Growth and Nutrient Removal Capacity of Chlorella Vulgaris Microalgae in High Ammonia Media

By

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

Abstract

Wastewater treatment is essential to remove toxic particulates before it is discharged to the surrounding environment. Recently, algal-based systems have been widely used for wastewater treatment. However, there is a lack of studies on algal growth's feasibility in high ammonia media. In this research, the growth of *Chlorella Vulgaris* microalgae in high ammonia media was investigated. In the first stage, 200, 400, 600, and 800 mg/l of ammonia (NH_4) levels were applied. The optimal growth and nutrient removal were achieved with 600 mg/l ammonia. Biomass concentration of 1268 mg/l and ammonia removal of 348 mg/l was obtained. Algae had low growth in a high-ammonia medium (800 mg/l). In the second stage, the buffering system of CO_2/ $NaHCO_3$ was applied. Different carbon dioxide concentrations (2, 4, and 6%) and sodium bicarbonate (1, 1.5 and 2 g/l) were investigated. The best results (biomass concentration and ammonia removal of 1740 and 417.33 mg/l, respectively) were obtained with 4% CO_2 and 1.5 gr/l $NaHCO_3$. This study shows the feasibility of *C. Vulgaris* growth in harsh low-pH conditions and its optimal conditions to remove nutrients from wastewater.

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Structure of the Thesis

Chapter 1 provides an introduction of the research, background, and objectives.

Chapter 2 reviews the literature on leachate, biological wastewater treatment by microalgae, culture systems, and the operational parameters, including light sources, mixing, and nutrients.

Chapter 3 provides information on the materials and methods used in this research, including a description of the measurement methods and a culture media description.

Chapter 4 presents the results and discussions, including data analysis.

Chapters 5 and 6 focus on the conclusion and recommendations for future work, respectively.

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List of Acronyms and Symbols

ANOVA	Analysis of Variance
BOD	Biological Oxygen Demand
BP	Biomass Productivity
COD	Chemical Oxygen Demand
d	Day
DI	Deionized Water
Factor A	Carbon Dioxide
Factor B	Sodium Bicarbonate
hr	Hour
HRAP	High Rate Algal Pond
L	Liter
LED	Light-emitting Diode
mg	Milligram
ml	Millilitre
N	Nitrogen
NH_3	Ammonia
NH_4	Ammonium
NPP	Normal Probability Plot
P	phosphorus

PBR	Photo Bioreactor
PPM	Parts Per Million
UTEX	University of Texas
UV	Ultraviolet
VVM	Volume/Volume/Minute

CHAPTER 1: INTRODUCTION

1.1 Background

Solid waste generation has been increasing dramatically over the past few decades (Karak, Bhagat, and Bhattacharyya 2012). Up to now, landfilling is the most common way of municipal solid waste disposal. Over time, these landfill sites generate a large quantity of leachate, which is toxic and causes other health and environmental problems (Beigl, Lebersorger, and Salhofer 2008). Leachate is a poisonous liquid with high ammonia concentration, biological oxygen demand (BOD), and chemical oxygen demand (COD) (Wiszniowski et al. 2006). The chemical composition of the landfill may vary by site. Different factors such as the age of the landfill, climate, and geographical data of the site affect the composition of landfill leachate. For instance, young landfill leachate typically has a higher amount of COD and a lower amount of Ammonia nitrogen (< 400 mg/l). In comparison, the old leachate has a higher amount of ammonia and a lower amount of COD (> 400 mg/l) (Renou et al. 2008).

Landfill leachate contaminates surface and groundwater sources, and also poses a threat to human health and other organisms (Naveen et al. 2017). Due to the health and environmental issues mentioned above, proper treatment of leachate before discharging is required. Different methods are being used for leachate treatment. However, current treatment technologies such as air stripping, pH adjustment, chemical precipitation, oxidation, and reduction are expensive and not efficient (Wiszniowski et al. 2006). Thus, there is a high demand for novel sustainable treatment methods with minimum environmental issues. Therefore, finding eco-friendly, low-cost, and innovative ways of leachate treatment is a necessity (Naveen et al. 2017). In recent years, algal-based systems have received substantial attention as they eliminate pollutants from

the environment and produce value-added products such as biomass (Abdel-Raouf, Al-Homaidan, and Ibraheem 2012). Those value-added bio-products can be utilized as organic fertilizer, animal feed, biofuel, and biogas (Mata, Martins, and Caetano 2010).

Compared to conventional methods of leachate treatment, algal-based methods are environmentally friendly and novel. Microalgae are found almost everywhere in nature and can tolerate very harsh conditions. Furthermore, their bioproducts have various applications. Algal biomass can be used as an alternative renewable source of energy to improve sustainability and resource recovery (Maity et al. 2014). The assimilation of CO_2 is another advantage of algal-based systems. Microalgae uptake CO_2 through photosynthesis; as a result, they allow carbon-sequestering (Satyanarayana, Mariano, and Vargas 2011). All of these benefits make microalgae-based systems a possible candidate for such a treatment scenario.

Although there are many reviews and research papers regarding conventional methods of leachate treatment, only a few studies exist on the use of algal systems for landfill leachate treatments. One of the most challenging leachate treatment problems with microalgae is the high concentration of ammonia nitrogen, which is toxic for algae due to the presence of free ammonia (Hernández-García et al. 2019). In this project, the growth and nutrient removal capacity of *Chlorella Vulgaris* microalgae under different conditions has been investigated.

1.2 Research Objectives

This first objective was to find the best microalgae species that can tolerate a high ammonia condition, which was chosen to be *Chlorella Vulgaris*. The second objective was to determine the highest amount of ammonia nitrogen that microalgae can grow. The last objective of this research was to investigate the effect of different levels of CO_2 and $NaHCO_3$ on growth and nutrient removal of *C. Vulgaris*.

CHAPTER 2: LITERATURE REVIEW

2.1. Ammonia removal by microalgae

The algae-based treatment has been investigated for different types of waste streams (Benemann, Tillett, and Weissman 1987). However, microalgae's ability to grow under high ammonia wastewater, such as leachate, requires further exploration. The high amount of nutrients, especially nitrogen in the form of ammonia and other compounds such as salts and organic matter in landfill leachate, makes it toxic to many microorganisms (Collos and Harrison 2014). The ammonia levels in these sources vary from 100 to more than 1000 mg/l (Naveen et al. 2017). The high concentration of ammonia (usually above 60 mg/l) in the medium is toxic and inhibits algal growth(Cheung, Chu, and Wong 1993). Besides that, uptake of ammonia leads to a sudden pH drop to 3, which acts as an inhibitory factor and stops algal growth. The ammonia and ammonium equilibrium is firmly pH-dependent (Körner et al. 2001). At low pH levels, microalgae are not inhibited by free ammonia; however, at high pH levels of 9 and higher, inhibition may occur. Tam (1996) investigated the effect of different ammonia concentrations on *C. Vulgaris* growth and found no significant difference was seen in specific growth rates for ammonia concentration ranging from 20-250 mg/l. After that, as ammonia increased, the growth of algae decreased. In cultures with ammonia levels above 80 mg/l, most of the initial ammonia remained in the culture (Tam and Wong 1996).

2.2. Microalgae

Phytoplanktons are the biggest producers of oxygen on the earth (Sebastiá 2014). Different types of algae such as single- or multi-celled forms are found in nature. Despite having diversity, microalgae are the most common type of algae (Molina Grima et al. 2003). Microalgae are unicellular autotrophic microorganisms found in many environments such as rivers, lakes, and oceans. They can also tolerate very harsh conditions and live in various climates (Benemann, Tillett, and Weissman 1987). They play an essential role in the food chain as they are considered the food chain base (Kim 2011). Besides their food supply role, they have been used for their lipid and protein content, specifically in Asia. Furthermore, algae are essential organisms as they have a vital role in CO_2 fixation and O_2 generation (Pires, Martins, and Simões 2012).

As autotroph organisms, they provide food via photosynthesis (Callieri and Stockner 2002). Except for blue-green algae, most types of microalgae are eukaryotic organisms. Compared to most terrestrial plants, microalgae growth rates are much higher and require less land (Alam, Xu, and Wang 2020).

Different species of microalgae have been studied pertaining to wastewater treatment. The seasonal fluctuations, environmental parameters, and wastewater characteristics are important factors that play a significant role in algal growth. Wiley et al. (2009) characterized algae species of wastewater ponds and found that Chlorella and Scenedesmus are the most prevalent species in waste streams . Chlorella species are one of the most tolerant and adaptive algae, which can grow under harsh physical and chemical conditions (Daliry et al. 2017).

2.3. COD uptake

Typically, microalgae are considered autotrophic microorganisms that utilize inorganic carbon sources. Carbon dioxide is the most common inorganic carbon source for the growth of microalgae (Oswald 1988). Although most of the algal species are autotrophs, some microalgae, namely Chlorella species, can be heterotrophs as well. In the absence of inorganic carbon and sunlight, they can use organic carbon sources such as acetate, sugar, and organic acids (Y. Wang et al. 2017). The microalgae can grow heterotrophically in two ways (M. A. Borowitzka 1999):

• Chemoheterotrophic: Without the presence of light and at a low level of carbon dioxide, they can utilize organic carbon as both carbon and energy source, which leads to a decrease in COD level.

• Photoheterotrophic: In the absence of carbon dioxide and under illumination, light and organic carbon can be utilized by microalgae as energy and carbon source, respectively.

2.4. O_2 release and CO_2 fixation

In wastewater treatment facilities, aeration is the most expensive part, reaching to 60% of the total costs (Changqing, Shuai, and Feng 2011). About 1 kWh energy is required for the removal of 1 Kg BOD (William J. Oswald 2003). However, BOD removal by algal systems needs less energy, and also by the production of biomass, it can make enough biogas for the generation of 1

kWh of electricity (William J. Oswald 2003). Therefore, algal-based systems can be used as an efficient way of aeration, which decreases the need for mechanical agitation.

2.5. Bacteria removal

Another advantage of algal-based systems is that they can remove indicator bacteria and pathogens in wastewater treatment ponds (El-Sheekh et al. 2016). Viruses, bacteria, parasites, and protozoa are different types of pathogens present in wastewater. Measuring indicator organisms such as total Coliform typically evaluates the efficiency of the disinfection process (Marazzi et al. 2020). Some studies have shown that microalgae are capable of decreasing coliform bacteria's population. Different factors such as physical and biological reactions, algae concentration, and solar irradiation influence the reduction of Coliform. Microalgae can reduce Coliform's population due to the following reasons (Schumacher, Blume, and Sekoulov 2003; Ansa et al. 2015).

- Some Antibacterial compounds released by microalgae such as Chlorellin, alpha-linolenic acid, methanolic and exanolic extracts and neophytadiene.
- High levels of pH that is common in algal-based treatment ponds, leads to a decrease the population of Coliform bacteria
- Some specific species of algae produce toxic materials

2.6. Impact of operational factors on microalgae growth

The efficiency of the treatment process is determined by the proper growth of algae. Understanding and controlling the key factors are crucial to obtain profitable growth. The major operational characteristics are bioreactor design, mixing, and aeration (Harun et al. 2010). Other operational factors such as nutrients, illumination, pH, and light/ dark cycle also affect microalgae growth(Abdel-Raouf, Al-Homaidan, and Ibraheem 2012).

2.6.1 Light

Presence of light is one of the most critical parameters impacting microalgae growth. Light provides energy for phototrophic organisms, such as algae. Saturated light in the environment leads to maximum algae growth (K. Lee and Lee 2001b). Converting light to energy determines the efficiency of food synthesis in algal cells. Thus, the energy coming from light is converted into chemical energy to be used by algal cells. However, such a conversion's efficiency is only 10%, and the remaining is released as heat (Satthong et al. 2019). Adenosine triphosphate (ATP), an energy source for cells, is produced through the photosynthesis process. Light intensity, wavelength, and light/dark cycles must be considered for algae's growth (Sorokin and Krauss 1958).

When the light intensity is below the compensation point, there is no growth. As the intensity increases, algae's growth increases and reaches the highest amount at the saturation point. Any further increase in light intensity results in a decrease in the growth of algae. Sunlight or artificial illumination can serve as a light source. The sunlight's intensity can reach 120,000 lux and includes all the wavelengths (Hsia and Yang 2015; Gim et al. 2016). For indoor experiments or

places with low intensity of sunlight, artificial lights can be used. Also, the depth of ponds and tanks is another crucial factor in the light regime. In deep vessels, light penetration is limited, which consequently inhibits photosynthesis. Self-shading is another problem that happens in dense cultures. A proper mixing system is an excellent way to overcome the mentioned problems (Evens et al. 2000; Zhang et al. 2017).

2.6.2 Temperature

Another factor that plays a vital role in the growth of microalgae is temperature. Different species have a diverse range of tolerances for temperatures. Very high or low temperatures can inhibit growth. The temperature ranges of 20-30℃ are typically considered a suitable range for most species. Increasing the culture temperature to reach the optimum point can increase the growth; however, any further increase may inhibit the growth. At temperatures below 15℃, algae stops growing (Kurpan Nogueira et al. 2015). For instance, Munoz et al. (2004) found that increasing the temperature from 25 to 30℃ doubled the removal efficiency of *C. Sorokiniana* and *R. Basilensis* strains (Muñoz et al. 2004). In humid climates with low evaporation, high temperatures can inhibit the growth of microalgae. Cold climates may decrease the growth of microalgae; however, some species can grow in those conditions too. Furthermore, high light intensity at low temperature may also inhibit growth (Renaudl et al. 1995). In contrast, at the optimum temperature, high intensities of light may not impact algae's growth (Singh and Singh 2015; Goldman 1974).

Growth of most algal species was not affected by a decrease of 15℃ below the optimum temperature (30℃), but a 2 to 4 ℃ increase from the optimum point can inhibit the growth (Razzak et al. 2013).

2.6.3 Mixing

The efficient mixing system is a key factor in algal treatment processes. Mixing decreases the boundary layers for nutrient delivery and improves mass transfer by the distribution of nutrients. Also, it prevents light limitation because of biomass shading, moves algae to different light regimes, and reduces the formation of dead zones (Carvalho, Meireles, and Malcata 2006). It maintains the uniform heat transfer in the culture (Koolivand et al. 2017). There are various methods of mixing such as turbulence, shear stress mixing, stirring, bubbling, etc; each has its advantages and disadvantages. Turbulence can inhibit the growth of microalgae whereas shear stress caused by a high rate of mixing can harm algal cells. In a study by Thomas and Gibson (1990), different algal groups' sensitivity to shear stress was investigated. They found that green algae's sensitivity to shear stress was less than that of diatoms and blue-green algae. Within a specific range of turbulence, microalgae growth rates could be improved with increased mixing (Thomas and Gibson 1990).

Shear rate and shear stress of turbulence are the most critical factors for the survival of algae (Warnaars, Hondzo, and Anthony 2006). There are different methods of mixing in algal cultures. To choose a proper mixing system, some essential elements such as type of species, the scale of the culture, culture system type, and the environment where the culture is operated must be considered (Weissman, Goebel, and Benemann 1988). Paddlewheels are typically selected for open systems such as raceways. These systems generate laminar regimes downstream and high turbulence at the wheel. For shallow systems, mechanical mixing is preferred (Huang et al. 2017). For small-scale laboratory vessels such as tubes and flasks, shaking is used. Bubbling is a common way of mixing in bubble column and flat plate photobioreactors (PBRs) (Qiang and Richmond 1996). Yang et al. (2014) examined the effect of different aeration rates ranging from

200 to 1600 ml/min. They reported that the best growth was achieved in a 1600 ml/min aeration test. They also noted that the size and number of nozzles impact algal growth (Yang et al. 2014). Figure 1 shows a flat-plate (PBR) with aeration.

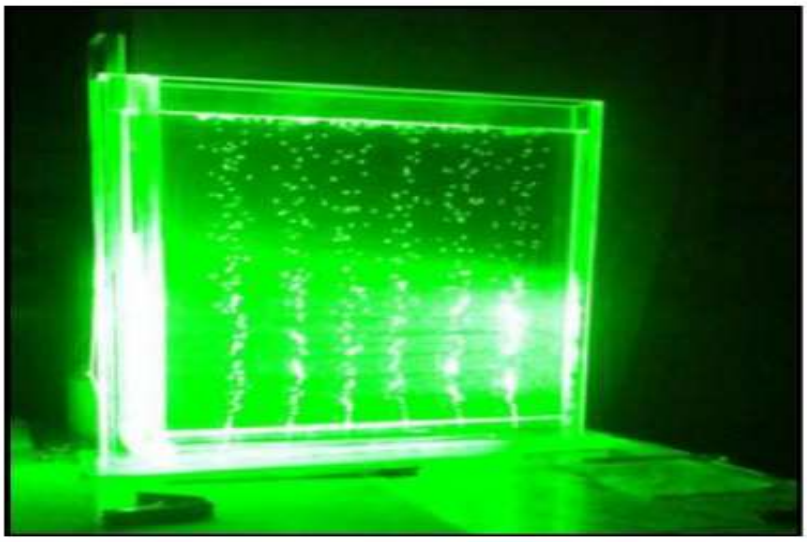


Figure 1- A flat-panel PBR with air bubbling (Yang et al. 2014)

2.6.4 Nutrients

Carbon is the most critical element in the growth of microalgae. As an autotrophic microorganism, microalgae utilize sunlight and CO_2 to make organic molecules (Goldman et al. 1972). Some microalgae species are capable of using organic carbon (Weissman, Goebel, and Benemann 1988). Different microalga species have a diverse range of optimum CO_2 concentrations. Chlorella species are commonly used for carbon sequestration (B. Wang et al. 2008). However, the suitable CO_2 level is controversial due to the underlying factors and various

studies found different optimal CO_2 concentrations. For instance, the growth of *Chlorella Vulgaris* at different CO_2 levels ranging from ambient to 8.5% was investigated and the maximum growth was reportedly achieved at 8.5% CO_2 (Sung et al. 1999). In another study, CO_2 levels from ambient to 20% were examined and best growth of *C. Vulgaris* was found at 6% CO_2 (Mejía Rendón, Colmenares Roldan, and Voroney 2013). On the other hand, in another study, the maximum growth of *C Vulgaris* was achieved at 10% CO_2 and had profitable growth until 50% (Tang et al. 2011).

Nitrogen: Nitrogen is another essential element required for the growth of microalgae. Microalgae can utilize nitrogen in the form of ammonia, nitrate, nitrite. However, NH_4 is the most favorable form of nitrogen for algae as it requires less energy to utilize. High levels of ammonia are a big challenge in algal treatment systems, typically for leachate (Alketife, Judd, and Znad 2017).

Phosphorus: Another important parameter in the growth of algae, which comprises about 1% cell dry weight, is phosphorus. Microalgae uptake the phosphorus from media in the form of orthophosphate. Low phosphorus concentrations (< 0.045 mg/l) in the media can inhibit microalgae growth (Liang et al. 2013; Xin et al. 2010).

2.6.5 pH

The pH of the medium can improve the metabolism of microorganisms. Each species thrives at a specific pH range. However, a neutral pH range between 6 to 8 is favorable for most algal species. Also, some microalgae species can grow under acidic or alkaline conditions (Press et al. 1982). Growth and biomass productivity of *Scenedesmus obliquus* is highly pH-dependent

(Hodaifa, Martínez, and Sánchez 2009). pH is considered a significant factor as it impacts many aspects of microalgae such as the solubility of mineral salts and oxygen in the media (Chen et al. 2011). Furthermore, a high pH level leads to phosphate precipitation, but it can redissolve again as pH drops (Press et al. 1982).

Algae consume CO_2 through the photosynthesis process. As a result, as microalgae grow, pH level increases in the media. This can be controlled by bacteria respiration as they release CO_2. Also, high pH helps the harvesting of microalgae by auto-flocculation (Axelsson, Marine, and Station 1988). However, in a medium with a high ammonia level, high pH might be a challenge as it leads to the formation of free ammonia, which is toxic for most microorganisms (Körner et al. 2001).

2.6.6 Effect of light/dark cycle

The light/dark cycle for phototrophic microorganisms such as microalgae and cyanobacteria is an important factor. The light/dark cycle directly affects the growth, lipid content, and nutrient removal capacity of microalgae (Liu et al. 2007). Photosynthesis is a two-step process. When cells are exposed to illumination, the light reaction happens. Carbon fixation occurs during both the light and the dark cycle Sunlight or artificial lights like fluorescents or LEDs serve as a light source (Elizabeth 1981). The light regime has a dramatic impact on the productivity and efficiency of treatment systems. Many studies have been conducted on the effects of the light period on algal growth. In one such study, Jacob et al. (2009) examined the impact of different light periods from 0:24 to 24:0 light/dark cycle. They concluded that the duration of the light period has a significant effect on the efficiency of PBRs. They also found a linear relationship between the light period and algal growth except for the cycle of 12:12. However, other studies

have found that algal growth increases as the light period increases to 16 hours As the light period increases from 16 to 24 hours, a reduction in algal growth was seen (Lee and Lee 2001a; Jacob-lopes et al. 2009).

2.7 Microalgae culture systems

Microalgae are cultivated for many purposes such as biomass & biofuel production and treatments. There are various methods of algal culturing. Open systems and closed systems are two ways of algal cultivation (Mata, Martins, and Caetano 2010).

2.7.1 Open systems

Lagoon, natural lake, and artificial ponds are examples of open systems. Open systems are typically classified into raceway ponds, high-rate algal ponds (HRAP), and unmixed ponds (Y. K. Lee 2001). HRAPs are stabilization ponds with uncomplicated mixing from a depth of 0.2 to 1 meter. Raceway ponds typically have a single loop (Mata, Martins, and Caetano 2010). Paddlewheels are typically used for mixing in raceways. Raceway pond is the most common form of open cultivation (Fig. 2). Open systems have an easy construction and operation and low costs (Singh and Dhar 2011); however, their efficiency is low. Moreover, there is no control over important parameters such as temperature and pH. The risk of contamination is also high. In addition, evaporation and low mass transfer rate are other problems of open systems (Benemann and Oswald 1996; Borowitzka 2005). Some open systems are shown in figures 2 and 3.

Figure 2 – A raceway pond with paddlewheels (New Mexico State University)

Figure 3 – High-rate algal pond (https://www.energy.gov/eere/bioenergy/algal-production)

2.7.2 Closed systems

Photobioreactors (PBRs) are the most efficient engineered systems for the cultivation of algae. They are specifically suitable for microalgae's solo cultures as the chance of contamination is low (Xu et al. 2009). In comparison with raceway ponds, PBRs are more efficient and productive. Other advantages of PBRs are better temperature control, mass transfer, and cycle control (Weissman, Goebel, and Benemann 1988). There are different types of PBRs. However, they are classified into three categories according to their design, tubular, bubble column, and flat plate. Each of these types has advantages and disadvantages (Harun et al. 2010). Figure 4 shows the different kinds of PBRs.

Tubular PBRs are made up of glass or plastic tubes. They are circulated with bubbles or pumps. These bioreactors are the preferred for the mass cultivation of microalgae and are suitable for outdoor use. However, having dead zones and the difficulty in cleaning are some of the disadvantages of tubular PBRs (Grobbelaar 2000).

Bubble column bioreactors are cylindrical vessels as their height is twice their diameter. They have several advantages, including low capital costs and a high surface-to-volume ratio. But they are not suitable for scaling up as light penetration is decreased by increasing the diameter (Grobbelaar 2000; Lee 2001).

Flateplate PBRs arecuboidal shape vessels with a low light path (Kurano 2001) and a high surface to volume ratio. Bubbling is used for agitation. Having dead zones and difficulty in cleaning are the problems associated with flateplate PBRs (Spiros and Design, n.d.; Ruiz et al. 2013).

a

b

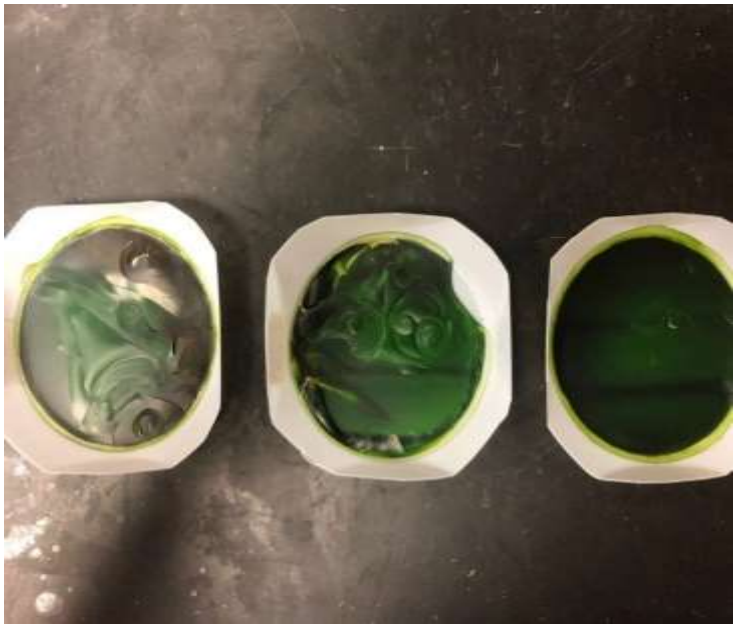
c

Figure 4 – a) Bubble column b) Flat plate c) Tubular (https://www.researchgate.net/figure/A-tubular-photobioreactor-designated-PBR-4000-G-IGV-Biotech_fig2_282301293)

CHAPTER 3: MATERIALS AND METHODS

The strain of *Chlorella Vulgaris* used in this survey was purchased from the University of Texas algae collection (UTEX). In the first step, the microalgae were sub cultivated in the Petri dish and incubated thereafter at 27±0.1 ˚C. To prepare a sufficient liquid bank, a petri dish was washed and transferred to a 500-ml bottle. The light/ dark cycle of 16:8 was applied at the room temperature. Then 100 ml of algae was inoculated in an 800-ml flask in a shaker. The BG 11 medium with the following composition was used for solid and liquid bank preparation (M. Borowitzka, n.d.): 1.5 g/l $NaNO_3$, 0.028 g/l K_2HPO_4, 0.075 g/l $MgSO_4$, 0.036 g/l $CaCl_2$, Citric acid, EDTA, $FeCl_3$, and trace element. The trace element solution was prepared using the following composition of 0.222 g/l $ZnSO_4$.7H2O, 0.039 g/l $Na_2MoO_4.2H_2O$, 0.079 g/l $CuSO_4$. $7H_2O$, 1.810 g/l $MnCl_2$. $4H_2O$ and 0.005 g/l $Co(NO_3)$. $6H_2O$. One millilitre of trace element was added to 1000 ml of media (Stanier 1979). For the main experiments, $NaNO_3$ was replaced by NH_4Cl. Sets of cool white LED lamps served as a light source. The airflow rate of 0.5 volume/volume/minute (VVM) was applied for mixing purposes. The airflow contained 2% carbon dioxide, and a 16:8 light /dark cycle was conducted.

b



a

Figure 5- a) dried biomass b) UTEX culture

3.1 Preliminary tests

Before cultivation, 100 ml of pure *C. Vulgaris* microalgae was centrifuged at 6000 rpm for 10 minutes, washed twice with deionized water (DI), and resuspended in 800 ml of artificial media in a 1-liter flask. After that, the flask was placed in the shaker. A set of white cool LED strips were placed on both sides of the shaker at the light intensity of 2500 lux. The media was mixed by aeration. Tests were carried out at room temperature (21±0.5C). The light/dark cycle of 16:8 was used.

3.2 Main tests

Before cultivation, 200 ml of pure algae was centrifuged at 6000 rpm for 10 minutes and washed twice with DI water. After that, it was inoculated in a bubble column PBR with a working volume of 3 liters. The light intensity was set at 4000 lux. Light/dark cycle of 16:8 was applied.

All the experiments were performed at room temperature (21±0.5C). No pH control was used after cultivation. Figure 6 shows the experimental setup.

Samples from bioreactors were taken daily. Concentrations of ammonium and orthophosphate were analyzed by using a flow injection analyzer (FIA) and a spectrophotometer, respectively. Ammonia removal was determined by a decrease in the amount of ammonia in the medium. The growth of microalgae was determined by using a UV-visible spectrophotometer at the wavelength of 680 nm. The pH of the reactor was measured by a pH meter. The experiments were carried out in three replicates. A control reactor with no microalga was also setup. A 2-level full factorial design was used to analyze the effect of parameters on growth and nutrient removal of *C. Vulgaris*. The amount of N and P was measured by running all the samples through a centrifuge and filtering through a 0.45 µm paper filter. To measure the biomass, samples were centrifuged and dried at 45°C for 24 hours until their weight became stable.

Figure 6 – Experimental setup

CHAPTER 4: RESULTS AND DISCUSSION

This research was done in two stages. In the first stage, the highest amount of ammonia nitrogen that *Chlorella Vulgaris* can tolerate was investigated. Four experiments with different ammonia levels of 200,400, 600, and 800 mg/l were performed for this step. In the second stage, the effect of carbon dioxide and sodium bicarbonate on growth and nutrient removal of microalgae was examined.

4.1 First stage

Four tests with ammonia levels of 200, 400, 600, and 800 mg/l were performed during this stage. These four experiments are called R1, R2, R3 and R4 respectively.

4.1.1 Growth of *C. Vulgaris*

Figure 7 shows *Chlorella Vulgaris* microalgae's growth of R1-R4. For R1 and R2, the lag phase of about 96 hours was observed. R3 with an ammonia level of 600 mg/l had the shortest lag phase and entered exponential growth after 45 hours. However, the lag phase with the ammonia level of 800 mg/l was the longest at 160 hours. This could be due to the high concentration of ammonia; thus, it takes longer to adapt to that harsh condition. R1 entered the logarithmic phase 100 hours after the beginning and continued its smooth growth, and reached the maximum optical density after 400 hours. It subsequently went to the stationary phase. The growth of R2 was similar to the R1. But it went to the death phase immediately after reaching the plateau, which took about 430 hours. The best and sharpest growth was seen at R3. It had a shorter lag phase and reached to exponential phase after 45 hours of cultivation. The maximum biomass

concentration of 1268 mg/l was achieved for the test with an ammonia level of 600 ppm. The biomass concentrations for R1, R2 and R4 were 782, 786, and 403 g/l, respectively.

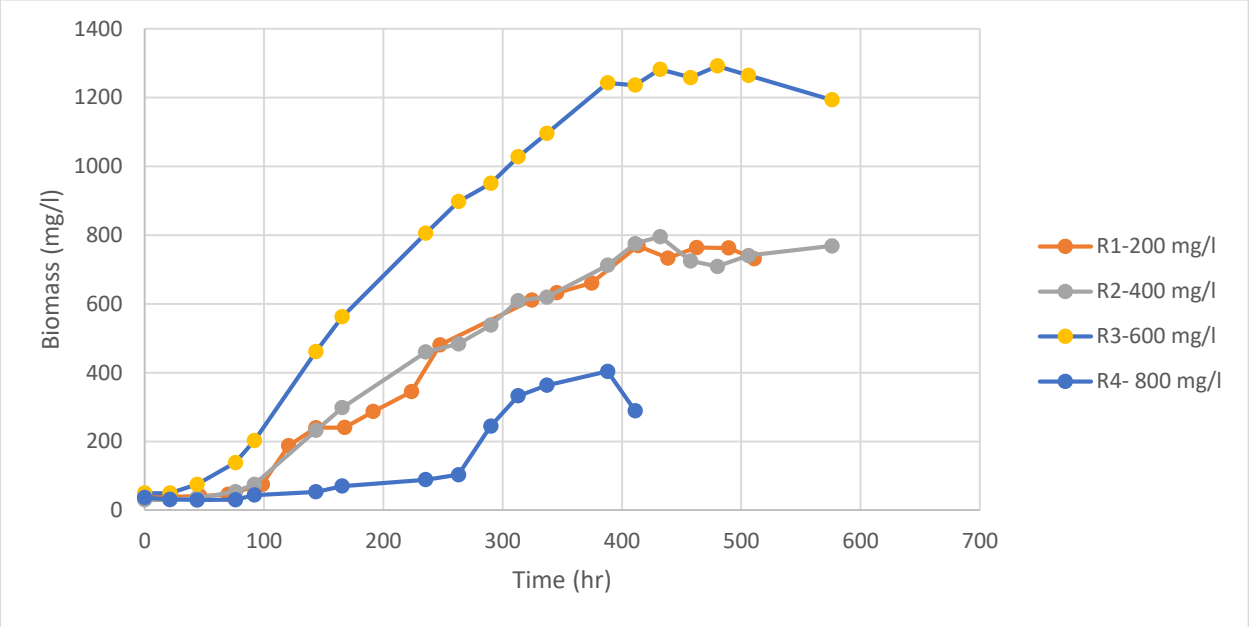


Figure 7 – The growth of 4 batch experiments vs. time, R1-R4

4.2.2 pH

Figure 8 shows the pH of 4 experiments. R1 had a higher pH level than other tests. It could be due to a low level of ammonia. During the trial, the pH was around 7.5, but at the end of the test, it started decreasing and stabilized around 7. The pH behavior for R2 and R3 was almost similar. However, the average pH level for the R3 was lower than that of R2. However, R4 showed a different pH behavior. The average pH of the culture was about 7 until the middle of the test. After that pH dropped dramatically and reached the lowest level of 4 by the end of the test. We can conclude that pH has an inverse relationship with ammonia concentration. As the ammonia level increased, the pH level decreased.

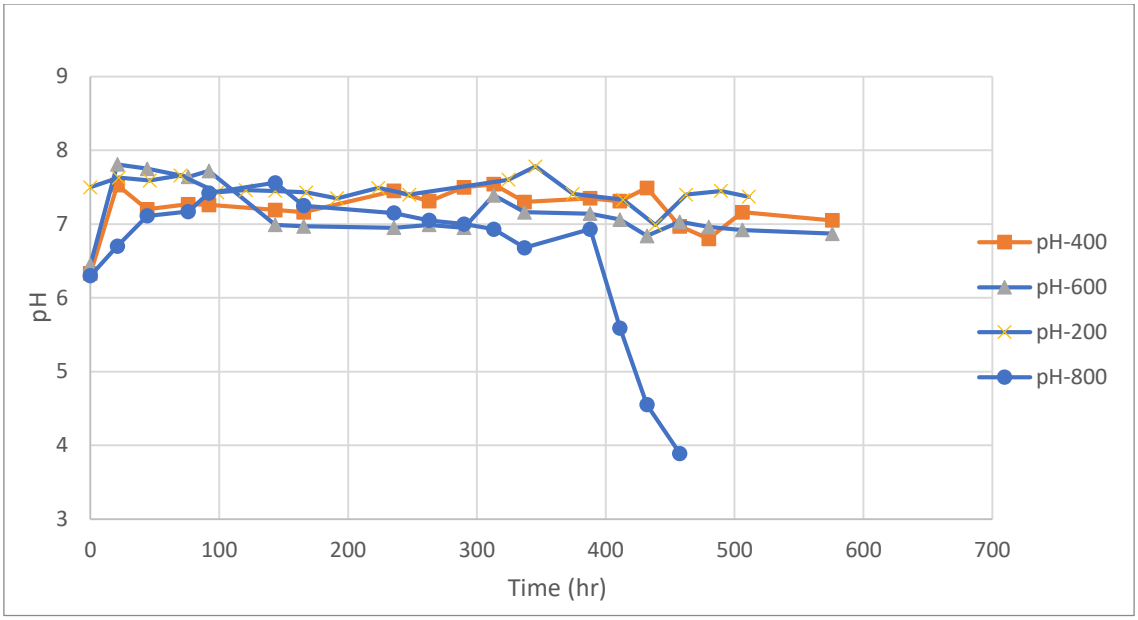


Figure 8 – pH level vs. time, R1-R4

4.1.3 Biomass Concentration

Figure 9 shows the biomass concentration of the 4 tests. For R1, the maximum biomass concentration of 782 mg/l was achieved; however, by doubling the amount of ammonia from 200 to 400 mg/l, no significant change was seen, and the biomass concentration of 786 mg/l was observed for R2. For R3, the highest biomass of 1268 mg/l, was achieved. By further increasing the amount of ammonia to 800 ppm, a significant reduction in biomass was seen and biomass concentration of 403 mg/l was recorded. That reduction was a result of a pH drop below 4. *Chlorella Vulgaris* microalgae had the best growth at the ammonia concentration of 600 ppm.

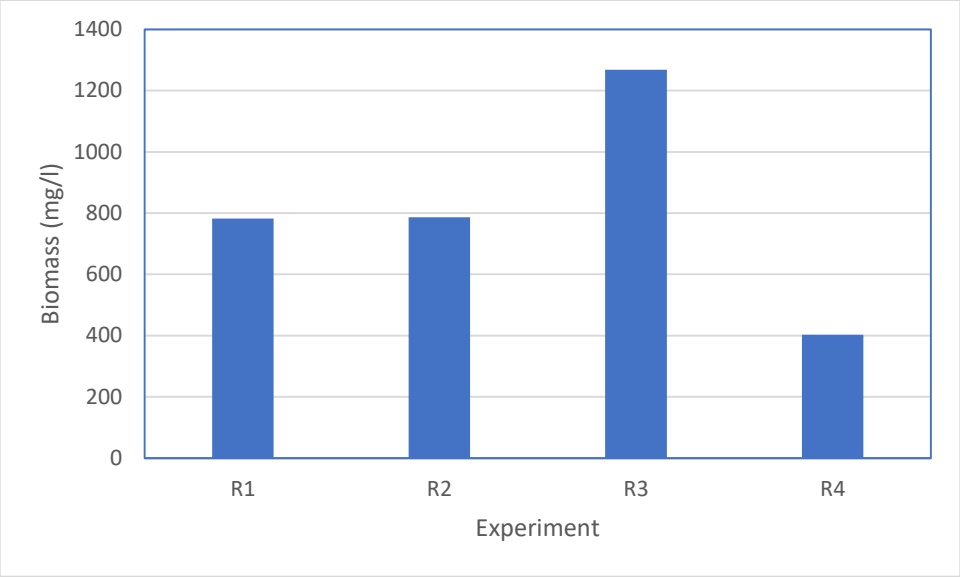


Figure 9- Biomass production of R1-R4

4.1.4 Biomass Productivity

The biomass productivity of tests is shown in figure 10. In the first test, the biomass productivity of 39.1 mg/l.d was achieved. For R2, a higher value of 43.68 mg/l.d was recorded. Although the biomass production of R1 and R2 was almost the same, R2 reached its highest biomass in a shorter time. The BP of 63.4 mg/l was obtained at an ammonia level of 600. However, the lowest BP of 25.18 mg/l.d was achieved at the ammonia level of 800 ppm.

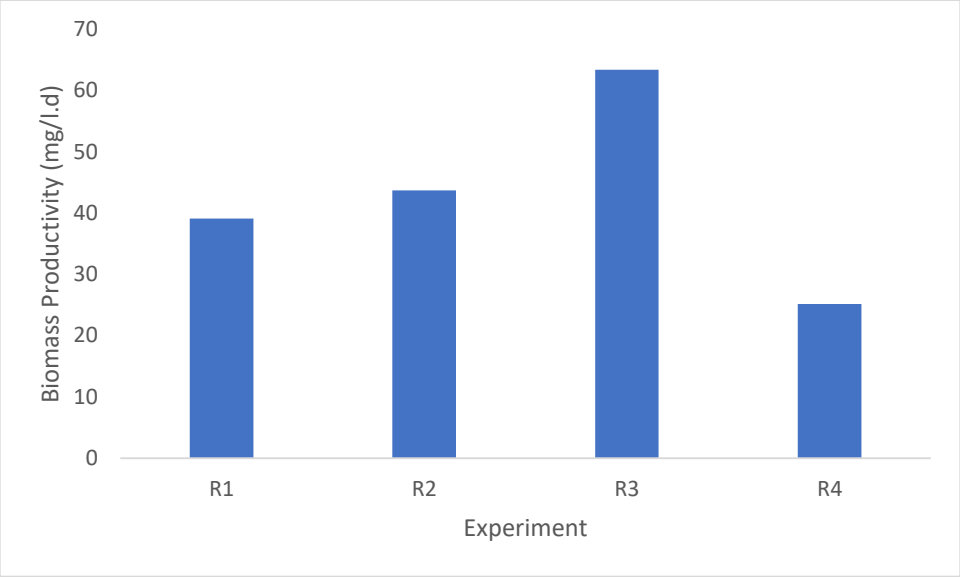


Figure 10 – Biomass productivity of R1-R4

4.1.5 Ammonia removal

The nutrient removal from each treatment is shown in figures 11 and 12. For the first test, most of the ammonia was consumed in 312 hours, and the ammonia level remained stable during that period. For R1, 170 mg/l ammonia was removed, which is about 85% of the initial level. For R2, ammonia was gradually consumed and reached 161 mg/l after 500 hours from the cultivation and then remained stable. For R3, 343 mg/l ammonia was consumed during the experiment. R4 was almost stable until 165 hours, representing the lag phase. Then ammonia was dramatically decreased during the exponential phase and reached 480 mg/l after 390 hours.

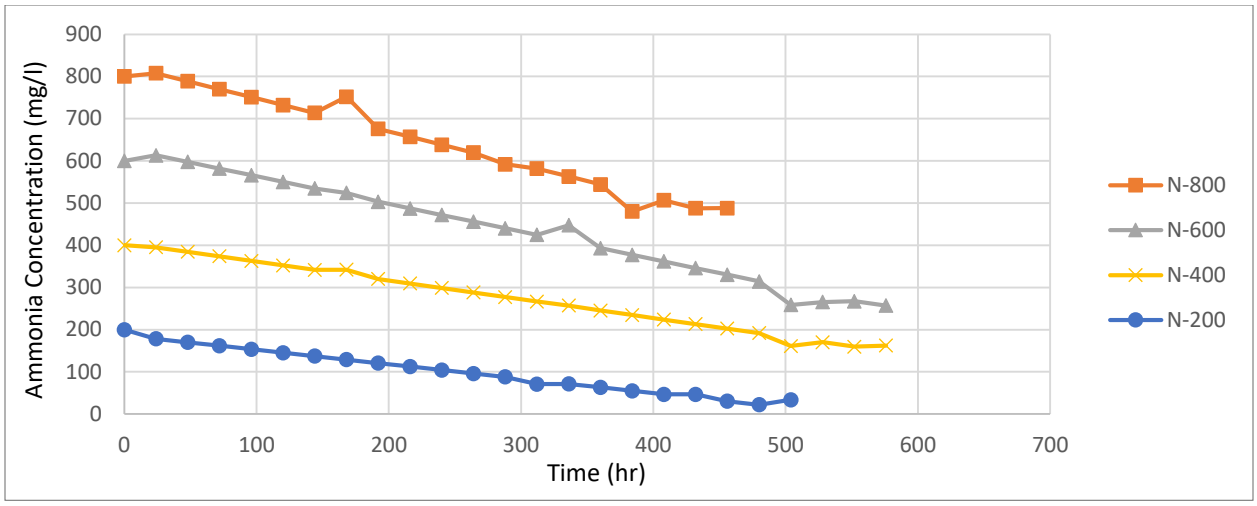


Figure 11 – Ammonia removal over time, R1-R4

4.1.6 Ammonia Removal Rate

The N-removal and N-removal rates of R1-R4 are shown in figure 12. For R1, about 170 mg/l of ammonia was consumed, which is 85% of the initial ammonia. The removal rate for R1 was 8.5 mg/l.d. For R2, 240 mg/l of ammonia was removed by microalgae. In R3, the best removal was achieved as microalgae consumed 348 mg/l of nitrogen. Removal rates of R3 and R4 were 17.4 and 20 mg/l.d respectively. The high removal rate of R4 is due to a shorter time.

4.1.7 Phosphorus removal

Figure 13 shows the phosphorus decrease over time. R1 and R4 removed about 98% of phosphorus (below detection limit). However, R2 and R3 removed 84% and 89%, respectively. Maximum P removal was achieved in the tests with the lowest (200 mg/l) and highest (800 mg/l) levels of ammonia. This is indicative of a relationship between P-removal and initial N levels. At low and high levels of ammonia, algae tend to consume more P than normal ranges.

Accordingly, *C. Vulgaris* microalga had the best performance at the ammonia level of 600 mg/l. As a result, this ammonia level was chosen for the second stage. Table 1 shows the results of first stage.

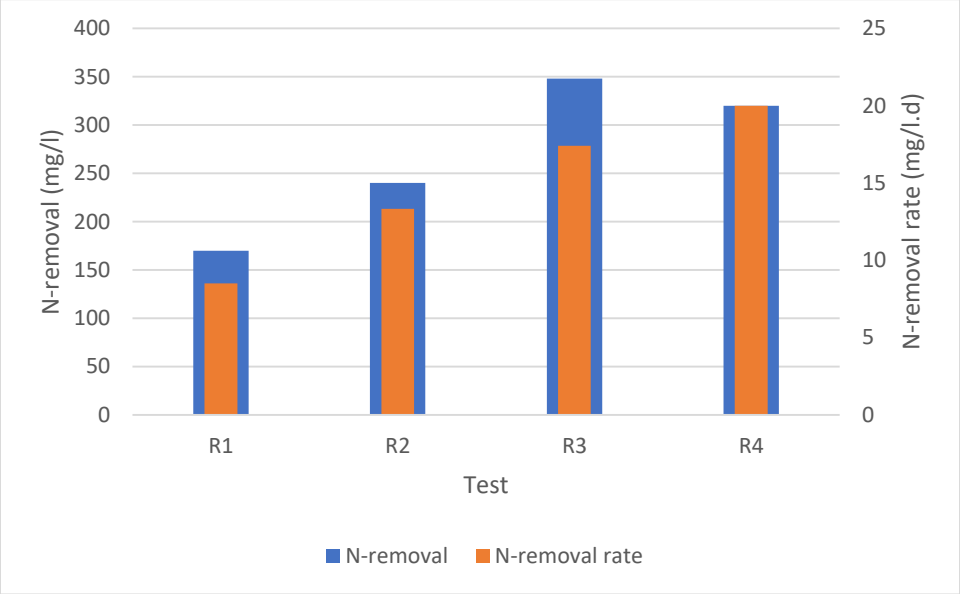


Figure 12- Ammonia removal and ammonia removal rate, R1-R4

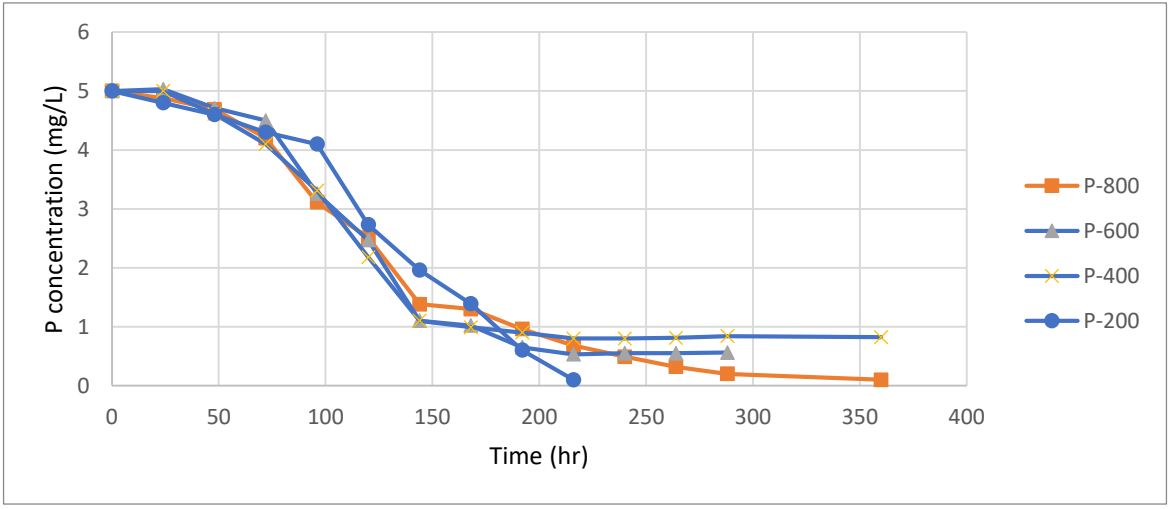


Figure 13- Phosphorus decrease over time, R1-R4

Table 1- Results of first stage

Initial N (mg/l)	**Biomass** (mg/l)	**BP** (mg/l.d)	**N-removal** (mg/l)	**N-removal rate** (mg/l.d)	**P-removal** (%)	**P-removal** (mg/l)	**P-removal rate** (mg/l.d)
200	782	39.1	170	8.5	>98	4.9	0.245
400	786	43.66	240	13.33	84	4.2	0.233
600	1268	63.4	348	17.4	89	4.45	0.222
800	403	25.18	320	20	>98	4.9	0.306

4.2. Second Stage

The importance of pH adjustment in algal culture was discussed earlier. With the algae growth in the media with ammonia, a drop in pH is observed. As a result, pH adjustment is associated with successful growth and nutrient removal. To adjust the pH, a buffering system of CO_2/ $NaHCO_3$ was applied. To investigate the effect of the buffering system of CO_2/ $NAHCO_3$ on the growth and removal capacity of the microalgae, a 2-level factorial experiment was designed. Other parameters such as pH, growth, biomass, biomass productivity and phosphorus, and nitrogen removal by microalgae were also determined. Table 2 shows the list of experiments in the second stage.

Table 2- Second phase experiments, S1-S5

Test	**Initial N** mg/l	**CO_2** %	**$NaHCO_3$** mg/l
S1	600	4	1.5
S2	600	6	1
S3	600	2	1
S4	600	2	2
S5	600	6	2

4.2.1 Growth

The growth for the second-phase experiments is shown in figure 14. For S1, the exponential phase started after approximately 24 hours of cultivation. As the pH of the culture was in a favorable range, a sharp rapid growth was seen. After about 220 hours, S1 entered the stationary phase and achieved the highest biomass concentration of 1740 mg/l. S2 showed poor growth despite a high level of CO_2 and low level of $NaHCO_3$. After 24 hours of lag phase, microalgae started growing but due to high CO_2 and ammonia utilization, a pH drop was noticed. The death phase commenced after 72 hours of cultivation without a stationary phase. Compared to S2, algal growth was sharper in S3; however, the death phase was reached at the same time. No stationary phase was observed herein as well. For S4, a lag phase of 24 hours was detected. After that, a sharp dramatic increase in the growth of algae was seen. After about 220 hours of cultivation, stationary phase of S4 was established. Although the performance of S5 was better than that of S2 and S3, compared to S1 and S4, its efficiency was low.

The maximum biomass concentration of each test is shown in figure 15. S1 and S4 had the highest biomass concentration of 1740 and 1631 mg/l, respectively. This is because the pH of these two tests remained in the tolerable range for optimum growth of *Chlorella Vulgaris*. S2, S3, and S5 reached the biomass concentration of 315, 474, and 639 mg/l, respectively. At high CO_2 concentrations, the culture became acidic, and as a result, algae went to death phase after a few days. Compared to the latter, S5 had better performance in terms of growth and biomass production.

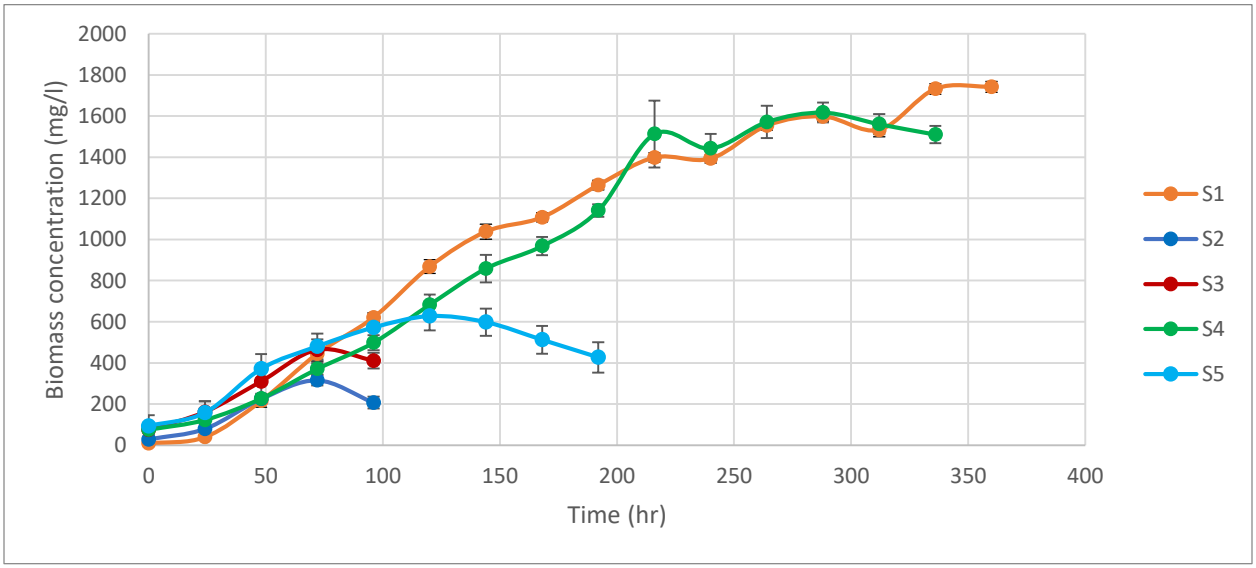


Figure 14 – Growth of S1-S5

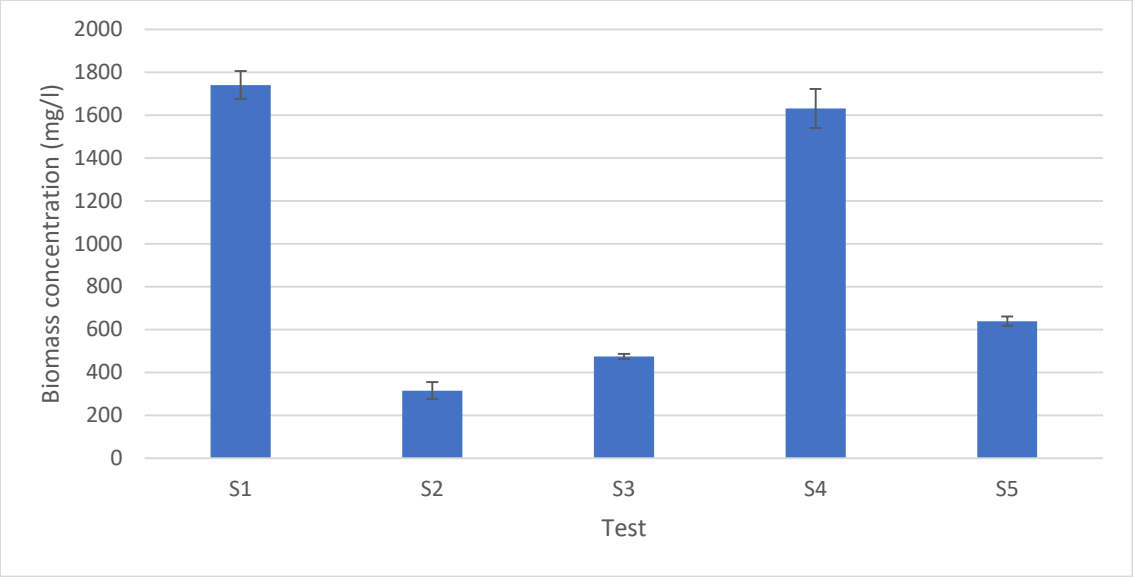


Figure 15 – Biomass concentration of tests S1-S5

4.2.2 Biomass Productivity

The biomass productivity (BP) of 5 tests is shown in figure 16. BP of S1 and S4 reached 116 and 116.5 mg/l, respectively. Despite having low biomass concentration, S3 reached the highest biomass productivity of 118.5 mg/l.d. It took 14 and 15 days for S1 and S4 to reached the peak, respectively, However, S3 only took 4 days. This shows the importance of other factors, such as time. Lower BPs of 78.75 and 79.87 mg/l.d were obtained for S2 and S5, respectively. The error bars in figure 16 show the average of the three replicates.

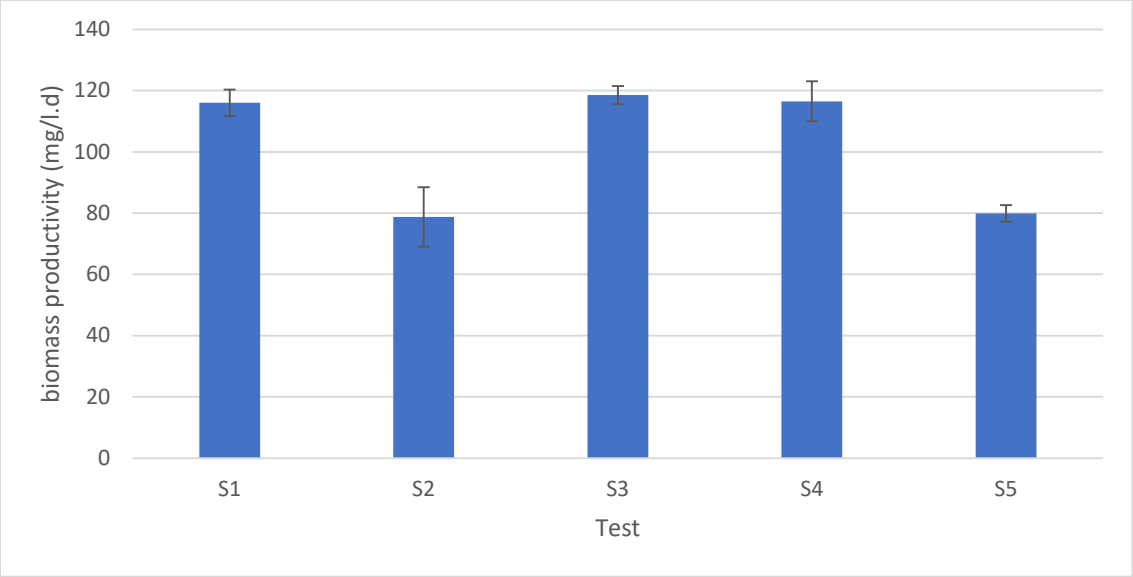


Figure 16 – Biomass productivity of S1- S5

4.2.3 pH

Figure 17 shows the pH profile of tests S1-S5. For S1 and S4, the pH profile decreased smoothly. For S1, pH dropped from 7.2 to about 6.6 after 200 hours of cultivation. For S4, pH decreased from 7.2 to 6. During experiments S1 and S4, pH remained within the range of 6-8, which is a favorable range for *C. Vulgaris* microalgae. The best growth, and nutrient removal were achieved for S1 and S4 as the buffering system of CO_2/ $NaHCO_3$ worked well. For S2 and S3, pH suddenly dropped to about 3.5 after 100 hours of cultivation. Due to the rapid pH decrease, dramatic growth was not seen in S2 and S3. As the pH dropped and the culture became acidic, algae went to the death phase rapidly. For S5, pH decreased smoothly during the first 48 hours of cultivation. However, as microalgae assimilated ammonia, pH dropped continuously, and reached about 4 after 200 hours of cultivation.

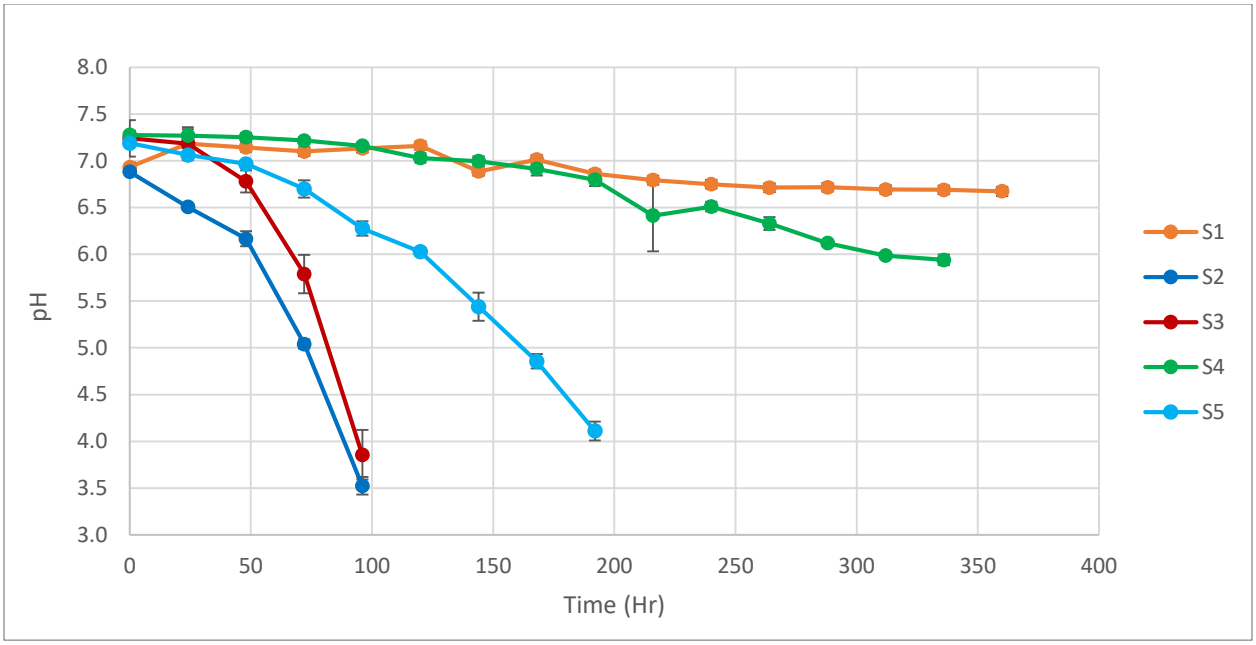


Figure 17– pH of S1- S5 over time

4.2.4 Nutrient removal

Figure 18 shows the phosphorus removal for tests S1-S5 over time. For almost all of the tests, rapid phosphorus consumption was observed during the first 72 hours of cultivation. For tests S1, S2, and S3, more than 85% of initial phosphorus was removed after 3 days. After 72 hours, the phosphorus level remained stable. However, P removals for S4 and S5 were higher than that of S1, S2, and S3. Phosphorus levels reached 1.25 and 0.910 mg/l for S4 and S5, respectively.

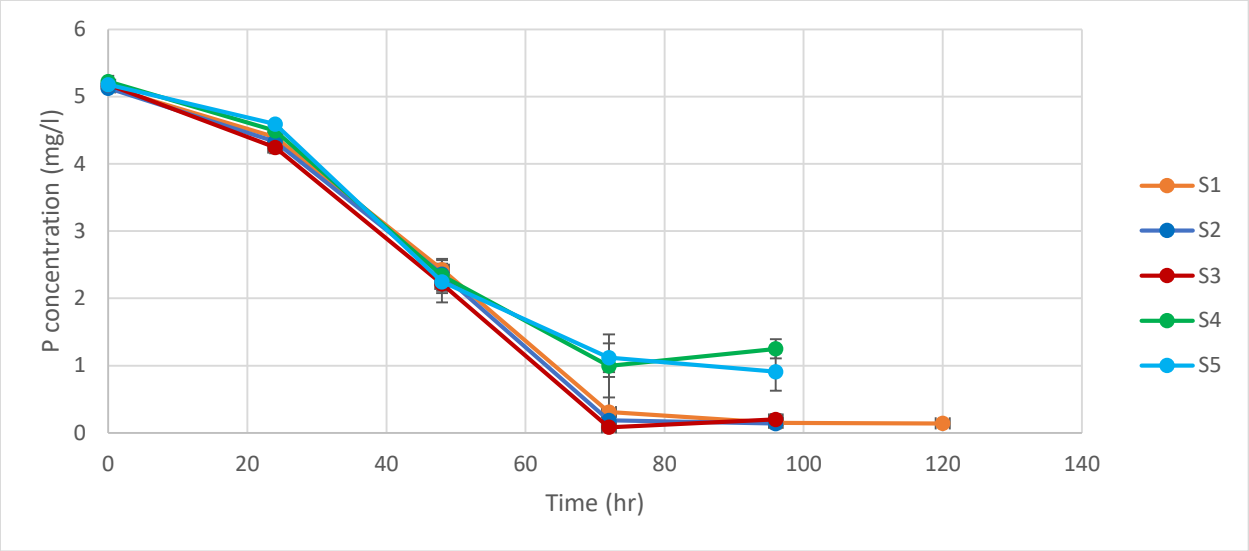


Figure 18 – P decrease for S1- S5 over time

Decrease in ammonia for the 5 experiments is shown in figure 19. Half-way into the experiment with CO_2 of 4% and 1.5 g/l of $NaHCO_3$, ammonia gradually decreased after 24 hours of cultivation. After 312 hours of cultivation, about 417 mg/l of ammonia was assimilated by microalgae. In tests S2 and S3, as pH dropped suddenly to about 4 and microalgae went to the death phase, only a small amount of ammonia decrease was observed. Initial amount of 94 and 103 mg/l were removed for S2 and S3, respectively. Same as other tests, no reduction was seen during the lag phase. After that, as algae grew, a gradual decrease in ammonia was observed. Ammonia concentration became stable after 312 hours of cultivation. *Chlorella Vulgaris* microalgae removed about 293 mg/l of the initial ammonia. During S5, 127 mg/l was consumed.

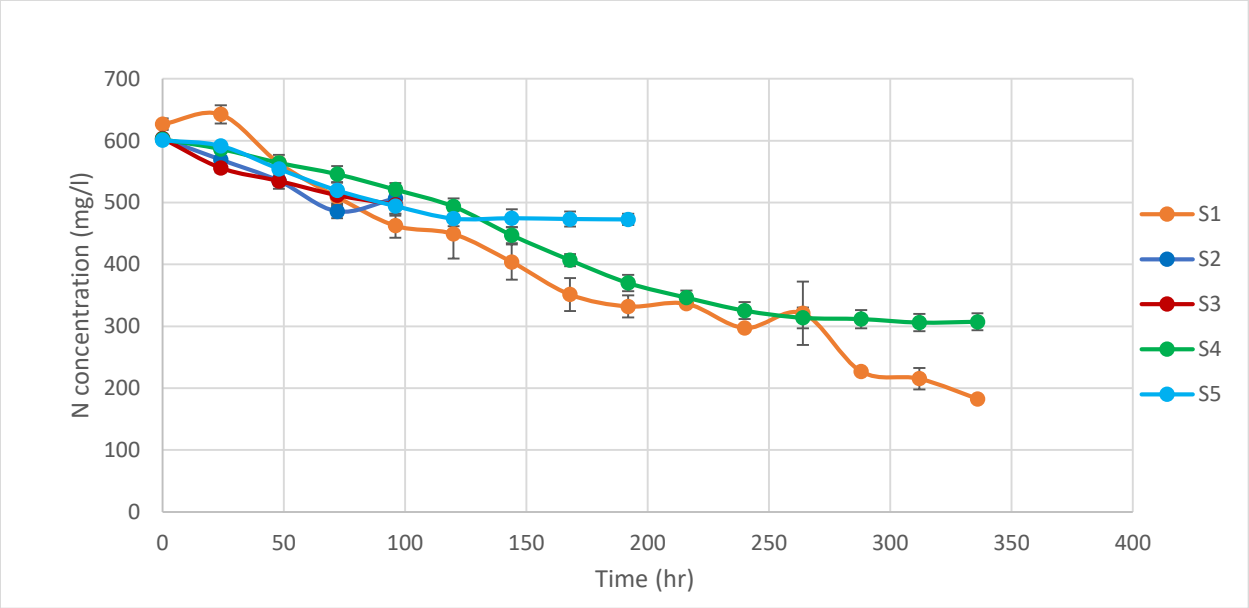


Figure 19 – Ammonia decrease for S1-S5 over time

Figures 20 and 21 show ammonia removal and ammonia removal rate, respectively. S1 had the highest ammonia removal. As the buffering system was well-maintained, the favorable pH range algae grew well and, thus, assimilated ammonia. For S4, almost 50% of initial ammonia was consumed. For S1 and S4, 417.33 and 292.67 mg/l ammonia was removed, respectively. However, for tests S2, S3, and S5, only 93.34, 103, and 127.33 mg/l ammonia was removed, respectively. For S1, the removal rate of 27.822 was achieved whereas S2, S3, S4, and S5 achieved 23.33, 25.75, 20.90, and 15.91 mg/l.d, respectively.

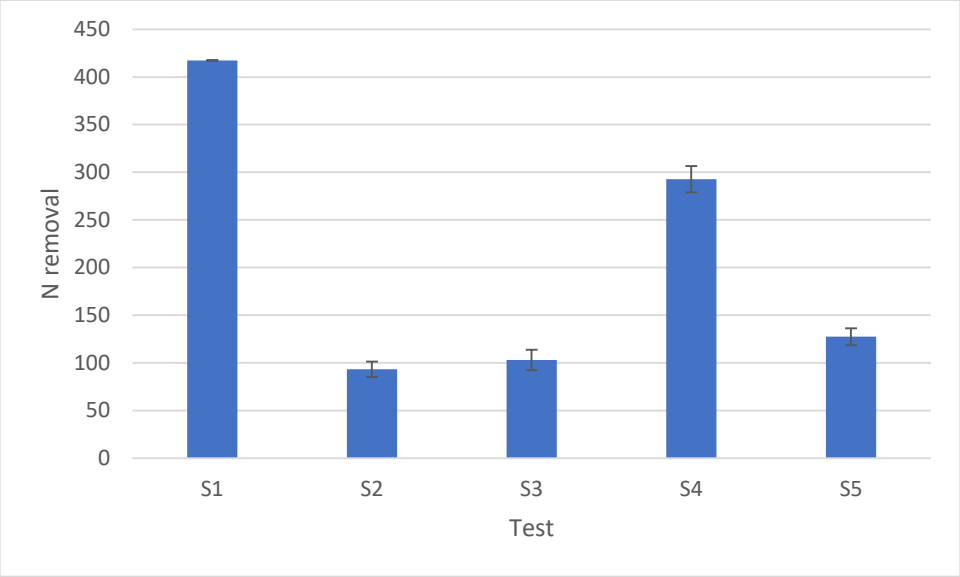


Figure 20 – Ammonia removals for S1-S5

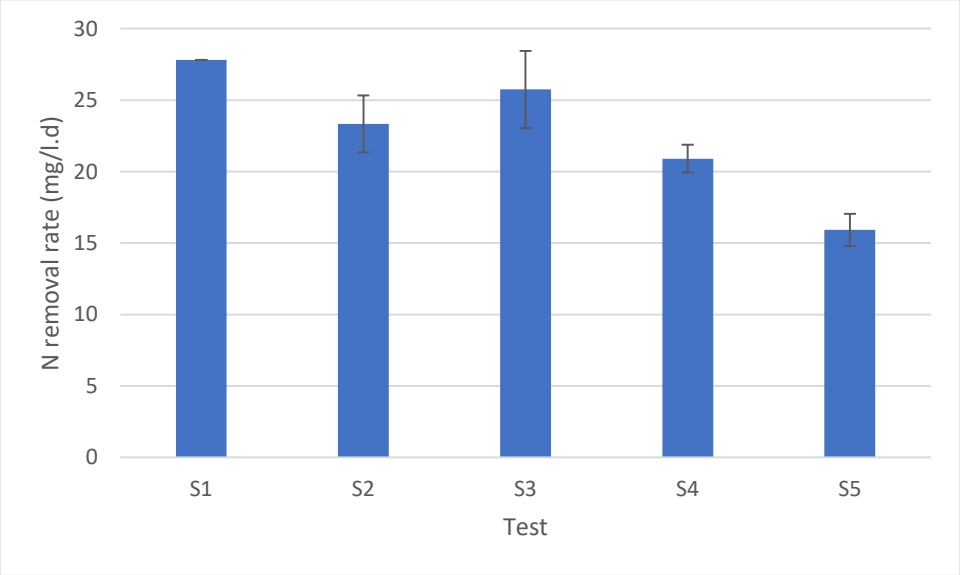


Figure 21 – Ammonia removal rates for S1-S5

4.2.5 Fed-batch examination in S2 and S3

To investigate the importance of pH and buffering system in algal growth and also the strength of *Chlorella Vulgaris* in harsh conditions, the pH of tests S2 and S3 were adjusted after going to the death phase. Figures 22 and 23 show the fed-batch growth of S2 and S3. In S2, microalgae reached the death phase after 96 hours of cultivation, and pH of 3.52 was recorded for 24 hours. At this point, 1g/l $NaHCO_3$ was added to the culture, and pH reached 6 consequently. No lag phase was seen. Microalgae started growing rapidly. After 72 hours, another 1 g/l of $NaHCO_3$ was added to raise the pH to 7. After 11 days, while microalgae were still in the exponential phase, 1301 mg/l biomass was achieved. Also, the pH stabilized at 6.80 and ammonia removal of 382 mg/l was achieved.

In S3, algae reached the death phase after 96 hours of cultivation, and the pH of 3.82 was recorded. Similar to S2, 1 g/l of $NaHCO_3$ was added, and after 72 hours, more sodium bicarbonate was added. pH increased sharply and reached about 6.5. A gradual growth in microalgae was noticed. After 12 days, a biomass concentration of 1401 mg/l was obtained. Approximately 390 mg/l ammonia was removed, and pH stabilized at 6.6. The results show that the buffering systems of CO_2/ $NaHCO_3$ worked well. Furthermore, the fed-batch system may be a good option for high ammonia media. *Chlorella Vulgaris* started growing after overcoming harsh conditions for 24 hours, which means this species could be the right candidate for leachate treatment.

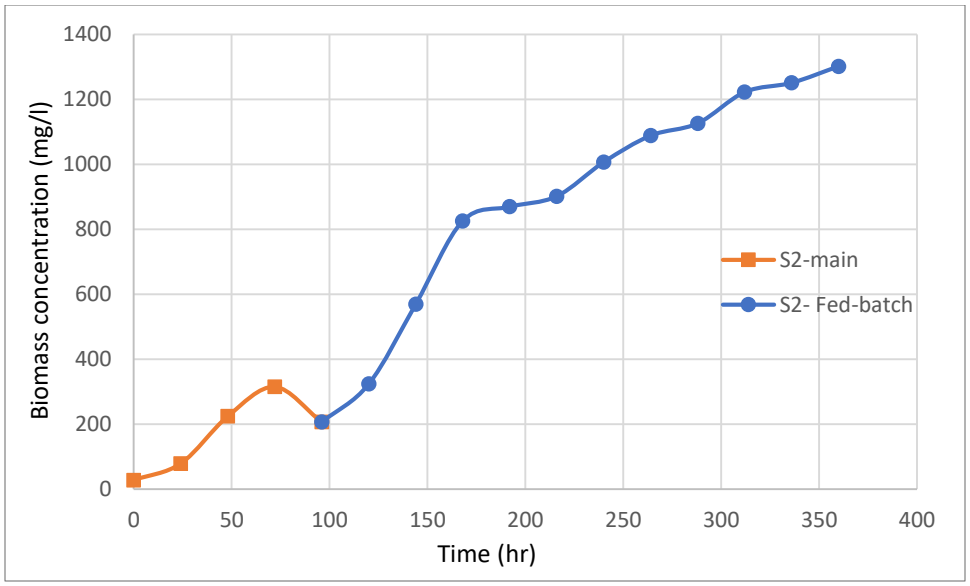


Figure 22 – Fed-batch testing of S2

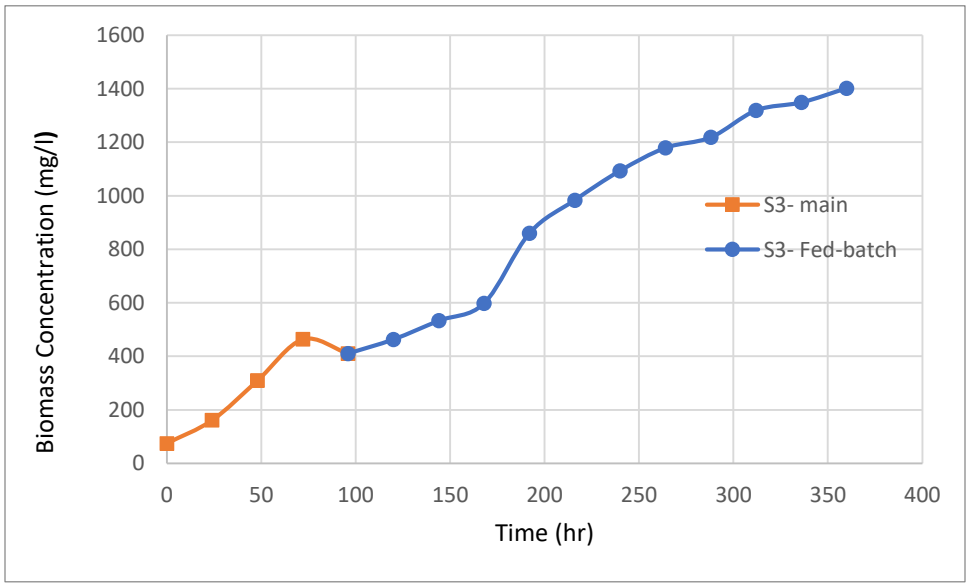


Figure 23 – Fed-batch testing of S3

A summary of results is shown in table 3.

Table 3- Second phase results

Test	Biomass mg/l	BP mg/l.d	N removal mg/l	N removal rate mg/l.d	P removal mg/l	P removal rate mg/l/d
S1	1740	116.00	417.33	27.822	4.86	0.97
S2	315	78.75	93.34	23.335	4.86	0.97
S3	474	118.50	103.00	25.75	4.8	0.96
S4	1631	116.50	292.67	20.90	3.75	0.75
S5	639	79.87	127.33	15.91	4.09	0.81

4.3. Statistical analysis

4.3.1 Biomass production

The ANOVA of biomass concentration is presented in figure 24. The P-value of <0.0001 showed that the 2FI model (Biomass =757.76-(279.0*A)+(364.24*B)-(204.26*AB))is significant. According to ANOVA, factors of A, B, and AB are important. This means that there is a significant difference between the concentrations of CO_2 and $NAHCO_3$. When P-value is smaller than 0.05, it means that the null hypothesis (there are no factor effects) is rejected. The R^2 of the model was 0.9927. To verify the ANOVA analysis, a normal plot of residuals was plotted. The distribution of residuals was normal. Figure 25 shows the normal plot of residuals for biomass.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3.027E+06	3	1.009E+06	456.22	< 0.0001	significant
A-CO2	9.346E+05	1	9.346E+05	422.53	< 0.0001	
B-NaHCO3	1.592E+06	1	1.592E+06	719.77	< 0.0001	
AB	5.007E+05	1	5.007E+05	226.35	< 0.0001	
Curvature	2.253E+06	1	2.253E+06	1018.62	< 0.0001	
Pure Error	22118.88	10	2211.89			
Cor Total	5.302E+06	14				

Figure 24 – ANOVA for biomass

Figure 26 shows the interaction between CO_2 and $NaHCO_3$. The amount of biomass was highly dependent on $NaHCO_3$ concentration. By increasing the amount of factor B from 1 to 2 g/l, biomass production increased sharply. However, no particular trend for the growth in CO_2 was found. As the CO_2 level increased from 2% to 4%, biomass concentration increased. However, a further increase from 4% to 6 % decreased the algal growth. At a low level of CO_2, increasing the amount of factor B increased biomass concentration. Besides that, the curvature is significant, which means a linear model is not adequate. As a result, a quadratic or higher order model can be used. This can be done by augmenting the design to a response surface design to better model the factors and responses.

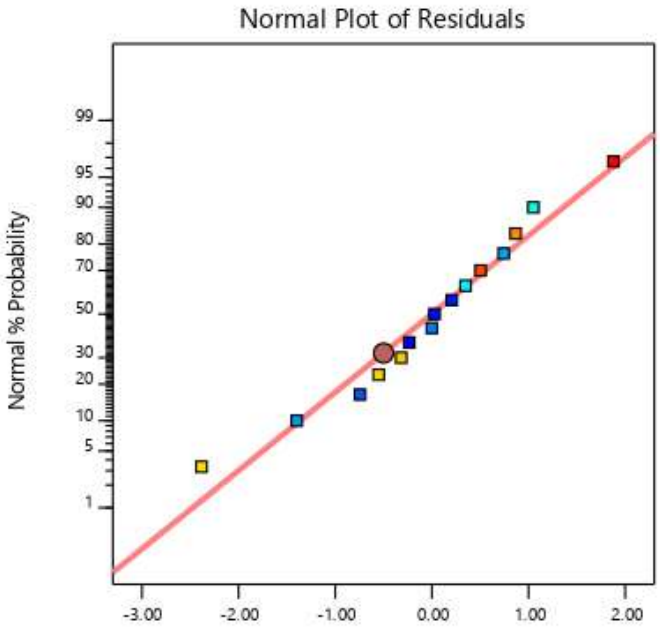


Figure 25 – Normal plot of residuals for biomass

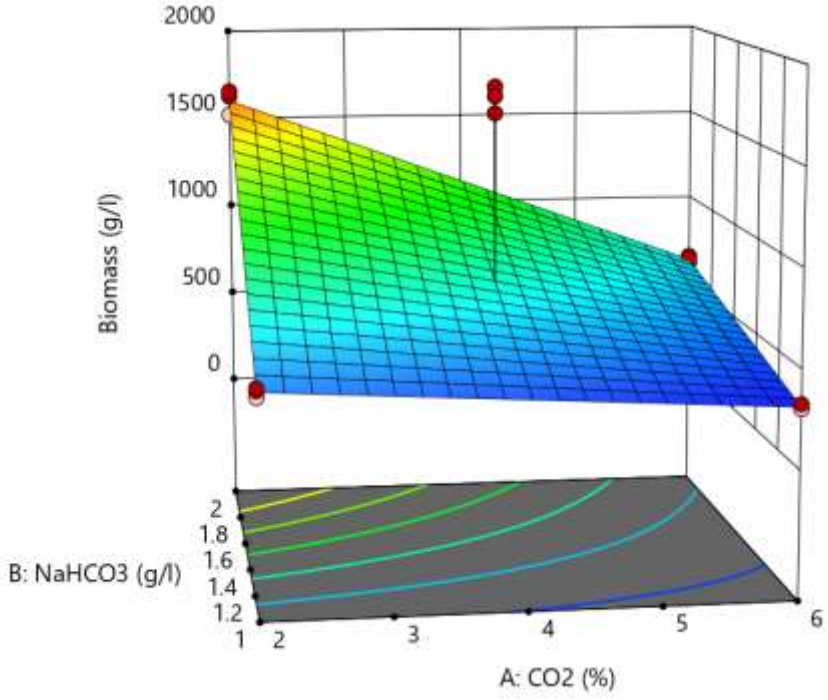


Figure 26 – The interaction of factors A and B for biomass

4.3.2 Biomass productivity

Figure 27 shows the ANOVA of biomass productivity including all factors. The P-values of 0.381 and 0.303 for Factor A and AB, respectively, indicated that these terms are not significant. Figure 28 shows the ANOVA table after eliminating non-significant terms. P-value of <0.001 indicated that the model is significant; this indicates that there is a significant different between different levels of Factor B A coded model of BP = 97.16 – 17.53*A was achieved.CO_2 was an essential factor in the biomass productivity of *C. Vulgaris*. The model R^2 value of 0.94 was achieved and the lack of fit was not significant. The normal plot of residuals is shown in figure 29. The 3-D interaction of factors is shown in figure 30. By increasing CO_2 from 2% to 4, no dramatic change in BP was observed. However, a further increase from 4 to, resulted in a significant drop in BP.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3721.54	3	1240.51	71.91	< 0.0001	significant
A-CO2	3686.73	1	3686.73	213.72	< 0.0001	
B-NaHCO3	14.47	1	14.47	0.8386	0.3814	
AB	20.35	1	20.35	1.18	0.3030	
Curvature	792.76	1	792.76	45.96	< 0.0001	
Pure Error	172.50	10	17.25			
Cor Total	4686.80	14				

Figure 27 – ANOVA analysis of BP including all terms

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3686.73	1	3686.73	213.40	< 0.0001	significant
A-CO2	3686.73	1	3686.73	213.40	< 0.0001	
Curvature	792.76	1	792.76	45.89	< 0.0001	
Residual	207.31	12	17.28			
Lack of Fit	34.81	2	17.41	1.01	0.3989	not significant
Pure Error	172.50	10	17.25			
Cor Total	4686.80	14				

Figure 28– ANOVA table of BP with significant terms

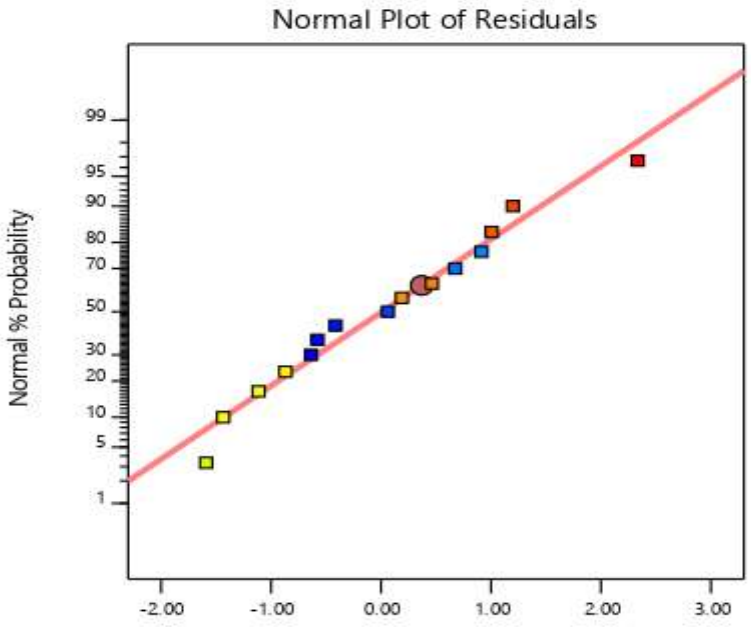


Figure 29 – Normal plot of residuals for BP

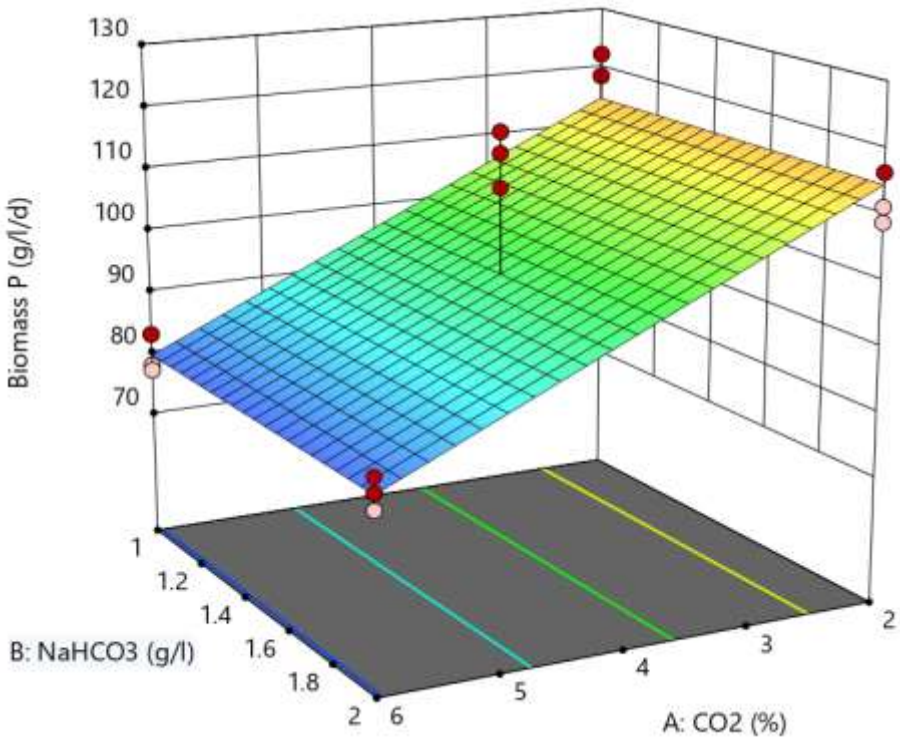


Figure 30 – 3-D interaction of A and B for BP

4.3.3 Ammonia Removal

Figure 31 shows the ANOVA table for ammonia removal. The P-value of <0.0001 shows that the model is significant. Also, factors A, B, and AB are significant that means the null hypothesis was rejected. The R^2 of the model was 0.95. The normal probability plot of the residuals is shown in figure 32. The coded equation for N removal was: N Removal = 159.55-50.22 *A +49.62 B – 32.05 AB

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	72135.41	3	24045.14	78.83	< 0.0001	significant
A-CO2	30260.56	1	30260.56	99.20	< 0.0001	
B-NaHCO3	29549.70	1	29549.70	96.87	< 0.0001	
AB	12325.15	1	12325.15	40.41	< 0.0001	
Curvature	1.502E+05	1	1.502E+05	492.50	< 0.0001	
Pure Error	3050.38	10	305.04			
Cor Total	2.254E+05	14				

Figure 31 – ANOVA table for ammonia removal

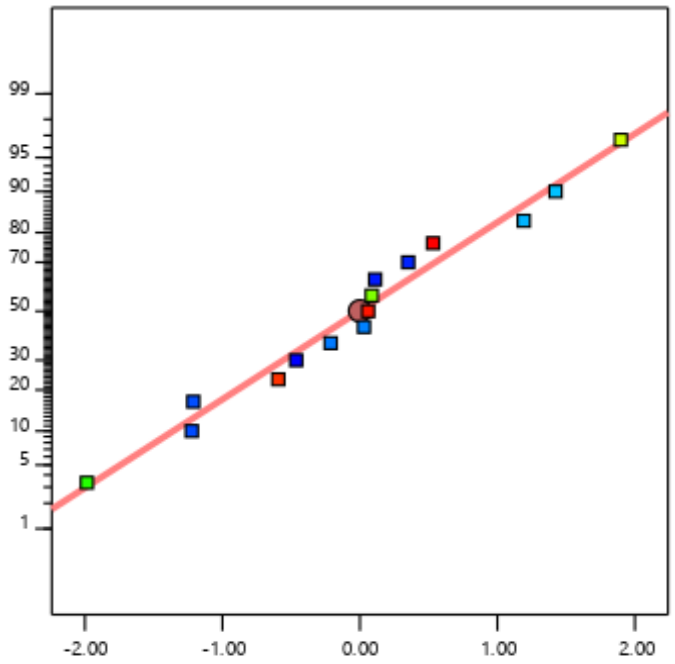


Figure 32 – The normal plot of residuals for ammonia

A 3-D diagram of the factors is shown in figure 33. At a low level of CO_2, ammonia removal increased sharply by increasing factor B. However, as Factor A increased, ammonia removal raised slowly. Moreover, factor A had a positive effect, while factor B negatively affected ammonia removal. The curvature is also significant, which indicates that the relationship

between factors and ammonia removal is not linear as confirmed by the 3-D plot. Therefore, a quadratic or higher order model could be used.

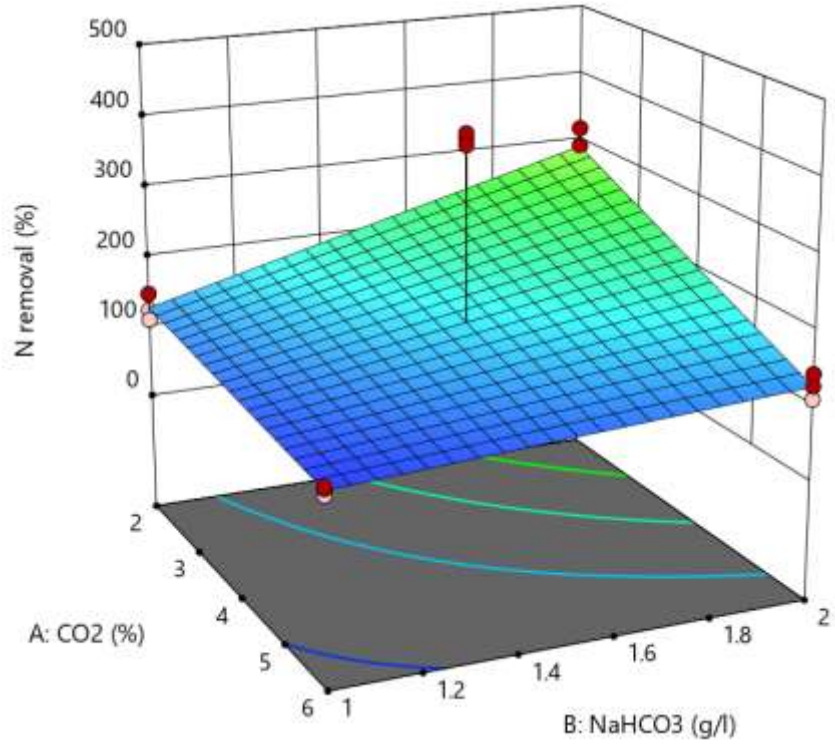


Figure 33 – 3-D diagram of the effect of A, B, and AB on ammonia removal

4.3.4 Phosphorus

ANOVA table containing all factors for phosphorus removal is shown in figure 34. P-value of 0.084 indicated that the term AB is not significant. Figure 35 showed the ANOVA table after removing AB while the model is significant and lack of fit is not significant. The model achieved an R^2 of 0.96. The normal probability plot of the residuals is shown in figure 36. An outlier was seen in the plot. An outlier is an observation that appears to deviate from other observations in the sample. This is usually due to data error. However, outliers sometimes might be an indication of something scientifically interesting. 3-D diagram of factors A and B is shown in figure 37.

According to this figure, sodium bicarbonate is the most important factor in phosphorus removal. A reverse relationship between factor B and P-removal was observed. By increasing the amount of B from 1 to 2 g/l, P-removal dropped dramatically. However, curvature was significant in this model.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2.42	3	0.8060	117.27	< 0.0001	significant
A-CO2	0.0784	1	0.0784	11.41	0.0070	
B-NaHCO3	2.31	1	2.31	336.72	< 0.0001	
AB	0.0252	1	0.0252	3.67	0.0845	
Curvature	0.4664	1	0.4664	67.86	< 0.0001	
Pure Error	0.0687	10	0.0069			
Cor Total	2.95	14				

Figure 34 – ANOVA table of P-removal, containing all factors

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2.39	2	1.20	140.09	< 0.0001	significant
A-CO2	0.0784	1	0.0784	9.18	0.0114	
B-NaHCO3	2.31	1	2.31	271.00	< 0.0001	
Curvature	0.4664	1	0.4664	54.61	< 0.0001	
Residual	0.0939	11	0.0085			
Lack of Fit	0.0252	1	0.0252	3.67	0.0845	not significant
Pure Error	0.0687	10	0.0069			
Cor Total	2.95	14				

Figure 35 – ANOVA table of P-removal, containing significant terms

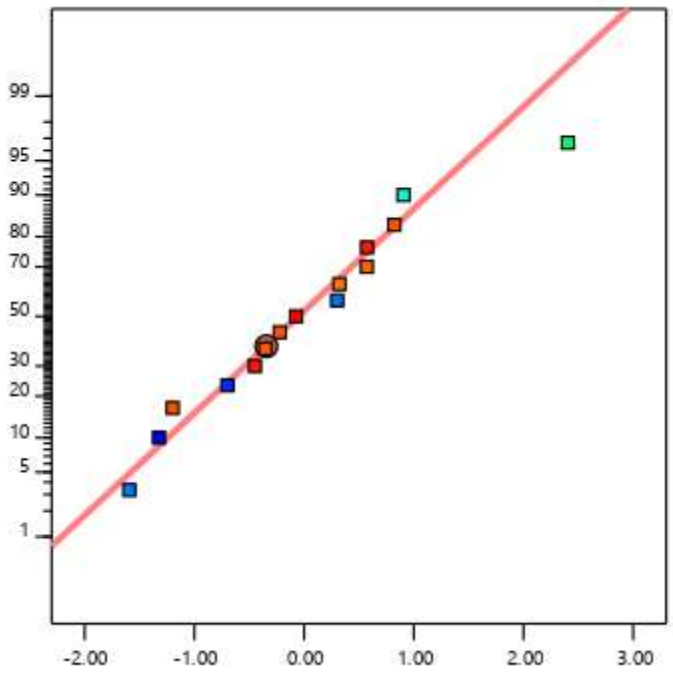


Figure 36 – the normal plot of residuals for phosphorus

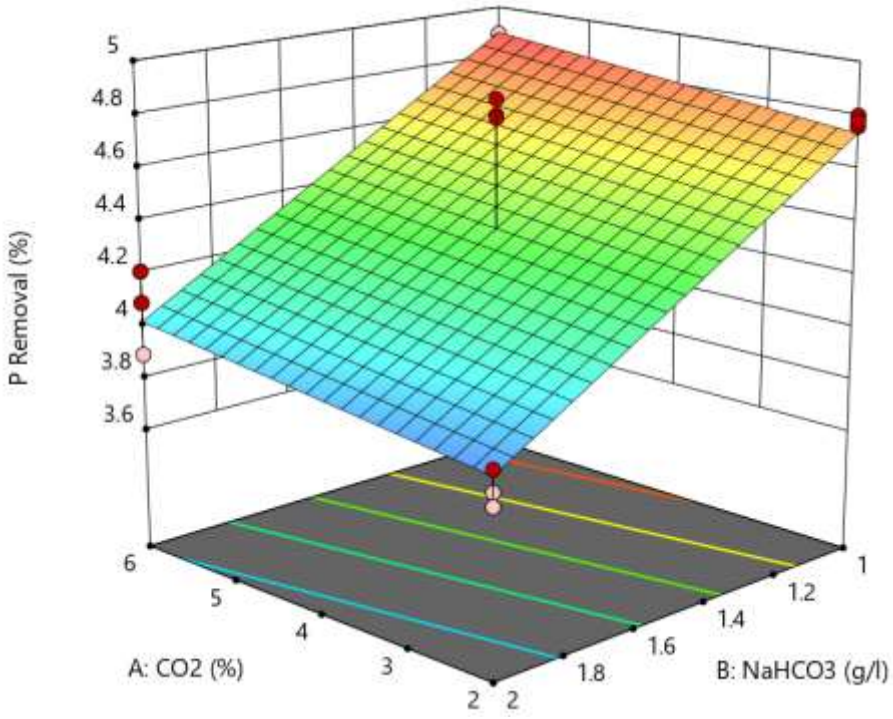


Figure 36 – 3-D diagram of factors A and b for P-removal

CHAPTER 5: SUMMARY AND CONCLUSION

Treatment of high ammonia streams such as leachate is challenging. Current methods are expensive and not efficient. This thesis investigated the growth and nutrient removal of *Chlorella Vulgaris* in high ammonia levels. The efficiency of the buffering system of CO_2/$NaHCO_3$ was also examined. According to the results, *Chlorella Vulgaris* microalgae is a good candidate for leachate treatment as it tolerated high ammonia levels and also survived harsh conditions of low pH. When the pH was maintained at a favorable range, 417 g/l of ammonia was removed, and 1740 mg/l biomass was produced. Further addition of $NaHCO_3$ after the death phase showed the importance of maintaining pH as microalgae were revatilized and started growing rapidly. Most phosphorus was removed in the first 72 hours of cultivation for all of the tests. This study shows the leachate treatment ability of *Chlorella Vulgaris* due to the high production of biomass, high ammonia and phosphorous removal rates, and it's at growth at the harsh low-pH conditions.

CHAPTER 6: RECOMMENDATIONS AND FUTURE RESEARCH

Based on the laboratory experiments, the following recommendations are made:

- ✓ The fed-batch process could be very successful for pH adjustment and leachate treatment.
- ✓ Due to the turbidity of leachate, flat panel PBRs can be further examined with a similar setup to find out the leachate removal capability.
- ✓ As the turbidity of leachate is high, light penetration may be decreased. A possible solution for this problem could be increasing the light period. The light/dark cycle of 18/6 or even more should be investigated.
- ✓ Future research should apply this buffering system in raw leachate and ½ raw leachate.

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