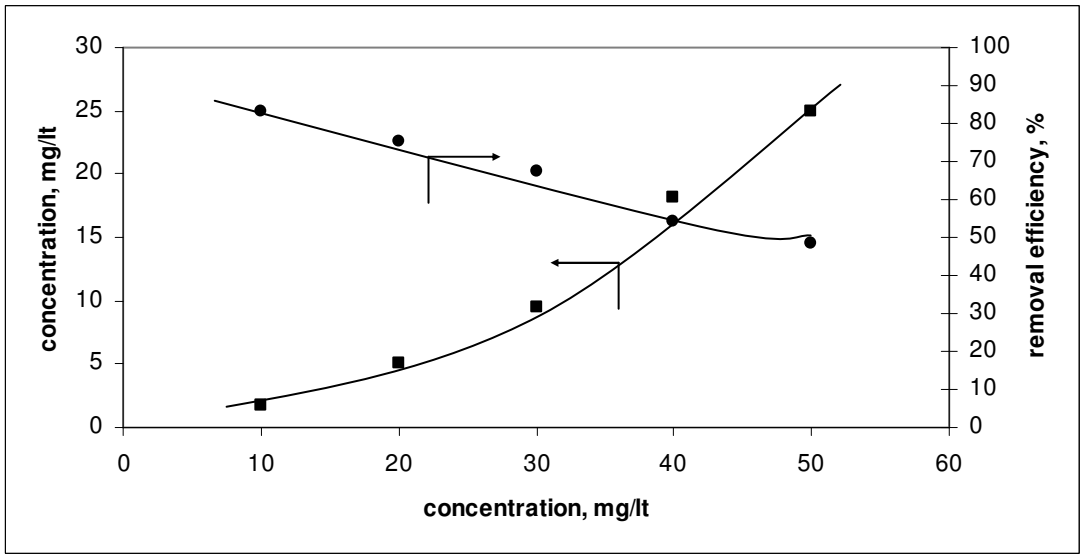


Figure 3.58 Variation of effluent NH_4-N concentrations and removal efficiencies with different initial NH_4-N concentration range (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

3.2.2. Effect of Hydraulic Retention Time on NH_4-N Removal Performance of Continuous Immobilized Photobioreactor System

In order to determine the effect of hydraulic retention time on nutrient removal performance of immobilized photobioreactor operated with *Chlorella vulgaris*, hydraulic retention time was gradually increased from 1,5 days to 5,5 day. Although the maximum NH_4-N removal was obtained at around $[NH_4\text{-}N]_o$=10 mg/lt, the initial NH_4-N concentration was adjusted to $[NH_4\text{-}N]_o$=20 mg/lt to evaluate the system performance at high NH_4-N concentrations. Light intensity, L/D cycle and N/P ratio was kept constant at about 2700 lux, 20h/4h and 8, respectively. The variation of effluent NH_4-N concentration and NH_4-N removal efficiencies with time at different hydraulic retention times were given through Figures 3.58-3.62, and results are summarized in Figure 3.63.

At the beginning of this part of experiment, nitrogen removing performance of *Chlorella vulgaris* at 1,5 days hydraulic retention time was examined. Variation of effluent NH_4-N concentration and NH_4-N removal efficiency at θ_H=1,5 days is showed in Figure 3.58. Initial NH_4-N concentration was adjusted to about $NH_4\text{-}N_o$=19±1 mg/lt. In the first 6 days of experiment, an increase in removal efficiency from 27% to 42% was observed. However, then a decrease occurred in removal rate as from 42% to 34%. As seen from the Figure, no significant removal was obtained at 1,5 days hydraulic retention time.

Figure 3.59 depicts variation of effluent NH_4-N concentration and NH_4-N removal efficiency with time at θ_H=2,5 days. Initial NH_4-N concentration was measured at about 20 mg/lt. As seen from the Figure, effluent NH_4-N decreased gradually from 20 mg/lt to 5 mg/lt. Depending on this decrease NH_4-N removal efficiency increased from 30% to 75,4%.

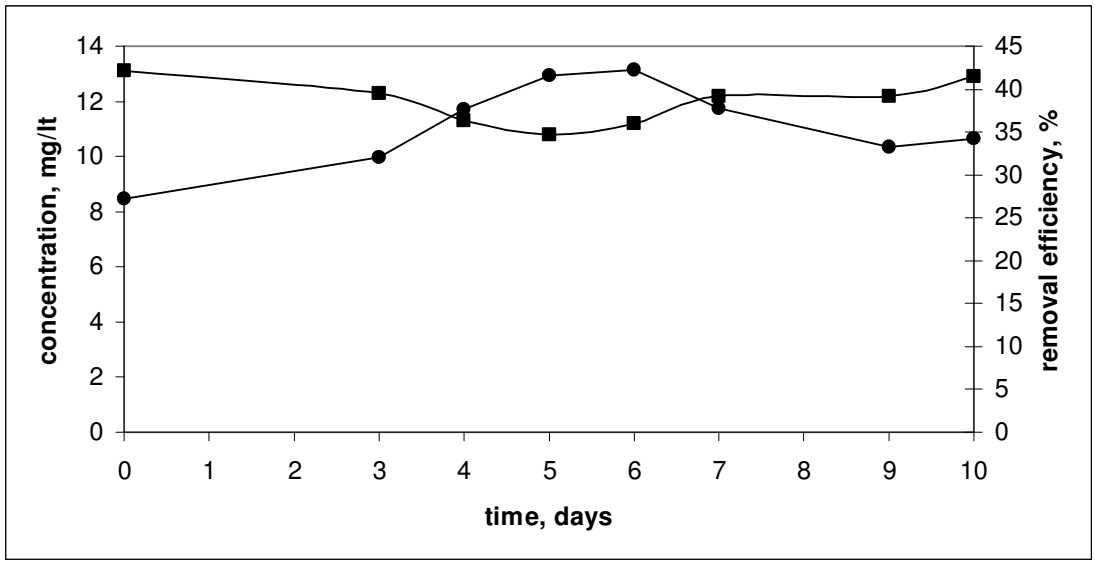


Figure 3.59 Variation of effluent NH_4-N concentration and removal efficiency with time at θ_H =1,5 days (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

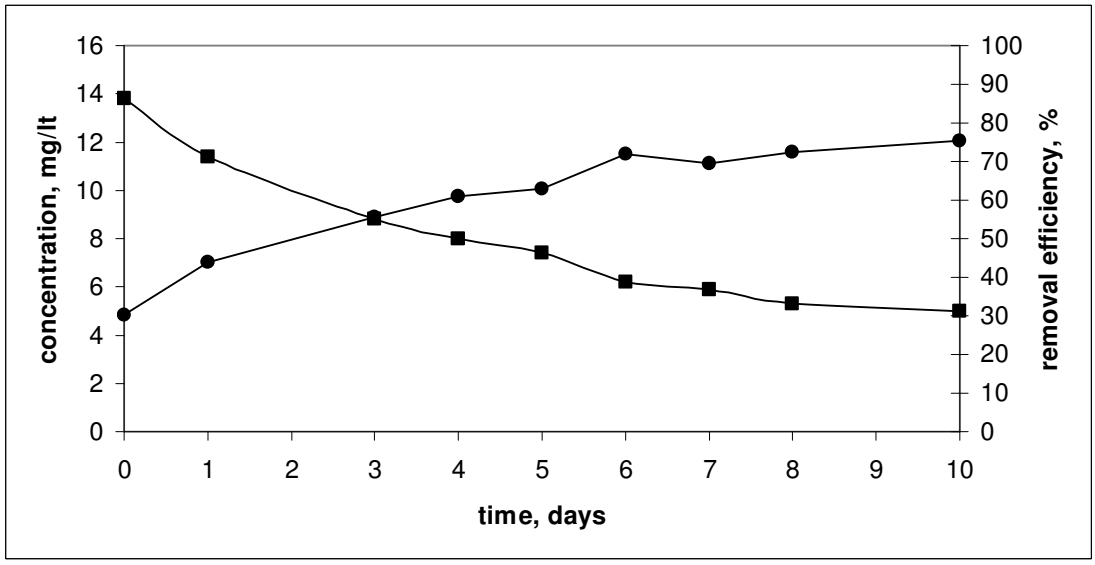


Figure 3.60 Variation of effluent NH_4-N concentration and removal efficiency with time at θ_H =2,5 days (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

Variation of effluent NH_4-N concentration and removal efficiency with time at 3,5 days hydraulic retention time are indicated in Figure 3.60. As seen in Figure, effluent NH_4-N concentration was almost constant at about 4,6 mg/lt. As a result, removal efficiencies varied between 75%-78%.

Hydraulic retention time increased to 4,5 days and the results are given in Figure 3.61. The concentration of ammonia nitrogen was reduced from 20 mg/lt to 2,3 mg/lt in the first 2 days of continuous operation. Then no significant variation in effluent nitrogen concentration was observed. Under the steady state condition, the removal efficiency was about 85%.

Final hydraulic retention time of this set of experiment was 5,5 days. Variation of effluent NH_4-N concentration and NH_4-N removal efficiency are shown in Figure 3.61 as seen in Figure 3.62, removal efficiency was almost stable at 85% in the first 5 days of batch experiment. For the rest of operation, removal efficiency was stable at about 93% removal efficiency. Final NH_4-N concentration decreased to 1,4 mg/lt from about 20 mg/lt.

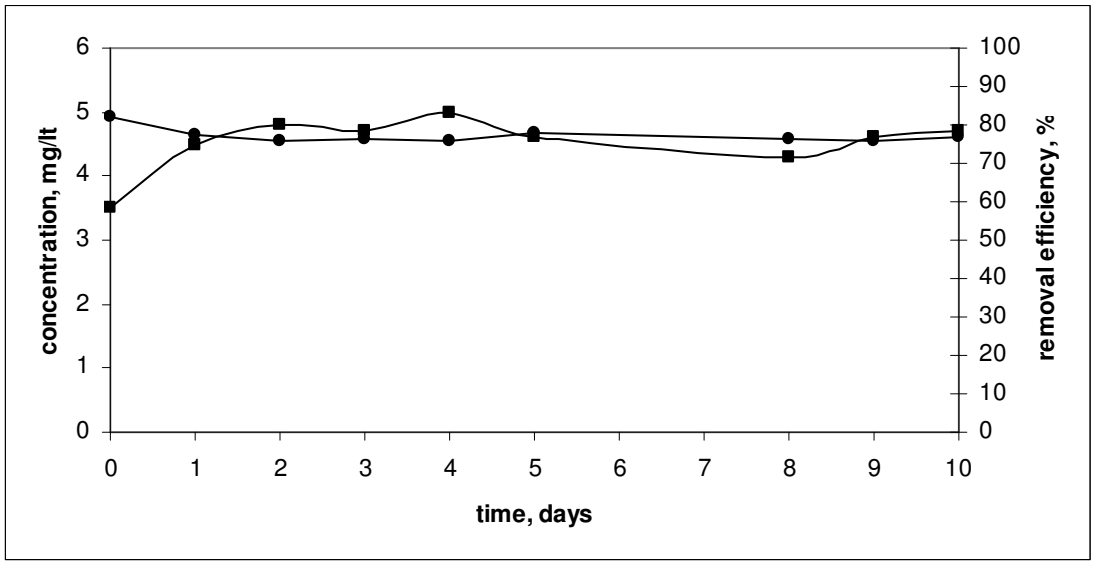


Figure 3.61 Variation of effluent NH_4-N concentration and removal efficiency with time at θ_H =3,5 days (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

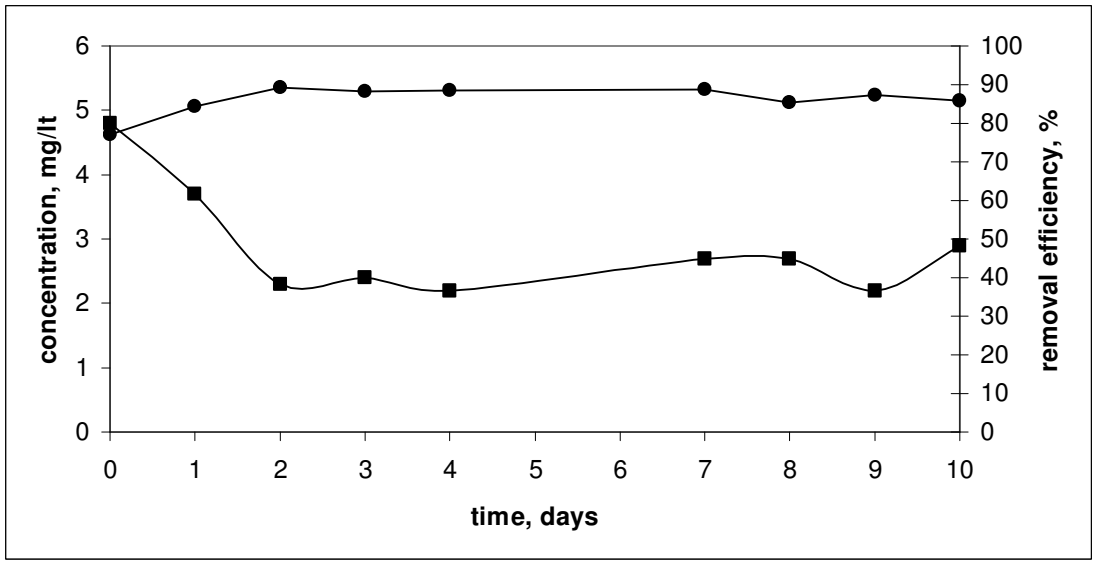


Figure 3.62 Variation of effluent NH_4-N concentration and removal efficiency with time at θ_H =4,5 days (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

The effect of hydraulic retention time on the NH_4-N removal in photobioreactor is summarized in Figure 3.63. Effluent NH_4-N concentration decreased from 12,9 mg/lt to 1,4 mg/lt for the hydraulic retention times between 1,5 days and 5,5 days. Depending on that variation in effluent NH_4-N, removal efficiency increases from 34,2% to 93,7%, respectively. These results indicate that, *Chlorella vulgaris* microalgal culture can efficiently remove NH_4-N in high hydraulic retention times ($\theta_H \geq 2,5$ days) with removal efficiency higher than 75%. However, lower hydraulic retention times ($\theta_H = 1,5$ days) significantly affect NH_4-N removal performance of the system resulting in decreasing in removal efficiency to around 34% with effluent NH_4-N concentration of 12,9 mg/lt. It can be concluded that *Chlorella vulgaris* containing immobilized photobioreactor should be operated with 4,5 days hydraulic retention time in order to obtain higher that 85% removal efficiency. On the other hand, 75% removal efficiency around 2,5 or 3,5 days retention time can be evaluated as high NH_4-N removal performance for the immobilized photobioreactor.

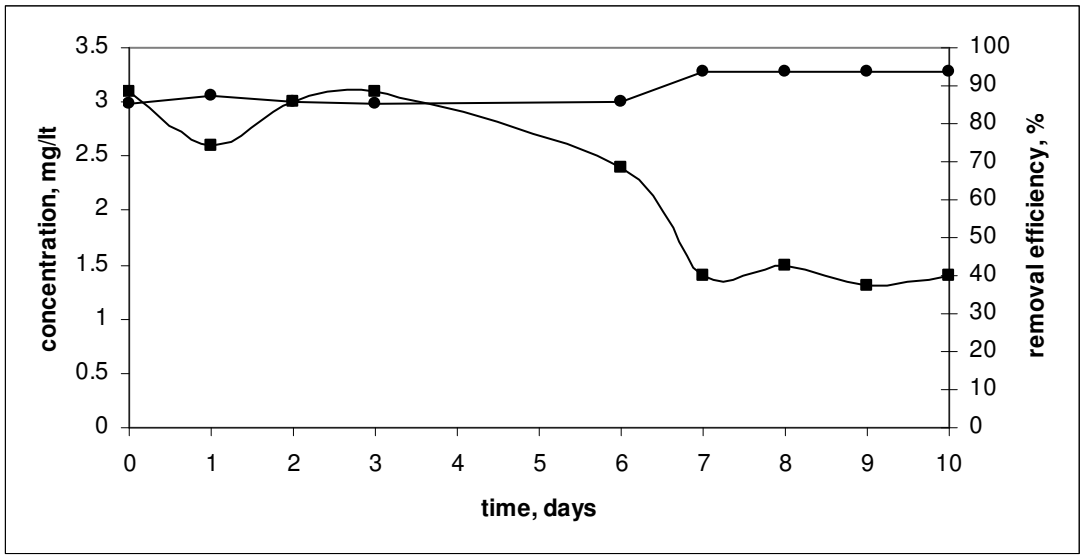


Figure 3.63 Variation of effluent NH_4-N concentration and removal efficiency with time at θ_H =5,5 days (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency).

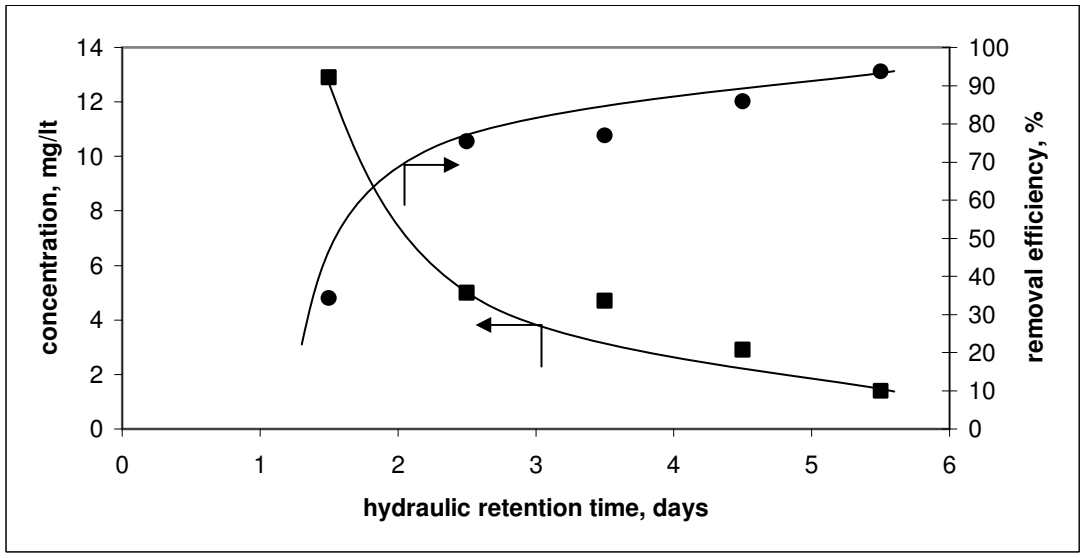


Figure 3.64 Variation of effluent NH_4-N concentrations and removal efficiencies with hydraulic retention time (—■— effluent NH_4-N concentration, —●— NH_4-N removal efficiency.

CHAPTER FOUR
CONCLUSIONS AND DISCUSSIONS

Treatment of synthetic wastewater in batch flasks and continuous immobilized photobioreactor by algal culture *C.vulgaris* was investigated. Batch experiments were conducted at different experimental conditions such as nutrient concentration, light/dark cycle, light intensity, nitrogen/phosphorus ratio and initial ChA concentration. Immobilized photobioreactor system was operated continuously under different NH_4-N concentrations and hydraulic retention times. Major variables considered in this thesis were NH_4-N, PO_4-P and ChA concentrations.

Effect of high ammonia nitrogen and phosphorus concentration on removal of these nutrients from synthetic wastewater by algae *C. vulgaris* in batch cultures were investigated at the beginning of the study and kinetic coefficients were determined. Experimental results indicated that *C. vulgaris* can completely remove up to 21,2 mg/lt NH_4-N ammonia- nitrogen concentration. However the tolerance for PO_4- P was lower. The culture was able to remove only 7,7 mg/lt initial PO_4-P concentration with 78% efficiency. The main reason for low removal performance at high nutrient concentrations could be low initial biomass or ChA concentrations, since algal treatment of wastewater is based on uptake of nutrients into biomass. The other reasons could be the sub-optimized conditions such as light intensity, N/P ratio. It was concluded that more studies are necessary to elucidate the mechanism of phosphorus metabolism such as phosphate transport, phosphate hydrolysis and ATP formation from phosphate in algae.

Based on the experimental data, batch kinetic coefficients of ammonia nitrogen removal by *C. vulgaris* were determined as k= 87,5 mg NH_4-N/g X d and K_m= 367 mg/lt. Similarly, kinetic coefficients for PO_4-P removal were found as k= 8,6 mg PO_4-P/g X d and K_m= 28 mg/lt. So, ammonia nitrogen removal rate is higher than that of phosphorus. This result is in agreement with the literature. Because nitrogen need is higher than phosphorus for algal growth. Yield coefficient for NH_4-N was Y_N =13,7 g X/g NH_4-N and Y_P= 52,3 g X/g PO_4-P for phosphorus.

In order to optimize the other growth conditions, batch operation was carried out at different L/D cycle, light intensity, N/P ratio, and ChA concentrations. The experimental results indicated that continuous illumination is not necessary to enhance nitrogen removal. The organisms need a kind of resting period to for metabolic activity.

High light intensities, up to 7250 lux, improve the effluent ammonia nitrogen concentration and initial specific removal rates, significantly. Increasing light intensity enhanced the phosphorus removal by *C. vulgaris*. However, the effect of light intensity on phosphorus uptake was not as strong as that is in ammonia nitrogen. No significant phosphorus uptake was observed up to 1030 lux. The highest removal was observed at 4100 lux with 61% removal efficiency.

The minimum N/P ratio for almost complete ammonia nitrogen removal was found to be N/P=6. Higher ratios gave approximately the same result, but they provide increasing in specific substrate removal rate. As a result, time period necessary for nitrogen removal is reduced from 10 days to 6 days in batch operation. Although, N/P= 2 resulted in around 94% nitrogen removal, specific ammonia nitrogen removal rate was significantly reduced resulting in longer operation period requirement for complete removal. The results indicated that alga is able to remove nitrogen efficiently under excess nitrogen but limited phosphate concentrations.

Phosphorus removal efficiency was lower compared to nitrogen removal by *C. vulgaris* in all experimental conditions. The maximum phosphorus removal efficiency was obtained as 79% at N/P=5,7, L/D= 20h/4h. Increasing ChA concentration did not provide any improvement in algal phosphorus uptake. However, higher light intensities (>7250 lux) and N/P ratios (>10) may enhance phosphorus removal. Therefore, further studies should be conducted to optimize the conditions for phosphorus removal.

In summary for batch experiments, *C. vulgaris* can remove up to 40 mg/lt ammonia nitrogen completely from synthetic wastewater under the optimized

experimental conditions of this study. The most important factors affecting algal nutrient uptake were determined as light intensity and N/P ratios among the examined. ChA concentration could be more effective in removing higher ammonia nitrogen concentrations (NH_4-N> 40 mg/lt).

Continuous operation of an immobilized photobioreactor system provided effective nutrient removal. Effluent NH_4-N concentrations increased from 1,7 mg/lt to 25 mg/lt with respect to increase in initial NH_4-N concentrations from 10 mg/lt to 45 mg/lt at θ_H= 2,5 days, as expected. The maximum removal efficiency was obtained as 83% at 10 mg /lt NH_4-N concentrations. These results indicate that, *Chlorella vulgaris* microalgal culture can efficiently remove low initial NH_4-N concentrations ($[NH_4\text{-}N]_o \leq$ 20 mg/lt). Higher concentrations ($[NH_4\text{-}N]_o$=50 mg/lt) significantly affect NH_4-N removal performance of the system resulting in decreasing in removal efficiency around 48,6%. It can be concluded that *Chlorella vulgaris* containing immobilized photobioreactor should be operated with 10 mg/lt initial NH_4-N concentration in order to obtain higher that 83% removal efficiency. However, 75% removal efficiency around 20 mg/lt initial NH_4-N concentration can be evaluated as high NH_4-N removal performance for the immobilized photobioreactor.

Increasing hydraulic retention time provided better effluent quality in terms of ammonia nitrogen. Effluent NH_4-N concentration decreased from 12,9 mg/lt to 1,4 mg/lt when the hydraulic retention times increased from 1,5 to 5,5. As a result over 93%, removal efficiency was obtained at θ_H= 5,5 days. However, lower hydraulic retention times (θ_H=1,5 days) significantly affect NH_4-N removal performance of the system resulting in decreasing in removal efficiency to around 34% with effluent NH_4-N concentration of 12,9 mg/lt. It can be concluded that *Chlorella vulgaris* containing immobilized photobioreactor should be operated with 4,5 days hydraulic retention time in order to obtain higher that 85% removal efficiency. On the other hand, 75% removal efficiency for 20 mg/lt NH_4-N around 2,5 or 3,5 days retention time can be evaluated as high NH_4-N removal performance for the immobilized

photobioreactor. The system performance can be improved by optimization of other parameters such as light intensity and liquid to surface ratio.

Phosphorus removal in photobioreactor system was not efficient. The effluent phosphate concentration fluctuated significantly and the system did not reach steady state in terms of phosphate. Effluent concentration exceeded the influent concentration in some experiments. We expect that phosphate was periodically released by the death algae and up-taken with the other living ones. Another explanation for this result could be precipitation and dissolution of the precipitate in the photobioreactor.

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.

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. However, the process needs further scientific research in order to optimize the process parameters and to evaluate the full scale application.

CHAPTER FIVE
RECOMMENDATIONS

Algal nutrient system is a quite well studied subject. But, the process needs kinetic coefficients determination studies and development of new photobioreactor systems. The thesis aimed to fulfill these two needs in the algal wastewater treatment process. Batch experiments gave satisfying results in understanding the process parameters. However, 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 batch experiments,
2. The effect of presence of carbon on nutrient removal by algae should be evaluated,
3. The batch operation can be conducted at higher light intensities to determine the maximum light intensity which causes photoinhibition.
4. The available light intensity by algal culture may change with the thickness of biofilm in the photobioreactor. Although this study was carried out at 7000 lux the light intensity in the reactor was around 2700 lux. Therefore, the effect of higher light intensities (>7000 lux) should be studied,
5. The effect immobilization surface area to liquid volume ratio on nutrient removal in photobioreactor should be investigated,
6. Different microorganism support materials with higher surface are could be used in the system,
7. Mixing conditions and CO_2 availability can be improved in the photobioreactor,
8. Nutrient content of the studied algal culture could be measured to evaluate if the waste algal sludge can be used as fertilizer and animal feed,

9. The other algal cultures which has higher nutrient uptake capacity can be used for this purpose,
10. Outdoor experiments at different seasons can be conducted to observe behavior of the system under natural conditions,
11. The system can be operated with real wastewater after the operation parameters are optimized.

REFERENCES

An, J., Sim, S., Lee, J.S. ,& Kim, B.W. (2003). Hydrocarbon production from secondarily treated piggery wastewater by the green alga Botryococcus brauni. *Journal of Applied Phycology*, 15, 185-191.

Anderson, G.A., Kommareddy, A.,& Schipull, M.A. (2002). Photobioreactor Design. *The society for Engineering in Agricultural, food and Biological Systems*. An ASAE/CSAE Meeting Presentation, Paper no: MBSK02-216.

Becker E.W, (1994). *Microalgae Biotechnology and Microbiology*. London, Cambridge University Press.

Bitch, N.N., Yaziz, M.I., & Baktı,N.A.K. (1999). Combination of Chlorella and Eichhornia Crassipes for wastewater nitrogen removal. *Water Research*, 33, 10, 2357-2362.

Cattaneo, C., Zolezzi, M., Rovatti, P., Fabiano, M., Povero, P., & Rovatti, M. (n.d.). Growth and nutrient uptake of microalgae (Nannochloropsis Sp.) in a tubular photobioreactor.

Craggs, R.J., McAuley, P.J., & Smith, V.J. (1997). Wastewater nutrient removal by marine microalgae grown on a corrugated raceway. Water Research, 31, 7, 1701-1707.

De-Bashan, L.E., Moreno, M., Hernandez, J.,& Bashan, Y. (2002). Removal of ammonium and phosphorus ions from synthetic wastewater by the microalgae

Chlorella vulgaris coimmobilized in alginate beads with the microalgae growth-promoting bacterium Azospirillum brasilense. *Water Research*, 36, 2941-2948.

De-Bashan, L:E:, Hernandez, J.P, Morey, T. & Bashan Y. (2004). Microalgae growth promoting bacteria as "helpers" for microalgae: a novel approach for removing ammonium and phosphorus from municipal wastewater. *Water Research*, 38, 466-474.

Dumas, A., Laliberte, G., Lessard, P., & de la Noüe, J. (1998). Biotreatment of fish farm effluents using the cyanobacterium phormidium bohneri. *Aquaculture Engineering* 17, 57-68.

Fuentes, M.M.R., Sanchez, J.L.G., Sevilla,J.M.F., Fernandez, F.G.A., Perez, J.A.S., & Grima, E.M. (1999). Outdoor continuous culture of Phorphydridium cruentum in a tubular photobioreactor: Quantitative analysis of the daily cyclic variation of culture parameters. *Journal of Biotechnology*, 70, 271-288.

Gitelson, A., Qiuang, H., & Richmond, A. (1996). Photic volume in photobioreactors supporting ultrahigh population densities of the photoautotroph Spirulina platensis. *Applied and Environmental Microbiology*, 1570-1573.

Gonzales, L.E., Canizares, R.O., & Baena, S. (1997). Efficiency of ammonia and phosphorus removal from a Colombian agroindustrial wastewater by the microalgae Chlorella vulgaris and Scenedesmus dimorphus. *Biosource Technology*, 60, 259-262.

Hu, Q., Westerhoff, P., & Vermaas, W. (2000). Removal of nitrate from groundwater by cyanobacteria: Quantitative assessment of factors influencing nitrate uptake. *Applied and Environmental Microbiology*, 133-139.

Kargi, F., & Uygur, A.(2003). Effect of carbon source on biological nutrient removal in a sequencing batch reactor. *Biosource Technology,* 89, 89-93.

Kim,J.K., Lee, B., Kim, S., & Moon, J. (1999). Characterization of denitrifying photosynthetic bacteria isolated from photosynthetic sludge. *Aquaculture Engineering*, 19, 179-193.

Laliberte, G., Lessard, P., de la Noüe, J., & Sylvestre, S. (1997). Effect of phosphorus addition on nutrient removal from wastewater with the cyanobacterium Phormidium bohneri. *Biosource Technology,* 59, 227-233.

Lee, K., & Lee, C. (2001). Effect of ligth/dark cycles on wastewater treatments by microalgae. biotechnology, *Biotechnology, Bioprocess Engineering*, 6, 194-199.

Martinez Sancho, M.E., Jimenez Castillo, J.M., & El Yousfi, F. (1999). Photoautotrophic consumption of phosphorus by Scenedesmus obliguus in a continuous culture. Influence of light intensity. *Process Biochemistry*, 34, 811-818.

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

Metcalf , & Eddy Inc., 1991. *Wastewater Engineering Treatment Disposal and Reuse*, (3rd ed.), Mc Graw-Hill, Singapore, 532-537.

Ogbonna, J.C., & Tanaka, H. (1997). Industrial-size photobioreactors. *Chemtech* 27, 43-49.

Olguin, E.J., Galicia, S., Angulo-Guerrero, O., & Hernandez,E. (2001). The effect of low light flux and nitrogen deficiency on the chemical composition of Spirulina sp. (Arthrospira) grown on digested pig waste. *Biosource Technology*, 77, 19-24.

Olguin, E.J., Galicia, S., Mercado, G., & Perez, T. (2003). Annual productivity of Spirulina (Arthrospira) and nutrient removal in a pig wastewater recycling process under tropical conditions. *Journal of Applied Phycology*,15, 249-257.

Picard, C.R., Fraser, L.H., & Steer, D., 2005. The interacting effects of temperature and plant community type on nutrient removal in wetland microcosms. *Bioresource Technology*, 96,1039-1047.

Pulz, O. (2001). Photobioreactors: production systems for phototrophic microorganisms. © *Springer-Verlag*. **Retrieved** September 2, 2005, **from** http://www.igv-gmbh.de/e/reference3.pdf

Tam, N.F.Y., & Wong, Y.S. (2000). Effect of immobilized microalgal bead concentrations on wastewater nutrient removal. *Environmental Pollution*,107, 145-151.

Travieso, L., Benitez, F., Weiland, P., Sanchez, E., Dupeyron, R., & Dominguez, A.R. (1996). Experiments on immobilization of microalgae for nutrient removal in wastewater treatments. Biosource Technology, 55, 181-186.

Valladerma, L.T., Del Campo, C.M., Rodriguez, C.M., de-Bashan, L.E., & Bashan Y. (2002). Treatment of recalcitrant wastewater from ethanol and citric acid production using the microalga Chlorella vulgaris and the macrophyte Lemna minuscula. *Water Research* 36, 4185-4192.

Villasclaras, S.S., Sancho, M.E.M., Caballero, M.T.E, & Perez, A.D. (1996). Production of microalgae from olive mill wastewater. *Internatinal Biodeterrioration & Biodegradation* 245-247.

APPENDICES

RAW DATA OF EXPERIMENTAL STUDIES

TableA.1 Raw data of batch experiment at NH_4-N=13,2 mg/lt

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-Pe (mg/lt)	E_P (%)
0	3,2034	0,05	13,2	13,2	0,0	7,7	7,7	0
1	3,2926		13,2	11,05	16,3	7,7	7,1	7,8
2	3,3019		13,2	9,9	25	7,7	6,8	11,7
3	3,7783		13,2	5,95	54,9	7,7	6,6	14,3
6	7,0123		13,2	2	84,8	7,7	3,5	54,6
7	7,5287		13,2	0,85	93,6	7,7	2,5	67,5
8	8,1454		13,2	0,52	96,1	7,7	1,7	77,9
9	8,1708		13,2	–	–	7,7	–	–
10	10,6709	–	13,2	–	–	7,7	–	–

Table A.2 Raw data of batch experiment at NH_4-N=21,2 mg/lt

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	–	–	21,2	21,2	0,0	15,4	15,4	0
1	3,6554		21,2	20	5,7	15,4	14	9,1
2	4,2038		21,2	16,9	20,3	15,4	13,7	11,04
3	5,0824		21,2	14,9	29,7	15,4	12,8	16,88
4	5,101		21,2	13,9	34,4	15,4	11,7	24,03
7	5,5681		21,2	11,5	45,8	15,4	11,5	25,33
8	6,9988		21,2	10	52,8	15,4	11,1	27,92
9	8,0386		21,2	8,8	58,5	15,4	11,3	26,62
10	8,1551	–	21,2	5,25	75,2	15,4	11,4	25,97

TableA.3 Raw data of batch experiment at NH_4-N=41,8 mg/lt

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	3,0641	0,07	41,8	41,8	0	21,4	21,4	0
1	3,4336		41,8	41,2	1,4	21,4	20,5	4,2
2	3,6216		41,8	38,8	7,2	21,4	20	6,5
3	5,9564		41,8	36,2	13,4	21,4	19,6	8,4
6	–		41,8	32,6	22	21,4	17,7	17,3
7	8,7483		41,8	27	35,4	21,4	15	30
8	9,7685		41,8	24	42,6	21,4	14,6	31,8
9	10,6709		41,8	20,8	50,2	21,4	12,9	39,7
10	10,7514	–	41,8	17,6	57,9	21,4	10,8	49,5

Table A.4 Raw data of batch experiment at NH_4-N=59 mg/lt

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-Ne (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-Pe (mg/lt)	E_P (%)
0	–	–	59	59	0,0	33	33	0,0
1	3,2352		59	58,6	0,7	33	31,8	3,6
2	5,314		59	58	1,7	33	29,5	10,6
3	5,4863		59	51,5	12,7	33	28,4	13,9
4	5,5321		59	48,25	18,2	33	28,4	13,9
7	6,7669		59	45,25	23,3	33	25,6	22,4
8	6,8844		59	44,25	25,0	33	26	21,2
9	6,9918		59	43	27,1	33	26	21,2
10	7,1513	–	59	39,6	32,9	33	26	21,2

TableA.5 Raw data of batch experiment at NH_4-N=74,7 mg/lt

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	–	–	74,7	74,7	0	41,8	41,8	0
1	4,6169		74,7	72	3,6	41,8	39,8	4,8
2	7,5273		74,7	–	–	41,8	–	–
3	8,0109		74,7	68,1	8,84	41,8	36,2	13,4
6	8,6795		74,7	67,8	9,24	41,8	35	16,3
7	10,8514		74,7	56,1	25	41,8	32,6	22
8	10,2882		74,7	51,9	30,5	41,8	33	21,1
9	11,9034		74,7	49,2	34,14	41,8	33	21,1
10	11,9358	–	74,7	46	38,4	41,8	32,6	22

Table A.6 Raw data of batch experiment at NH_4-N=92,8 mg/lt

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	–	–	92,8	92,8	0	50	50	0
1	4,5182		92,8	89,6	3,45	50	49,8	0,4
2	7,5273		92,8	–	–	50	–	–
3	8,0109		92,8	86,8	6,5	50	45,2	9,6
4	8,2238		92,8	82,8	10,8	50	44,4	11,2
7	10,1191		92,8	79,2	14,7	50	42	16
8	11,0342		92,8	70	24,6	50	41,4	17,2
9	11,8758		92,8	66	28,9	50	40,6	18,8
10	11,9345	–	92,8	59,1	36,3	50	41,6	16,8

TableA.7 Raw data of batch experiment at NH_4-N=130 mg/lt

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	3,1068	0,1	130	130	0	60,4	60,4	0
1	3,6389		130	124,2	4,2	60,4	60,4	0
2	3,9411		130	123,6	4,6	60,4	59,2	2
3	6,0059		130	121,8	6,0	60,4	58,6	3
6	–		130	115,2	11,1	60,4	57,6	4,6
7	10,6291		130	108	16,7	60,4	55,2	8,6
8	11,1228		130	107,4	17,1	60,4	52,8	12,6
9	11,558		130	104,4	19,4	60,4	52	14
10	12,1462	–	130	98,4	24,1	60,4	49,2	18,54

Table A.8 Raw data of batch experiment at NH_4-N=149,3 mg/lt

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	3,0575	0,13	149,3	149,3	0	71,6	71,6	0
1	3,6718		149,3	149,1	0,1	71,6	70,4	1,7
2	5,0927		149,3	142,8	4,4	71,6	70,4	1,7
3	5,9506		149,3	142,8	4,4	71,6	68,8	4
4	–		149,3	136,5	8,6	71,6	65,2	9
7	11,8715		149,3	128,8	13,7	71,6	61,6	14
8	12,2450		149,3	124,6	16,5	71,6	60,8	15
9	12,6717		149,3	120,4	19,4	71,6	58,4	18,4
10	12,7925	–	149,3	110,6	25,9	71,6	56,4	21,2

TableA.9 Raw data of batch experiment at NH_4-N=192 mg/lt

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	3,1209	0,16	192	192	0	93,5	93,5	0
1	3,56		192	182,4	5	93,5	91,5	2,14
2	5,2721		192	176,8	7,9	93,5	92,5	1,1
3	8,5391		192	169,2	11,9	93,5	92,5	1,1
6	–		192	159,8	16,8	93,5	85	9,1
7	12,7116		192	158,4	17,5	93,5	83,5	10,7
8	12,7256		192	157,2	18,1	93,5	82	12,3
9	12,7686		192	156,8	18,3	93,5	81,5	12,8
10	12,8866	–	192	152	20,8	93,5	79	15,5

Table A.10 Raw data of batch experiment at NH_4-N=282 mg/lt

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	4,3825	0,07	282	282	0	139	139	0
1	4,8492		282	270	4,3	139	131	5,8
2	5,4608		282	264	6,4	139	127	8,6
3	8,9928		282	239	15,2	139	121	13
4	13,0761		282	224	20,6	139	120	13,7
7	18,5168		282	215	23,8	139	120	13,7
8	18,7886		282	211	25,2	139	120	13,7
9	19,3936		282	205	27,3	139	119	14,4
10	23,0898	–	282	196	30,5	139	118	15,1

TableA.11 Raw data of batch experiment at NH_4-N=316 mg/lt

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	3,8796	0,06	316	316	0	149	149	0
1	4,4947		316	302	4,4	149	147	1,34
2	5,5713		316	294	7	149	145	2,7
3	8,3748		316	287	9,2	149	144	3,4
6	12,9881		316	264	16,5	149	142	4,7
7	17,5084		316	245	22,5	149	142	4,7
8	17,9698		316	243	23,1	149	141	5,4
9	18,3538		316	238	24,7	149	140	6
10	18,2884	–	316	237	25	149	140	6

Table A.12 Raw data of batch experiment at NH_4-N=388 mg/lt

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	3,2544	0,07	388	388	0	175	175	0
1	3,6558		388	356	8,2	175	173	1,1
2	5,4005		388	336	13,4	175	170	2,9
3	8,0383		388	330	14,9	175	168	4
4	18,5692		388	308	20,6	175	166	5
7	20,257		388	300	22,7	175	165	5,7
8	23,746		388	299	22,9	175	164	6,3
9	23,306		388	266	31,4	175	163	6,9
10	23,2965	–	388	265	31,7	175	161	8

Table A.13 Raw data of batch experiment at NH_4-N=410 mg/lt

Time (days)	**ChA (mg/lt)**	**Biomass (g/lt)**	**NH_4-N_o (mg/lt)**	**NH_4-N_e (mg/lt)**	**E_N (%)**	**PO_4-P_o (mg/lt)**	**PO_4-P_e (mg/lt)**	**E_P (%)**
0	3,4495	0,06	410	410	0	199	199	0
1	4,0423		410	406	1	199	191	4
2	5,5392		410	388	5,4	199	190	4,5
3	8,3008		410	346	15,6	199	188	5,5
4	12,9146		410	343	16,3	199	187	6
7	18,6114		410	341	16,8	199	186	6,5
8	19,5674		410	326	20,5	199	185	7
9	20,896		410	311	24,1	199	183	8
10	22,7972	–	410	298	27,3	199	182	8,5

Table A.14. Raw data of batch experiment at different initial NH_4-N concentrations.

NH_4-N_o (mg/lt)	**NH_4-N_e (mg/lt)**	**E_P (%)**
10	0,52	96,1
20	5,25	73,8
40	17,6	57,9
60	39,6	32,8
70	46	34,3
90	59,1	34,3
125	98,4	24,1
150	110,6	25,9
190	152	20,8
280	196	30,5
316	237	25,0
388	265	31,7
410	298	27,3

Table A.15 Raw data of batch experiment at light/dark cycle=12h/12h

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	2,7439	0,082	39,4	39,4	0,0	19,4	19,4	0
1	3,8524		39,4	35	11,2	19,4	18,1	6,70
2	5,9332		39,4	31	21,3	19,4	16	17,53
3	7,5277		39,4	26,62	32,4	19,4	15,2	21,65
6	8,6584		39,4	25,4	35,5	19,4	14,8	23,71
8	10,0254		39,4	23,4	40,6	19,4	14,4	25,77
9	10,2364		39,4	20,5	48,0	19,4	13	33
10	10,3362	0,3	39,4	18,2	53,8	19,4	12,8	34,02

Table A.16 Raw data of batch experiment at light/dark cycle=18h/6h

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	2,5767	0,09	43,5	43,5	0,0	19,4	19,4	0
1	5,3984		43,5	37,6	13,6	19,4	17,8	8,25
2	7,1839		43,5	34,6	20,5	19,4	17,8	8,25
3	8,4733		43,5	32,4	25,5	19,4	17,6	9,28
6	9,7049		43,5	25,2	42,1	19,4	15	22,68
8	10,445		43,5	26	40,2	19,4	14,8	23,71
9	9,5523		43,5	22,3	48,7	19,4	14	27,84
10	9,6367	0,3	43,5	19,1	56,1	19,4	12,8	34,02

Table A.17 Raw data of batch experiment at light/dark cycle=20h/4h

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-Pi (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	2,02	0,18	47,2	47,2	0,0	22	22	0
1	4,6832		47,2	46,2	2,1	22	21,2	3,64
2	7,0195		47,2	35	25,8	22	20,6	6,36
3	8,3497		47,2	33,8	28,4	22	19,2	12,73
6	9,0458		47,2	26,2	44,5	22	15,6	29,09
7	10,1645		47,2	23,8	49,6	22	14,8	32,73
8	10,9624		47,2	17,6	62,7	22	12,6	42,73
9	11,8562		47,2	13,8	70,8	22	11,4	48,18
10	12,1698		47,2	10	78,8	22	8,6	60,91

Table A.18 Raw data of batch experiment at light/dark cycle=24h/0h

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	3,0641	0,07	41,8	41,8	0,0	21,4	21,4	0
1	3,4336		41,8	41,2	1,4	21,4	20,5	4,21
2	3,6216		41,8	38,8	7,2	21,4	20	6,54
3	5,9564		41,8	36,2	13,4	21,4	19,6	8,41
6	–		41,8	32,6	22,0	21,4	17,7	17,29
7	8,7483		41,8	27	35,4	21,4	15	29,91
8	9,7685		41,8	24	42,6	21,4	14,6	31,78
9	10,6709		41,8	20,8	50,2	21,4	12,9	39,72
10	10,7514	–	41,8	17,6	57,9	21,4	10,8	49,53

Table A.19. Raw data of batch experiment at different initial L/D cycles.

Light Exposure Time (h)	**NH_4-N_e (mg/lt)**	**E_N (%)**
12	18,2	53,8
18	19,1	56,1
20	10,0	78,8
24	17,6	57,9

Table A.20 Raw data of batch experiment at light intensity=550 lux

Time (days)	**ChA (mg/lt)**	**Biomass (g/lt)**	**NH_4-N_o (mg/lt)**	**NH_4-N_e (mg/lt)**	**E_N (%)**	**PO_4-P_o (mg/lt)**	**PO_4-P_e (mg/lt)**	**E_P (%)**
0	2,1921	0,12	48,4	48,4	0,0	22,4	22,4	0
1	4,3648		48,4	46,6	3,7	22,4	22	1,79
2	5,4		48,4	43,8	9,5	22,4	21,8	2,68
3	6,0359		48,4	42,8	11,6	22,4	21,6	3,57
6	7,3076		48,4	40,8	15,7	22,4	20,8	7,14
7	8,583		48,4	38,4	20,7	22,4	20,6	8,04
8	9,5126		48,4	37,8	21,9	22,4	20,4	8,92
9	10,5396		48,4	37,4	22,7	22,4	20	10,71
10	11,0256	–	48,4	35,8	26,0	22,4	19,8	11,61

Table A.21 Raw data of batch experiment at light intensity=740 lux

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	2,0276	0,1	48	48	0	22,2	22,2	0
1	4,0768		48	46,6	2,9	22,2	22	0,9
2	5,3007		48	42,4	11,7	22,2	21,8	1,8
3	5,5648		48	42	12,5	22,2	21,8	1,8
6	7,1591		48	41,8	12,9	22,2	20,8	6,31
7	9,0041		48	38	20,8	22,2	20,2	9
8	9,5632		48	34,6	27,9	22,2	20	9,91
9	10,9908		48	33,6	30,0	22,2	20	9,91
10	11,3562	–	48	32,2	32,9	22,2	19,6	11,71

Table A.22 Raw data of batch experiment at light intensity=1030 lux

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	2,095	0,08	48,6	48,6	0	22,8	22,8	0
1	4,1507		48,6	46,6	4,1	22,8	22,4	1,75
2	6,5113		48,6	42,6	12,3	22,8	21,8	4,39
3	7,1279		48,6	37,6	22,6	22,8	21,6	5,26
6	9,8154		48,6	36,4	25,1	22,8	20	12,28
7	10,8523		48,6	34	30,0	22,8	19,8	13,16
8	11,2352		48,6	32,2	33,7	22,8	19,8	13,16
9	11,8579		48,6	30,2	37,9	22,8	19,6	14,04
10	12,2451	–	48,6	27,6	43,2	22,8	19,6	14,03

Table A.23 Raw data of batch experiment at light intensity=1610 lux

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	2,0769	0,14	48,4	48,4	0,0	22,4	22,4	0
1	4,8576		48,4	46,6	3,7	22,4	21,6	3,57
2	5,8203		48,4	39,4	18,6	22,4	21,4	4,46
3	6,6339		48,4	36,8	24,0	22,4	20,8	7,14
6	8,4484		48,4	37,4	22,7	22,4	19,4	13,39
7	10,6956		48,4	32,8	32,2	22,4	18,8	16,07
8	11,1252		48,4	31,2	35,5	22,4	18,2	18,75
9	11,5692		48,4	29	40,1	22,4	18	19,64
10	12,2044	–	48,4	24	50,4	22,4	17,6	21,43

Table A.24 Raw data of batch experiment at light intensity=2600 lux

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	2,2492	0,14	48	48	0,0	22,6	22,6	0
1	4,0435		48	46,8	2,5	22,6	22,2	1,77
2	6,181		48	39,6	17,5	22,6	21,4	5,31
3	7,7624		48	37,6	21,7	22,6	21	7,08
6	9,6575		48	35,2	26,7	22,6	19,2	15,04
7	11,3114		48	32,2	32,9	22,6	18,2	19,47
8	11,8562		48	28,2	41,3	22,6	17,8	21,24
9	12,0032		48	26,2	45,4	22,6	17	24,78
10	12,342	–	48	23	52,1	22,6	15,4	31,86

Table A.25 Raw data of batch experiment at light intensity=3380 lux

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	2,2492	0,16	50	50	0,0	22	22	0
1	4,4785		50	46	8,0	22	21,8	0,91
2	6,7818		50	36,2	27,6	22	20,8	5,45
3	7,3065		50	33,4	33,2	22	19,8	10
6	8,539		50	32,2	35,6	22	17,4	20,91
7	10,7782		50	31,4	37,2	22	16,6	24,54
8	11,3254		50	28,2	43,6	22	14,8	32,73
9	11,9654		50	21,2	57,6	22	13,8	37,27
10	12,2541	–	50	14,8	70,4	22	12,6	42,72

Table A.26 Raw data of batch experiment at light intensity=4100 lux

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	1,7805	0,18	47,2	47,2	0,0	22	22	0
1	4,6832		47,2	46,2	2,1	22	21,2	3,64
2	7,0195		47,2	35	25,8	22	20,6	6,36
3	8,3497		47,2	33,8	28,4	22	19,2	12,73
6	9,0458		47,2	26,2	44,5	22	15,6	29,09
7	10,1645		47,2	23,8	49,6	22	14,8	32,73
8	10,9624		47,2	17,6	62,7	22	12,6	42,73
9	11,8562		47,2	13,8	70,8	22	11,4	48,18
10	12,1698	–	47,2	10	78,8	22	8,6	60,91

Table A.27 Raw data of batch experiment at light intensity=7250 lux

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	2,0211	0,21	42,6	42,6	0,0	18,6	18,6	0
1	4,125		42,6	40,6	4,7	18,6	18,6	0
2	5,8769		42,6	29	31,9	18,6	18,4	1,075
3	7,394		42,6	25	41,3	18,6	16,8	9,68
6	9,1536		42,6	22	48,4	18,6	14,2	23,66
7	11,0192		42,6	16,6	61,0	18,6	12,8	31,18
8	11,5623		42,6	10,2	76,1	18,6	11,2	39,78
9	12,0312		42,6	5,2	87,8	18,6	9,9	46,77
10	12,5421	–	42,6	2,6	93,9	18,6	8,2	55,91

Table A.28 Raw data of batch experiment at different light intensities

Light intensity (Lux)	NH_4-N_e (mg/lt)	E_N (%)
550	35,8	26,0
740	32,2	32,9
1030	27,6	43,2
1610	24	50,4
2600	23	52,1
3380	14,8	70,4
4100	10	78,8
7250	2,6	93,9

Table A.29 Raw data of batch experiment at N/P ratio=2/1

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-Ne (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-Pe (mg/lt)	E_P (%)
0	2,0211	0,21	42,6	42,6	0,0	18,6	18,6	0
1	4,125		42,6	40,6	4,7	18,6	18,6	0
2	5,8769		42,6	29	31,9	18,6	18,4	1,1
3	7,394		42,6	25	41,3	18,6	16,8	9,68
6	9,1536		42,6	22	48,4	18,6	14,2	23,66
7	11,0192		42,6	16,6	61,0	18,6	12,8	31,18
8	11,5623		42,6	10,2	76,1	18,6	11,2	39,78
9	12,0312		42,6	5,2	87,8	18,6	9,9	46,77
10	12,5421		42,6	2,6	93,9	18,6	8,2	55,91

Table A.30 Raw data of batch experiment at N/P ratio=3,5/1

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	2,8814	0,08	41,4	41,4	0,0	12,1	12,1	0
1	4,6862		41,4	39	5,8	12,1	11,5	4,96
2	6,7724		41,4	34,2	17,4	12,1	10,7	11,57
5	10,6824		41,4	10	75,8	12,1	8,3	31,41
6	19,9802		41,4	3,9	90,6	12,1	7,5	38,02
7	20,371		41,4	1,1	97,3	12,1	7,4	38,84
8	21,652		41,4	0,7	98,3	12,1	6,9	42,98
9	22,917	0,32	41,4	0,29	99,3	12,1	6,5	46,28

Table A.31 Raw data of batch experiment at N/P ratio=5,7/1

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	3,0556	0,07	41,6	41,6	0,0	7,3	7,3	0
1	4,6618		41,6	38,6	7,2	7,3	6,8	6,85
2	6,4089		41,6	34	18,3	7,3	5,9	19,18
5	12,3255		41,6	8,2	80,3	7,3	2,9	60,27
6	19,5644		41,6	1,6	96,2	7,3	2	72,6
7	20,799		41,6	1,1	97,4	7,3	1,9	73,97
8	22,0476		41,6	0,7	98,3	7,3	1,7	76,7
9	23,0162	0,59	41,6	0,1	99,8	7,3	1,5	79,45

Table A.32 Raw data of batch experiment at N/P ratio=6,8/1

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	2,8845	0,08	41,6	41,6	0,0	6,1	6,1	0
1	3,9112		41,6	38,4	7,7	6,1	5,7	6,56
2	5,8647		41,6	33,6	19,2	6,1	4,9	19,67
5	12,8214		41,6	8,1	80,5	6,1	2	67,21
6	19,4324		41,6	1,3	96,9	6,1	1,9	68,85
7	21,4088		41,6	0,9	97,8	6,1	1,7	72,13
8	22,823		41,6	0,7	98,3	6,1	1,5	75,41
9	23,167	0,62	41,6	0,1	99,8	6,1	1,5	75,41

Table A.33 Raw data of batch experiment at N/P ratio=8/1

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	2,7354	0,06	41,6	41,6	0,0	5,2	5,2	0
1	4,3309		41,6	38,6	7,2	5,2	4,7	9,62
2	6,7052		41,6	32,4	22,1	5,2	3,6	30,77
5	13,2822		41,6	5,7	86,3	5,2	1,9	63,46
6	20,5836		41,6	0,7	98,3	5,2	1,5	71,15
7	22,1298		41,6	0,24	99,4	5,2	1,3	75
8	23,0656		41,6	0,2	99,5	5,2	1,3	75
9	22,703	0,69	41,6	0,06	99,9	5,2	1,2	76,92

Table A.34 Raw data of batch experiment at N/P ratio=10/1

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	2,8019	0,07	41,8	41,8	0,0	4,1	4,1	0
1	3,9522		41,8	39,6	5,3	4,1	3,6	12,2
2	5,8325		41,8	36,4	12,9	4,1	3	26,83
5	13,1376		41,8	7,9	81,1	4,1	1,8	56,1
6	20,205		41,8	0,7	98,3	4,1	1,6	60,98
7	21,2762		41,8	0,2	99,5	4,1	1,6	60,98
8	21,4036		41,8	0,1	99,8	4,1	1,4	65,85
9	21,3344	0,62	41,8	0,1	99,8	4,1	1,4	65,85

Table A.35 Raw data of batch experiment at different N/P ratios

N/P Ratio	NH_4-N_e (mg/lt)	E_N (%)
2	2,6	93,9
3	0,29	99,3
5,7	0,1	99,8
6,8	0,1	99,8
8	0,06	99,9
10	0,1	99,8

Table A.36 Raw data of batch experiment at initial ChA concentration=3,5 g/lt

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	2,8814	0,08	41,4	41,4	0,0	12,1	12,1	0
1	4,6862		41,4	39	5,8	12,1	11,5	4,96
2	6,7724		41,4	34,2	17,4	12,1	10,7	11,57
5	10,6824		41,4	10	75,8	12,1	8,3	31,41
6	19,9802		41,4	3,9	90,6	12,1	7,5	38,02
7	20,371		41,4	1,1	97,3	12,1	7,4	38,84
8	21,652		41,4	0,7	98,3	12,1	6,9	42,98
9	22,917	0,32	41,4	0,29	99,3	12,1	6,5	46,28

Table A.37 Raw data of batch experiment at initial ChA concentration=4 g/lt

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	4,0504	0,065	42	42	0,0	10,7	10,7	0
1	4,5768		42	37,2	11,4	10,7	10	6,54
2	6,88		42	30,8	26,7	10,7	9,2	14,02
3	8,6214		42	23,5	44,0	10,7	8,2	23,36
6	13,3539		42	7,6	81,9	10,7	6,9	35,5
7	13,9526		42	6,3	85,0	10,7	6,7	37,38
8	19,4174		42	0,56	98,7	10,7	6,5	39,25
9	19,8652		42	0,52	98,8	10,7	6,4	40,2
10	20,7388	–	42	0,2	99,5	10,7	6,3	41,12

Table A.38 Raw data of batch experiment at initial ChA concentration=4,6 g/lt

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	4,5954	0,08	41,8	41,8	0,0	10,8	10,8	0
1	4,8562		41,8	38,4	8,1	10,8	10,5	2,78
2	6,0174		41,8	34,8	16,7	10,8	9,8	9,26
3	8,9624		41,8	27,5	34,2	10,8	9	16,67
6	13,649		41,8	11,8	71,8	10,8	7,5	30,56
7	14,5264		41,8	9,1	78,2	10,8	7,4	31,48
8	20,1338		41,8	5,44	87,0	10,8	7,1	34,26
9	20,6522		41,8	3,32	92,1	10,8	7	35,19
10	21,3122	–	41,8	0,65	98,4	10,8	6,9	36,11

Table A.39 Raw data of batch experiment at initial ChA concentration=5 g/lt

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	5,1435	0,09	41,8	41,8	0,0	10,7	10,7	0
1	6,2226		41,8	35,8	14,4	10,7	10,1	5,61
2	8,0527		41,8	28,6	31,6	10,7	9	15,89
3	9,1236		41,8	21,2	49,3	10,7	8,1	24,3
6	13,3558		41,8	4,1	90,2	10,7	6,8	36,45
7	13,9654		41,8	0,9	97,8	10,7	6,6	38,32
8	22,1472		41,8	0,36	99,1	10,7	6,5	39,25
9	24,5652		41,8	0,13	99,7	10,7	6,2	42,06
10	–	–	41,8	–	–	10,7	–	–

Table A.40 Raw data of batch experiment at initial ChA concentration=5,4 g/lt

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	5,4329	0,1	42,2	42,2	0,0	11,1	11,1	0
1	5,7185		42,2	35,8	15,2	11,1	10,4	6,31
2	9,2052		42,2	28,8	31,8	11,1	9,2	17,12
3	10,5663		42,2	17,9	57,6	11,1	8,1	27,03
6	13,2115		42,2	1,6	96,2	11,1	6,5	41,44
7	14,2154		42,2	0,8	98,1	11,1	6	45,95
8	22,1472		42,2	0,24	99,4	11,1	5,8	47,75
9	28,8652		42,2	–	–	11,1	–	–
10	–	–	42,2	–	–	11,1	–	–

Table A.41 Raw data of batch experiment at initial ChA concentration=6 g/lt

Time (days)	ChA (mg/lt)	Biomass (g/lt)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)	PO_4-P_o (mg/lt)	PO_4-P_e (mg/lt)	E_P (%)
0	6	0,12	42,2	42,2	0,0	11,2	11,2	0
1	6,747		42,2	34	19,4	11,2	10,3	8,05
2	10,2663		42,2	27,2	35,5	11,2	9,1	18,75
3	10,9689		42,2	17,6	58,3	11,2	7,9	29,46
6	13,3689		42,2	0,9	97,9	11,2	6,8	39,29
7	13,8654		42,2	0,18	99,6	11,2	6,3	43,75
8	39,2398		42,2	–	–	11,2	–	–
9	40,5264		42,2	–	–	11,2	–	–
10	–	–	–	–	–	–	–	–

Table A.42 Raw data of batch experiment at different initial ChA concentrations

Initial ChA (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)
2,88	0,29	99,3
4	0,2	99,5
4,6	0,65	98,4
5	0,13	99,7
5,4	0,24	99,4
6	0,18	99,6

Table A.43 Raw data of continuous experiment at initial NH_4-N concentration=10 mg/lt, θ_H=2,5 days.

Time (days)	**Q (lt/day)**	**θ_H (days)**	**NH_4-N_o (mg/lt)**	**NH_4-N_e (mg/lt)**	**E_N (%)**
0	13,1	2,3	8,8	5,7	35,23
1	11	2,73	8,7	4,9	43,7
3	11,5	2,61	10,1	3,6	64,36
4	12,0	2,5	9,9	3,4	65,66
6	10,7	2,8	10,3	3,6	65,1
7	12,	2,5	10,7	2,9	73
8	12,3	2,44	10,3	2,1	79,6
9	12,4	2,42	10	1,8	82
10	11,6	2,59	10	1,7	83

Table A.44 Raw data of continuous experiment at initial NH_4-N concentration=20 mg/lt, θ_H=2,5 days.

Time (days)	**Q (lt/day)**	**θ_H (days)**	**NH_4-N_o (mg/lt)**	**NH_4-N_e (mg/lt)**	**E_N (%)**
0	10	3	19,8	13,8	30,3
1	9	3,3	20,3	11,4	43,84
3	10,4	2,8	19,8	8,8	55,6
4	9,3	3,2	20,5	8	61
5	11,1	2,7	19,9	7,4	62,8
6	10,3	2,9	22,2	6,2	72,1
7	12	2,5	19,4	5,9	69,6
8	11,8	2,59	19,2	5,3	72,4
10	11,7	2,5	20,3	5	75,4

Table A.45 Raw data of continuous experiment at initial NH_4-N concentration=30 mg/lt

Time (days)	Q (lt/day)	θ_H (days)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)
0	13,1	2,29	31,2	17,2	44,9
1	10,6	2,83	33,8	15,2	55,03
2	11,50	2,61	29,2	9,9	66,1
3	12,00	2,5	29,9	7,3	73,6
4	10,70	2,80	30,6	6,5	78,8
7	12,00	2,5	30,8	7,9	74,4
8	12,30	2,44	28,4	8,6	69,72
9	12,40	2,42	29,4	9,2	68,71
10	11,60	2,59	29	9,4	67,6

Table A.46 Raw data of continuous experiment at initial NH_4-N concentration=40 mg/lt

Time (days)	Q (lt/day)	θ_H (days)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)
0	10	3	41	10	75,61
1	12	2,5	36,6	13	64,48
3	9,00	3,33	39,4	13,5	65,74
4	12,50	2,4	42,2	18,2	56,87
5	14,70	2,04	39,8	18,6	53,27
6	14,00	2,14	38,6	18	53,37
8	8,70	3,45	42	18,1	56,91
9	8,90	3,37	40,8	17,7	56,62
10	8,90	3,37	39,6	18,2	54,04

Table A.47 Raw data of continuous experiment at initial NH_4-N concentration=50 mg/lt

Time (days)	**Q (lt/day)**	**θ_H (days)**	**NH_4-N_o (mg/lt)**	**NH_4-N_e (mg/lt)**	**E_N (%)**
0	15,8	1,9	45,6	32,4	29
1	13,4	2,3	46,2	32,2	30,3
2	11,5	2,61	46,8	32,2	31,2
3	9,1	3,3	49,8	34	31,7
6	12,2	2,5	50	32,8	34,4
7	11,2	2,7	48,2	29,2	39,4
8	12,1	2,5	46	26,8	41,7
9	10,4	2,9	50,8	27,4	46,1
10	10,1	3	48,6	25	48,6

Table A.48 Raw data of continuous experiment at different initial NH_4-N concentrations

NH_4-N_o (mg/lt)	**NH_4-N_e (mg/lt)**	**E_N (%)**
10	1,7	83
20	5	75,4
30	9,4	67,6
40	18,2	54,04
50	25	48,56

Table A.49 Raw data of continuous experiment at initial θ_H=1,5 days

Time (days)	Q (lt/day)	θ_H (days)	NH_4-N_o (mg/lt)	NH_4-N_e (mg/lt)	E_N (%)
0	21,12	1,36	18	13,1	27,2
3	19	1,6	18,1	12,3	32
4	18	1,7	18,1	11,3	37,6
5	13	2,31	18,5	10,8	41,6
6	17	1,76	19,4	11,2	42,3
7	12	2,5	19,6	12,2	37,8
9	15,1	2	18,3	12,2	33,3
10	17,9	1,7	19,6	12,9	34,2

Table A.50 Raw data of continuous experiment at initial θ_H=2,5 days

Time (days)	Q (lt/day)	θ_H (days)	N_o (mg/lt)	N_e (mg/lt)	E_N (%)
0	10,00	3	19,8	13,8	30,3
1	9,00	3,3	20,3	11,4	43,8
3	10,40	2,8	19,8	8,8	55,56
4	9,30	3,2	20,5	8	60,98
5	11,10	2,7	19,9	7,4	62,8
6	10,30	2,9	22,2	6,2	72,07
7	12,00	2,5	19,4	5,9	69,59
8	11,80	2,59	19,2	5,3	72,4
10	11,70	2,5	20,3	5	75,4

Table A.51 Raw data of continuous experiment at initial θ_H=3,5 days

Time (days)	Q (lt/day)	θ_H (days)	N_o (mg/lt)	N_e (mg/lt)	E_N (%)
0	8,1	3,70	19,3	3,5	81,87
1	8,2	3,66	20,1	4,5	77,61
2	8,00	3,75	19,7	4,8	75,64
3	8,00	3,75	19,9	4,7	76,38
4	8,00	3,75	20,7	5	75,85
5	8,30	3,61	21	4,6	78,1
8	8,20	3,66	18	4,3	76,1
9	8,30	3,61	19,2	4,6	76,04
10	8,10	3,70	20,3	4,7	76,85

Table A.52 Raw data of continuous experiment at initial θ_H=4,5 days

Time (days)	Q (lt/day)	θ_H (days)	N_o (mg/lt)	N_e (mg/lt)	E_N (%)
0	7,1	4,23	21	4,8	77,14
1	7	4,29	23,8	3,7	84,45
2	7,20	4,17	21,2	2,3	89,15
3	7,10	4,23	20,4	2,4	88,24
4	7,00	4,29	19	2,2	88,4
7	7,10	4,23	24	2,7	88,75
8	7,10	4,23	18,5	2,7	85,41
9	7,00	4,29	17,5	2,2	87,43
10	7,10	4,23	20,5	2,9	85,9

Table A.53 Raw data of continuous experiment at initial θ_H=5,5 days

Time (days)	**Q (lt/day)**	**θ_H (day)**	**N_o (mg/lt)**	**N_e (mg/lt)**	**E_N (%)**
0	5,14	5,84	21,4	3,1	85,5
1	4,91	6,11	20,6	2,6	87,38
2	5,80	5,17	21,2	3	85,85
3	5,80	5,17	21,2	3,1	85,38
6	5,20	5,77	16,6	2,4	85,5
7	5,20	5,77	22,7	1,4	93,8
8	5,30	5,66	23,2	1,5	93,54
9	5,20	5,77	21,4	1,3	93,93
10	5,20	5,77	22,1	1,4	93,67

Table A.48 Raw data of continuous experiment at different hydraulic retention times

Hydraulic Retention Time (days)	**NH_4-N_e (mg/lt)**	**E_N (%)**
1,5	12,9	34,2
2,5	5	75,4
3,5	4,7	76,9
4,5	2,9	85,9
5,5	1,4	93,7