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Nutrient Removal by Microalgae from Pre-Treated Municipal Wastewater

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BOD	Biological Oxygen Demand
C	Carbon
C/N	Carbon/Nitrogen
$Ca(OH)_2$	Calcium Hydroxide
$CaCO_3$	Calcium Carbonate
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
EC	European Commission
EEA	European Environmental Agency
EPBR	Enhanced Biological Phosphorus Removal
$FeSO_4$	Ferrous Sulphate
H_2PO_4, HPO_4^{2-}	Orthophosphate ions
HRAP	High Rate Algal Pond
HRT	Hydraulic Retention Time
ISO	International Organization for Standardization
K_2SO_4	Potassium sulphate
KOH	Potassium hydroxide
MBWWT	Microalgal Based Wastewater Treatment
N	Nitrogen
NaOH	Sodium Hydroxide
NH_3, NH_4^+	Ammonia, Ammonium
NO_2, NO_3	Nitrite, Nitrate
O_2	Oxygen
OD	Optical Density
P	Phosphorus
RPM	Revolutions per minute
TN	Total Nitrogen
TP	Total Phosphorus
WWT, WWTP	Wastewater Treatment, Wastewater Treatment Plant

1.0. INTRODUCTION

1.1 Wastewater and wastewater treatment

The importance of wastewater treatment as a research area has significantly increased in recent years due to the growing concerns about environmental sustainability and the need to develop innovative and sustainable solutions for effective wastewater management. This shift in focus has been driven by factors such as population growth, urbanization, industrialization, and changes in consumption patterns, which have all contributed to a rising demand for freshwater resources worldwide (Connor 2015, Li et al. 2019). Unfortunately, if the current business-as-usual scenario persists, it is projected that the global water deficit will reach 40% by 2030 (Douglas 2009). Moreover, large amounts of wastewater containing excessive nutrients, such as nitrogen and phosphorus, are generated every year by urban, industrial, and agricultural practices, which can lead to eutrophication in aquatic environments (Le et al. 2010, Li et al. 2019, Sukačová et al. 2015). Traditional wastewater treatment methods are designed to treat water and safely return it back to the environment. Within the 27 member states of the European Union, approximately 69% of the wastewater generated by the population is subjected to tertiary treatment, whereas only 13% undergo primary and secondary treatments (Geremia et al. 2021). In Hungary, 72.08% of wastewater undergoes tertiary treatment, secondary process was 7.19% undergoes secondary and primary treatment while 2.18% remains untreated (EEA 2020)

Over the past four decades, all European Union countries have extensively developed centralized wastewater treatment infrastructure, which has been instrumental in promoting high levels of urbanization in the region (EEA 2021, Büttner et al. 2022). However, these systems are far from efficient and sustainable because of high energy consumption, high operational costs, and limited resource recovery (Jain 2021). OECD (2020) reported that the cost of investment and operation of these systems in Europe amounted to approximately €39 billion between 2011 and 2015. Therefore, it is necessary to develop novel approaches for treating wastewater that can remove excess nutrients in a manner that promotes circular economy and sustainability.

1.2. Wastewater treatment with microalgae

Microalgae are microscopic photosynthesising organisms that thrive in various aquatic environments, as well as in wastewater from different sources (Zhou, 2014). They can grow rapidly and reproduce exponentially every few hours when provided favourable autotrophic or mixotrophic conditions. Microalgae can withstand varying levels of temperatures, pH, salinities, light intensities alone or in symbiosis with other organisms. The concept of using microalgae to treat wastewater was conceived by William Oswald in California in the 1950s (Oswald & Gotaas 1957, Oswald 1963). The purpose of using microalgae in the process was to both facilitate the absorption of plant nutrients and supply bacteria with oxygen. The bacteria, on the other hand, were involved in the breakdown of organic components in wastewater, which is the same process used in activated sludge. The process was first developed in shallow ponds, less than 1m deep with continuous stirring by paddle wheels. The high rate algal ponds (HRAPs) are aerobic throughout their entire volumes unlike the facultative ponds, which are anoxic at their lower depths. HRAPs are constructed in a meandering configuration known as raceways. A properly designed and operated HRAP can remove over 90% of the BOD and nearly 80% of the nitrogen and phosphorus present (Oswald 1988). The necessity to increase productivity and maintain monoculture of algae have led to the cultivation of microalgae in closed photobioreactors like tubes and flat panels (Pushparaj et al. 1997, Mirón et al. 1999, Kwietniewska et al. 2012). Photobioreactors possess better light penetration properties that enable the maintenance of higher biomass and productivity levels with less hydraulic retention time (HRT) compared to open ponds (Borowitzka 1998). However, photobioreactors are generally more sophisticated and operationally challenging than ponds. Therefore, photobioreactors are primarily employed for commercial cultivation of microalgae rather than for treatment of wastewater.

Microalgae have been employed to treat wastewater for many years in countries, including U.S.A, Australia, China, Mexico, Thailand (Sun et al. 2019), and presently also in Europe (Camia et al. 2018). Recently, the microalgae-based wastewater treatment (MBWWT) process has gained increasing attention as a promising alternative technology for the advanced treatment and nutrient recovery in wastewater (Li et al. 2019). Many research works have established the feasibility of incorporating microalgae into wastewater treatment as an additional means of tertiary treatment due to its high efficiency in eliminating nutrients, which makes them useful in the advanced treatment of municipal, agricultural, and industrial wastewaters (Abdelfattah et al. 2022, Alcántara et al. 2015, Jia & Yuan 2016, Li et al. 2019, Olguín 2003, Oswald 1963, Oswald 1988, Sukačová et al. 2015, Whitton et al. 2015). However, the efficiency of MBWWT could be challenged by seasonal changes in light intensity and temperature levels, absence of external CO_2 supply, sudden exposure to higher concentrations of ammonia and other contaminants that could negatively affect the growth of microalgae, and as a result undermine the efficiency of the treatment process.

This research aims to evaluate the potential of microalgae, specifically *Chlorella vulgaris*, to remove nutrients, typically N and P, from pre-treated municipal wastewater. Specifically, we ask the question: "Can microalgae be used effectively to remove nutrients from pre-treated municipal wastewater?" To answer this question, we assessed the efficacy of nutrient removal through dilution rates of different concentrations using the photobioreactor method. The study also identifies the most efficient chemical flocculants for harvesting the microalgal biomass for water quality restoration and biofertilizer production. The sedimentation technique was employed to evaluate six chemical flocculants for the recovery of microalgal biomass.

2.0. General Objective

The aim of this study is to determine the nutrient uptake efficiency, particularly N & P, and to evaluate the most efficient harvesting technique of the microalga biomass.

2.1. Specific Objectives

- To explore the cultivation of the microalga, *Chlorella vulgaris*, in pre-treated municipal wastewater using treatment of dilution ratios and investigate the potential value of biomass obtained.
- Investigate on the efficiency of microalgal removal of nutrient and other contaminants in municipal wastewater.
- To assess the most efficient harvesting technique of the microalga suitable for water quality restoration, biofertilizer, biofuel and production

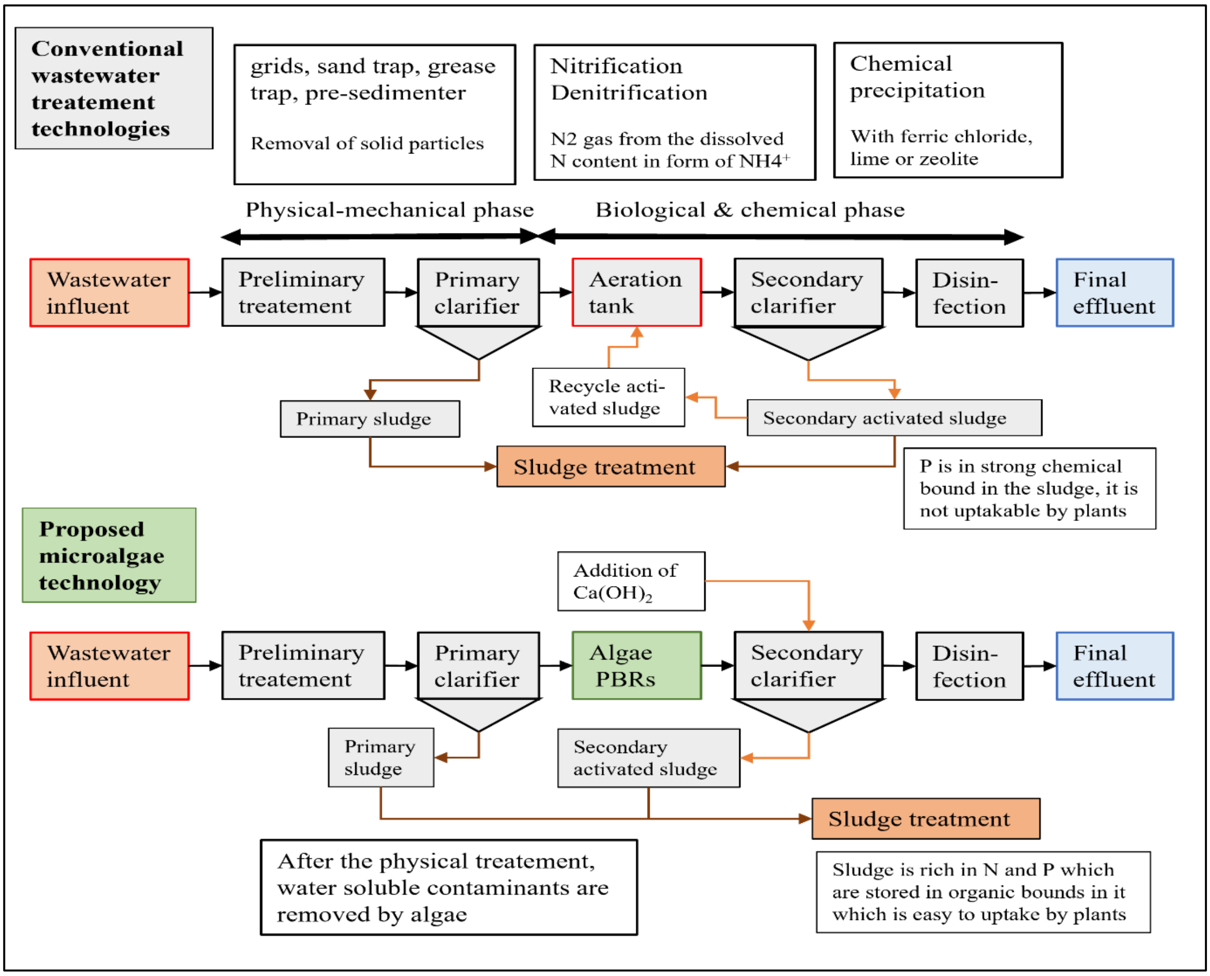

Figure 1. Conventional vs. Microalgae-Based Wastewater Treatment: A Comparative Analysis

2.0. LITERATURE REVIEW

2.1. The current status of wastewater treatment

Wastewater treatment involves a series of stages that includes physical (settling for the removal of coarse suspended materials), biological (to minimise biodegradable organic matter dissolved or in colloidal suspension), and chemical treatment steps (mainly to remove N, P, and potentially harmful microorganisms). However, conventional wastewater treatment plants (WWTPs) are struggling due to stringent pollutant limitations imposed by circular economy principles on wastewater discharge and reuse. The discharge of nitrogen and phosphorus into surface water resources has been on the rise due to human activities, notably agriculture and urbanization. This influx of nutrients has resulted in eutrophication, which can significantly impact the structure and functioning of aquatic ecosystems, posing a threat to human health, biodiversity, and the long-term sustainability of ecosystems (Wang et al. 2013). The primary sources of nutrient inputs into rivers are wastewater discharge and agricultural run-offs with approximately 70% being attributed to wastewater discharges. This has resulted in the implementation of strict nutrient discharge standards and increased pressure on the water treatment industry to reduce nutrient loads entering rivers, especially in ecologically sensitive areas (Bowes et al. 2015, UKTAG 2013). Studies have shown that wastewater contains nearly five times the amount of energy needed for its treatment (Tarallo et al. 2015). Consequently, the treatment process requires more energy, leading to increased energy consumption and operation costs. Additionally, the excess energy can disrupt nutrient removal processes, potentially reducing the effectiveness of nutrient recovery. Large urban wastewater treatment plants (WWTPs) have increasingly implemented nitrogen and phosphorus removal to reduce the negative impacts of these nutrients on sensitive watercourses. However, smaller WWTPs in remote locations may also discharge nitrogen and phosphorus into these watercourses, often with less rigorous treatment, leading to potential underestimation of the negative impacts of these discharges (Bowes et al. 2015, Molinos-Senante et al. 2014, Lutterbeck et al. 2017).

2.1.1. Nitrogen Removal

Until today, conventional nitrification-denitrification processes have been used as nitrogen removal methods from wastewater. Despite being economical, effective, and easy to operate, they have been criticized for their environmental and economic unsustainability due to high energy consumption, sludge production, greenhouse gas emissions, and poor nutrient recovery. To address these challenges, alternative methods such as ammonia stripping, magnesium ammonium phosphate hexahydrate precipitation, and membrane technology have been developed for nitrogen recovery. Although these methods are similarly effective, they face challenges such as secondary pollution, the need for additional phosphorus and magnesium sources, and high energy consumption, leading to increased operational costs (Zhou et al. 2023).

2.1.2 Phosphorus Removal

After carbon and nitrogen, phosphorus is also an essential and indispensable element that performs vital functions in biological processes of all living organisms. Unlike N, which is renewable and extractable from the atmosphere, P is derived from phosphate rock that is a finite resource and is currently limiting (Li et al. 2018). Therefore, it is crucial to promote sustainable P recycling and increased resource efficiency. Chemical precipitation, involving the use of aluminium sulphate, ferric chloride, and ferric sulphate remains the most widely utilized method for P removal by WWTPs, but its application is usually associated with the generation of large amounts of contaminated sludge, which necessitates proper handling and disposal, leading to increased costs and detrimental environmental impact. Biological phosporus removal by Enhanced Biological Phosphorus Removal (EPBR) through activated sludge systems is an economically feasible and environmentally sustainable substitute for chemical treatment (Bunce et al. 2018). Nevertheless, its reputation is marred by fluctuating performance and high sludge contamination (Nguyen et al. 2013). Membrane filtration is also used, but membrane fouling reduces its efficiency and increases operation costs with regular membrane changing. Although membrane filtration method is favoured by on chemical addition and less volume of sludge generation, its efficiency is reduced due to membrane fouling, leading to increased operation costs associated with regular membrane replacement (Morse et al. 1998, Graziani & McLean 2006)

2.2. The Need for Microalgae-based Wastewater Treatment

Microalgae-based wastewater treatment (MBWWT) is an emerging technology that offers a promising solution to the challenges associated with nutrient recovery from conventional wastewater treatment methods despite many studies has been extensively conducted on a small scale. Inefficient nutrient recovery and high costs are common challenges in conventional wastewater treatment methods, which make it imperative to explore and utilize alternative technologies. Microalgae-based wastewater treatment, therefore, presents a more sustainable and cost-effective approach to remove pollutants from wastewater, while producing valuable biomass for soil amendment and biofuels. Additionally, microalgae can be used as efficient bioremediators of organic wastes and wastewater, which makes it a safe producer of biomass for recycling in other bioproductive industrial uses (Paniagua-Michel et al. 1987).Incorporating MBWWT into traditional wastewater treatment practices can meet the evolving needs for sustainable wastewater treatment in this modern era. Until now, researchers have demonstrated the viability of employing microalgae in wastewater treatment as a supplement for tertiary wastewater treatment, due to its high efficiency in removing nutrients and restoring water quality (Lee et al. 2015, Whitton et al. 2015). Therefore, MBWWT process is considered an efficient and effective technology that can successfully meet the increased need for wastewater treatment.

2.3. Why Micro-algae?

Micro-algae, including eukaryotic autotrophic protist-algae and prokaryotic cyanobacteria, have proven to be an environmentally friendly and substitute to conventional energy-intensive and traditional biological treatment methods (Mohsenpour et al. 2021, Singh et al. 2015). The metabolic versatility of microalgae, which allows them to carry out photoautotrophic, mixotrophic, and/or heterotrophic metabolism, makes them a desirable option for treating various types of wastewaters (Hu et al. 2018, Subashchandrabose et al. 2013). Microalgae are able to utilize inorganic N and P in wastewater, as well as organic and inorganic carbon for growth, thereby decreasing the concentration of these components in the water and making it safe for reuse. They can remove certain heavy metals and hazardous organic compounds, preventing subsequent water pollution (Arora et al. 2021, Dhanker et al. 2021). Microalgae have developed a wide resilience for environmental circumstances, including high nutrient concentrations. This advantage is evident in recent studies that support nutrient removal in wastewater (Chawla et al. 2020). Additionally, microalgae biomass obtained from wastewater streams offers a great potential for the production of sustainable bioproducts (depending on national regulations regarding the re-use of microalgae biomass/bioproducts), including proteins (Soto-Sierra et al. 2018), fatty acids (Kumar et al. 2019), pigments (D’Alessandro & Antoniosi Filho 2016), biofertilizers/biochar (Yu et al. 2017), and animal feed (Madeira et al. 2017). The use of microalgae for wastewater treatment represents a significant step towards a more sustainable and environmentally-friendly approach to water management.

2.4. Economic Perspective of Microalgae-Based Wastewater Treatment

Despite the numerous benefits of MBWWT, its large-scale adoption is hindered by practical and economic constraints. One of the challenges relate to the energy consumed during the cultivation process. Aeration is required in microalgae production to provide carbon in the form of CO_2 and enable the digestion of inorganic nitrogen and phosphorus (Liu et al. 2020).

However, the energy necessary to compress the air, whether enhanced with CO_2 or not, is an energy-intensive process and a major contributor to high operating costs (Davis et al. 2016). The majority of operational energy is expended during the culture stage and mixing in photobioreactors using pumping and/or aeration requires almost 10 times more energy than mixing by paddlewheels in high-rate algal ponds (Stephenson et al. 2010). According to a case study conducted in Almeria, Spain, recirculation, and aeration pumps are the largest energy consumers (Acién et al. 2012). Plappally & Lienhard (2012) reported that recirculation and aeration pumps consume 24 kWh d^{-1} and 96 kWh d^{-1} of energy per unit, respectively. The total energy usage is 15 kWh m^{-3}, which is 100 times larger than conventional wastewater treatment systems that use mechanical and/or aerated mixing (0.15 to 0.62 kWh m^{-3}). Similarly, Gouveia et al. (2016) found that microalgae wastewater treatment in a photobioreactor would cost around €95 to treat $1m^3$ of wastewater under continuous operation for 14 days, with energy consumption being the most expensive factor. This is not favourable compared to the treatment cost of between 0.1 and 0.2€ per m^3 for traditional wastewater treatment systems (Cashman et al. 2014).

Despite the economic challenges associated with MBWWT, it still holds immense potential as an environmentally friendly substitute for conventional wastewater treatment systems. Ongoing research and innovation have led to the development of more energy-efficient cultivation techniques that promise to lower operating costs significantly. Additionally, the use of microalgae biomass for the production of high-value products, such as biofuels and nutraceuticals, can generate revenue streams that offset the treatment cost. Overall, microalgae-based wastewater treatment presents a sustainable solution that not only treats wastewater but also contributes to the circular economy by generating value from waste resources.

2.5. Municipal Wastewater

The increase in human population directly correlates with the quantity of municipal wastewater generated. Municipal wastewaters are primarily composed by water used for personal hygiene and toilets within households, containing predominantly easily degradable organic matter (Plöhn et al. 2021). Depending on the composition of the wastewater, supplementary measures may be required for further treatment (Qadir et al. 2020). Microalgae can be used to remove excess nutrients and other contaminants, including heavy metals and pathogens, from the wastewater. While municipal wastewater is laden with nitrogen, phosphorus, and organic carbon, it also contains a range of additional contaminants in micro-concentrations, including pharmaceuticals, hormones, surfactants, plasticizers, flame retardants, pesticides, and heavy metals (Luo et al. 2014). Despite the presence of such micropollutants, municipal wastewater treatment primarily focuses on removing organic matter, nitrogen, and phosphorus, as they are the primary causes of eutrophication (Sriram & Seenivasan 2012). Recently, municipal wastewater is gaining attention within the Water–Energy–Food nexus, as it is now considered as more than just waste that needs to be treated and disposed of into the environment. Instead, it presents an opportunity to retrieve all three resources—water, energy and nutrients, which algins with the principles of circular economy (Qadir et al. 2020). The reason behind this is not only by supplying water, but also reclaiming resources from wastewater can yield financial and economic benefits (Otoo & Drechsel 2018).

2.6. Selection of Microalgae Strain

Microalgal strains come in a wide variety, and many of them have the potential to remove contaminants from wastewater. However, only a subset of these strains are actually used, likely because of their rapid growth rate, low production cost, and high resistance to extreme, and potentially challenging environmental conditions, such as low or high temperature, pH and light intensity (Plöhn et al. 2021). Single-celled, non-motile, spherical, green microalgae measuring 2–10μm in diameter are what make up the genus *Chlorella*. *Chlorella* is the most extensively researched and grown microalgae in the world at present times due to its high photosynthetic efficiency and high nutritional value (Masojdek et al. 2008). *Chlorella* species have repeatedly demonstrated their ability to remove contaminants efficiently and effectively from a wide range of aqueous solutions due to their high biosorption capabilities. It was shown that *Chlorella vulgaris* had a total phosphorus (TP) removal efficiency of about 85% and a total nitrogen (TN) removal efficiency of about 89% (Doherty et al. 2015). *Chlorella minutissima* and *Chlorella sorokiniana* were shown to eliminate 41% and 34% of the TN and 70% of the TP, respectively (Singh et al. 2011). In addition to nitrogen and phosphorous, Chlorella *spp.* has been demonstrated to be an effective biosorbent of a wide variety of metal ions, including Al^{3+}, $Ca^{2+,}$ Fe^{2+}, Mg^{2+}, Mn^{2+}, and $Zn^{2+.}$ In particular, *Chlorella spp*. was able to remove up to 100% of Fe^{2+} and $Mn^{2+,}$ 70-87% of Al^{3+} and 80-98.6% of Mg^{2+} (Wang et al. 2010). Due to its versatility and efficiency in filtering out a wide range of pollutants, the *Chlorella* genus has become one of the most widely employed microalgal species for wastewater treatment (Wu et al. 2019).

2.7. Why Chlorella vulgaris?

Chlorella vulgaris, a microalga belonging to the order Chlorococcales, Oocytaceae family, and genus *Chlorella*, has a spherical shape that ranges in size from 1 to 10 microns and is characteristically green due to the presence of chloroplasts. Alongside *Haematococcus pluvialis* and *Arthrospira (Spirulina) platensis*, *Chlorella vulgaris* has been observed to grow under photoautotrophic, heterotrophic, and mixotrophic conditions (Chojnacka & Marquez-Rocha 2004). This microalga is known for its significant intracellular content of proteins (51-58%), carbohydrates (12-17%), lipids (14-22%), nucleic acids (4-5%), as well as vitamin C, β-carotenes, and B vitamins (B1, B2, B6, and B12) (EGEE 2018). Moreover, *C. vulgaris* is effective at removing nutrients from its substrate, making it a viable option for treating wastewater. It reduces nitrogen, phosphorus, BOD, and COD from wastewater with retention times ranging from 10 hours to 42 days (Wang et al. 2010). Pittman et al. (2011) mentioned that *C. vulgaris* can remove more than 90% of N and 80% of P content from primary treated municipal wastewater. Aside its nutrient removal ability, the biomass produced can be harvested for biofuel and biofertilizer production. Although algae-based biodiesel is found to be expensive, it can be made more affordable by cultivating algae in wastewater, which contains all essential nutrients for algal growth (Santa Barbara 2007). Studies have shown that the biodiesel produced is of good quality and can be used as fuel in vehicles (Ahmad et al. 2013). Pooja et al. (2022) used *C. vulgaris* to remove toxic pollutants and nutrients from wastewater and found that it efficiently reduced nutrient concentrations like nitrates, COD, and BOD by up to 93%, 95%, and 92%, respectively. After applying the treated sewage effluent as a bio-fertilizer to cultivate *Solanum lycopersicum*, they found that it showed efficient growth and productivity as good as the chemical fertilizer-aided yields. This further proved that sewage can be successfully replaced with chemical fertilizers, thereby conforming to the concept of a circular economy.

2.8. Nitrogen removal in algal cultures

Nitrogen removal is largely achieved through assimilation to algal cells in microalgal wastewater treatment processes. Nitrogen, the second most important nutrient after carbon, can make up over 10% of the algal biomass (Becker 1994). Microalgae assimilate nitrogen compounds primarily as ammonium (NH_4^+) and nitrate (NO_3), preferably and solely, as ammonium (NH_4^+) especially when NH_4^+ is available (Larsdotter 2006, Oliver & Ganf 2000). Nevertheless, high concentrations of ammonium, exceeding 20 mg NH_4^+-N L^{-1}, can be detrimental to the cells when combined with high pH values, as this may lead to ammonia toxicity (Borowitzka 1998). Other nitrogen sources, such as urea ($(NH_2)_2CO$) & nitrite (NO_2^-), can also be used, but the toxicity of nitrite at higher concentrations renders it less desirable (Becker 1994). Some species of cyanobacteria can assimilate the amino acids arginine, glutamine, and asparagine, and fix nitrogen gas (N_2); however, this process is the most energy-demanding of all nitrogen sources and only occurs in some cyanobacteria when no other nitrogen compounds are available in sufficient quantities (Bhaya et al. 2000, Benemann 1979). Furthermore, some microalgae can take up excess nitrogen beyond their immediate metabolic requirements, allowing them to store it for later use in the event of nitrogen starvation.

Table 1: Efficiency of algal treatments on nitrogen removal in different wastewater streams (Jia & Yuan, 2016)

Medium Source	Algae species	Initial nitrogen (mg/L)	Removal efficiency (%)	Reference
Secondary effluent	*Chlorella vulgaris*	N-NH_4^+: 8.05±0.16; 18.31 ± 0.53	100 % within 2 days	(Kim et al. 2013)
Secondary effluent	*Phormidium sp., C. reinhardtii, C. vulgaris and S. rubescens* separately.	TKN: 26.4 ± 0.7	100 % within 4 days for *Phormidium sp.,* & *C.reinhardtii;* within 6 days for the other two	(Su et al. 2012)
Piggery wastewater	*Chlorella zofingiensis*	TN: 63.96-82.7	65 %- 80 % within 4 days	(Zhu et al. 2013)
Source-separated urine	*Chlorella sorokiniana*	TN: 4300-7100	20- 30 % per day	(Tuantet et al. 2014)
Reject water	Microalgae consortium	TN: 220	65 % per day	(Halfhide et al. 2015)

Note: TKN: total Kieldahl nitrogen; TN: total nitrogen

2.9. Phosphorus removal in algal cultures

2.9.1.1. Assimilation

Phosphorus is a critical nutrient needed for the growth of microalgae just like all living organisms. It is used in microalgae cells primarily for the production of essential molecules such as phospholipids, ATP and nucleic acids. Microalgae assimilate phosphorus in inorganic forms, preferably as orthophosphate ions ($H_2PO_4^-$ or HPO_4^{2-}) and it undergoes an active and energy-requiring uptake process (Becker 1994). In situations where orthophosphates are limited, microalgae can breakdown and convert organic phosphates to orthophosphates using phosphatases at the cell surface. In addition, microalgae have the ability to assimilate excess phosphorus, resulting in the accumulation of polyphosphate (volutin) granules within the cells (Solovchenko et al. 2016). These reserves can support growth during times of limited phosphorus availability (Oliver & Ganf 2000). Interestingly, changes in external phosphorus concentration do not always have an immediate effect on the growth rate of microalgae, in contrast to the prompt responses to changes in temperature and light (Larsdotter 2006). Mostert and Grobbelaar (1987) discovered that the concentration of phosphorus in cells varies with supply concentration, ranging from around 1 mg P per g dry mass at a supply concentration of 0.1 mg P L^{-1} to 100 mg P per g dry mass at supplies of 5 mg P L^{-1} or higher. On average, an algal cell contains 13 mg P per g dry weight. Cultivation of microalgae in wastewater, which typically has a concentration of between 10 and 20 mg P L^{-1}, can lead to the accumulation of far more than required for growth. These findings highlight the potential of microalgae to effectively remove phosphorus from wastewater while also accumulating valuable resources for reuse. Optimal growth conditions for microalgae are critical for maximizing phosphorus assimilation as it is contingent on carbon assimilation and thus, algal growth. Since phosphorus assimilation depends on carbon assimilation and algal growth, it is essential to optimize conditions for algal growth.

2.9.1.2. Precipitation

During photosynthesis, microalgae take up inorganic carbon in the form of CO_2 and bicarbonate (HCO_3^-) from the water (Oswald, 1988). The bicarbonate then requires the enzyme carbonic anhydrase to convert it to CO_2. The use of HCO_3^- as a carbon source results in an increase in the pH of the medium due to the reaction:

$$HCO_3^- \rightarrow CO_2 + OH^-.$$

This pH increase can cause a significant impact on water chemistry if the pH exceeds 11 in algal cultures. Phosphorus may then precipitate with available calcium cations to form calcium phosphates, which is the most commonly formed. This precipitation is favoured by high pH values, elevated temperatures, and high concentrations levels of calcium and phosphorus (Song et al. 2002). Precipitation from wastewater is possible even at neutral pH values, provided the phosphate concentration is at a minimum of 50 mg P L^{-1} and the calcium concentration is at least 100 mgCa L^{-1} (Carlsson et al. 1997). Nevertheless, even higher phosphate concentration can induce precipitation in soft water not more than 50 mg L^{-1} calcium concentration. However, calcium phosphates precipitate may redissolve at low aqueous calcium and phosphate concentrations (Larsdotter 2006).

2.10. Factors Influencing Microalgae Culturing

To achieve successful nutrient removal from wastewater using microalgae, it is crucial to have a good understanding of the factors that affect growth, nutrient removal efficiency, and biomass productivity. These factors include physical, chemical, and biological factors, and their impact on the growth rate of microalgae is significant. Additionally, operational factors play a crucial role in the process (Gupta & Bux 2019, Li et al. 2019, Mohsenpour et al. 2021).

2.10.1 Physical Factors

2.10.1.1 Light

Light is essential for the activity and growth of photosynthetic organisms such as microalgae, which efficiently capture and convert light energy into chemical energy (ATP) through photosynthesis, producing oxygen and reducing agents (Jia & Yuan 2016). This energy is used to build cell structures and reproduce, resulting in increased biomass and oxygen production (Zuccaro et al. 2020) However, high algal density can reduce light penetration and cause inefficient use of light (Park & Lee, 2001). Excess light, particularly in combination with sub-optimal temperature or high oxygen levels, can also damage the photosynthetic apparatus. Therefore, it is crucial to optimize light supply through adequate design of the cultivation system. (Tredici et al 2015).

The variation of light wavelength also has significant impact in the microalgal growth. Satthong et al. (2019) studied the effect of white LED, red LED, and fluorescence light on the growth of *C. vulgaris TISTR8580*. The algae population remained stable during the initial period of the experiment but showed a notable increase in cell density from day 2 to day 3, thereby indicating exponential growth. The population reached a stationary phase on day 4 and remained constant thereafter. White LED light resulted in the highest biomass density on day 14, while the fluorescent light produced the highest density in total. Red LED light had the highest density on day 3 but declined more than that under the white LED light by day 4.

The duration and intensity of light exposure greatly affect algal growth and the efficiency of removing carbon, nitrogen, and phosphorus from wastewater. Increasing light intensity, along with exogenous CO_2, not only boosts biomass production but also enhances COD and nitrogen removal in municipal wastewater treatment (Li et al. 2012). Sukačová et al. (2015) found that algae exposed to artificial illumination for 24 hours removed significantly more total phosphorus (TP) than those exposed to a 12-hour light-12-hour dark cycle by solar radiation. However, Powell et al. (2008) observed that increased light intensity could adversely affect the ability of microalgae to absorb phosphorus, which consumes energy from photosynthesis, as shown by Hessen et al. (2002). Algae-based waste treatment's nutrient absorption and biomass production are regulated by light and dark cycles. *Chlorella kessleri*, for example, absorbs more nitrate and grows faster when exposed to continuous light, but its carbon-removal efficiency is higher when light and dark cycles are regulated (Lee & Lee 2001). Only a fraction of the solar energy absorbed by microalgae is converted into biomass (Mehrabadi et al. 2015), and the light intensity available to microalgae is positively correlated with their biomass production ability (Li et al. 2012, Zhou et al. 2012).

2.10.1.2 Temperature

The growth of microalgae, including their photosynthesis and carbon fixation, is directly dependent on temperature. Each microalgal species has a specific temperature range that is optimal for growth. Based on this range, microalgae can be classified as psychrophiles (<15°C), mesophiles (<50°C), and thermophiles (>50°C). These classifications are based on the physiological and morphological responses of the species to temperature (Zuccaro et al. 2020). *C. vulgaris* was shown to remove nutrients more efficiently in a shorter period at a lower temperature (Filippino et al. 2015). The growth of microalgae is affected by both seasonal and daily temperature changes. The optimal temperature range for microalgae growth is 20-35℃, and cooling equipment can be expensive to maintain ideal growth temperatures (Eustance et al. 2016). Therefore, micro-algal species selected for wastewater treatment should be able to survive in the prevalent environmental conditions at the treatment plant. Low and high temperatures can both inhibit algae growth, and the biochemical composition of algal biomass is also influenced by temperature (Hu et al. 2008). The temperature is a crucial factor in algal development and the biochemical composition of biomass, making it a vital determinant in the bioremediation process of wastewater.

2.10.2 Chemical Factors

2.10.2.1 Water Characteristics

The interaction between wastewater and microalgae is the most important element in microalgae-based wastewater treatment systems. Wastewaters come in a wide variety (Li et al., 2019) and each one is distinct in terms of its physical and chemical characteristics. Algal growth, nutrient recovery rates, and biomass productivity may be significantly impacted by nutrient levels, pH, temperature, chromaticity, N & P concentrations, hazardous chemicals (including heavy metals, aldehydic, and phenolic compounds), and other factors (López

Barreiro et al. 2015). Most research has focused on municipal wastewater and animal wastewater since they are readily available and somewhat consistent.

The profile of wastewater relies heavily on nutrient concentrations and nutrient concentration strengths, which reflect the type and amount of pollutants. However, algae have their own ideal conditions for production. The macro and micronutrients in the artificial media packages were designed to provide the optimal growing conditions. In practise, it is uncommon for wastewater nutrients to meet the ideal profile of nutrients for algae development. Li et al (2019) mentioned methods that were employed to address this mismatch. The technique is to use screened or trained algae to acclimatize to the wastewater environment, and then modify the wastewater to meet the growth requirements of the algae. Frequently, both tactics were employed concurrently to improve the outcomes.

2.10.2.2 pH

Many cellular activities, such as energy metabolism, organelle structure and function, enzyme activity, and protein synthesis, are affected by the pH of the cell's environment. In general, culture media for algae have a pH between 7 and 9 (Pandey et al. 2013). Maintaining a constant pH in algal growth media necessitates the presence of specific components that either prevent the precipitation of metal ions, slow the growth of pollutants (microbial inhibitor) or serving other functions both (Brand et al. 2013). The different algae species have a range of pH tolerance. While some species are confined to a small range, others are able to thrive in a wide range (Moss 1973). *Chlorella ellipsoidea* may thrive in conditions where the pH ranges between 4 to 10. Mayo (1997) believed *that C. vulgaris* grows best at a pH of 6.5 but (Gong et al. 2014) argued that its best growth performance was observed at pH 10.

Nutrient absorption is also affected by pH. Zhou et al. (2015) examined the of *Chlorella vulgaris* to degrade nutrients at different pH levels and found that 7–8 was best for ammonia and nitrogen decomposition. However, Liang et al (2022) found contrasting outcomes. The ideal pH for $N\text{-}NH_4^+$ removal in a co-culture system of *Bacillus licheniformis* and *C. vulgaris* was found to be 7, but for phosphorus removal pH had no significant impact (Liang et al. 2022, Zhou et al. 2015). However, pH may have an impact on nutrient concentration. Calcium phosphate may precipitate out at high pH levels, leading to a rise in free ammonia concentration (Cai et al. 2013). Meanwhile, algae photosynthesis is inhibited by high levels of free ammonia, reducing their growth (Abeliovich & Azov 1976). However, algal activities including CO_2 consumption and $N\text{-}NH_4^+$ uptake may also contribute to pH shifts. Microalgae are known to enhance their OH^- concentration and $N\text{-}NH_4^+$ absorption during photosynthesis, which in turn releases H^+ (Knud-Hansen 1998). A reduction in pH owing to nitrification is caused by both algae and nitrifying bacteria. Furthermore, microalgae growth can cause pH changes due to nitrification, while photosynthetic activity can reduce pH over time through CO_2 consumption (Mousavi et al. 2019, Sayadi et al. 2016).

2.10.2.3 CO_2 supply

Exogenous CO_2 is beneficial to microalgae treatment efficiency and autotrophic development (Hu et al. 2012, Park & Craggs, 2010) when other carbon sources are limited (Min et al. 2011). In addition, CO_2 addition during photosynthesis can regulate water pH, which can have a major impact on nutrient uptake or loss (Park & Craggs 2010). Phosphorus precipitation often occurs at pH > 9, while a pH 8 would restrict NH_3 volatilization (Park & Craggs, 2010). Evidence from the previous studies suggests that the addition of CO_2 can have significant effects on phosphorus removal but lesser effects on COD and nitrogen removal (Hu et al. 2012). Moreover, CO_2 can raise the C/N ratio in wastewater, it can boost microalgae's biomass productivity and bioenergy generation (Park et al. 2013). Some researchers have discovered that for certain species of algae, a high organic carbon content can help improve nutrient removal (Hongyang et al. 2011). Saturation levels of fatty acids are also affected by the CO_2 content in wastewater. Total saturated lipid content of Chlamydomonas species grown at varying CO_2 concentrations was reported to be 65.3% under 4% CO_2 and 58.1% under 2% CO_2 conditions (Nakanishi et al. 2014). Kong et al. (2021) established that blue-green algae are most applicable to fix CO_2 in treated wastewater, more screening of microalgal strains with superior anti-fouling performance, high CO_2 tolerance, and NH_3-N performance is required.

2.10.2.4 Dissolved Oxygen

Microalgae growth relies on various factors, such as solar radiation, nutrient availability (e.g., C, N, P), and inhibitory factors (e.g., excess oxygen). Photosynthesis during the day increases the dissolved oxygen content, which can impede algae development when levels surpass 200% of saturation (Garcia et al. 2000). Adequate dissolved oxygen concentration stabilizes the rate of photosynthesis and biomass production. However, photosynthesis is inhibited when dissolved oxygen concentration exceeds 470% of air saturation, but not lethal to cells (Molina et al. 2001). High rate algal ponds (HRAPs) promote the growth of mixed microalgae and bacteria consortiums. Heterotrophic bacteria use O_2 produced by microalgae during photosynthesis to decompose organic matter, and the microalgae use O_2 during dark respiration (Falkowski & Raven 2007). Bacteria's heterotrophic metabolism of organic matter mineralizes

CO_2 to inorganic molecules like $NH4^+$ and PO_4^{3-}, which are directly absorbed by micro-photoautotrophic algae for development (de Godos et al. 2010). Photosynthetic oxygenation can supply the necessary dissolved O_2 for a treatment system without mechanical aeration or mixing, reducing the energy requirements and overall cost of algae culturing (Mohsenpour et al. 2021).

2.10.2.5 Nutrients (C/N & N/P ratios)

Nutrient absorption is influenced by the C/N and N/P ratios, both of which are crucial to the potential productivity and the dominance of candidate species in culture (Richmond 2013). The C/N and N/P ratios in wastewater tend to be less favourable than the ratios typically found in environments where microalgae thrive (Woertz et al. 2009). When bacteria and microalgae can consume organic carbon through mixotrophic or heterotrophic metabolism, it is believed that carbon will be the limiting element in wastewater treatment (Su et al. 2012). However, when the C/N ratio is not optimal for microalgae reproduction, they can get the CO_2 they need from the air. In addition, maintaining a healthy C/N ratio is a simple and successful strategy for facilitating microalgal nitrogen uptake. Ji et al. (2018) found that microalgae showed the potential of reaching a productivity level of 2.85 g/L while eliminating NH_4-N, TN, TP, and COD at rates of 100%, 96%, 90%, and 93%, respectively, by mixing piggery effluent with brewery wastewater to produce a balanced C/N ratio of 7.9.

The biochemical composition of algal biomass is affected by the nitrogen content in wastewater. At high nitrogen concentrations, the concentration of carbohydrates and lipids in the algal cell was reduced by around 20%. Some species of microalgae even increase their triacylglycerol content when phosphate is limited (Hu et al. 2008).

2.10.3. Operational Factors

2.10.3.1 Mixing

Mixing is important in algal ponds as it fosters an even distribution of light and nutrients, which promotes consistent photosynthetic activity. Stagnation periods can create a boundary layer that hinders the microalgae's ability to assimilate nutrients, exchange gases, and produce oxygen. Mixing can be achieved through stirring, shaking or bubbling air through the culture. Ogbonna et al. (1997) found that mixing can enhance the performance of algal cells by increasing cell density in the mixed system, but only when cell density is high. Mechanical mixers, such as paddlewheels, are installed in open pond systems to ensure frequent aeration during microalgal culturing. Nutrient uptake, biomass productivity and culture health may be dependent on efficient distribution of resources in the media (Doria et al. 2012).

2.10.3.2 Hydraulic Retention Time (HRT)

The regulation of cell density, species composition, algal/bacteria ratio, and nutrient removal efficiency in algal ponds heavily relies on hydraulic retention time. The dynamics of algal populations and the biochemical composition of total algal biomass are also affected by the varying growth rates of different algal species, which are in turn influenced by HRT (Mehrabadi et al., 2015). To ensure optimal conditions, standardization of retention time is crucial, as longer HRTs can impede algal development due to shading and nutrient deficiency, while shorter HRTs hinder nutrient absorption from effluent and may result in algal cell washout if below the minimum production time of an algal cell (Larsdotter, 2006). For wastewater-treating algal ponds, the typical HRT ranges from 2 to 7 days. Whitton et al. (2018) studied the efficacy and remediation mechanisms of immobilized microalgae for continuous wastewater treatment at various HRTs. They found that PO_4-P removal rates improved with increasing HRT, but ammonium remediation was related to HRT or $NH4^+$ concentration at concentrations of <0.001 mg/L, and NO_3^--N reduction improved as HRT increased with few residual concentrations.

2.10.4 Biological Factors

2.10.4.1. Microalgae-Bacteria Interactions

Co-culturing microalgae with bacteria or a consortium of different bacterial species is found to be essential for the growth of microalgae and nutrient recovery during wastewater treatment. The interaction between microalgae and bacteria is efficient such that the photosynthetic O_2 produced microalgae is essential for bacterial respiration and growth. In turn, microalgae use sunlight to fix CO_2 released by bacteria to ensure a self-regulating and ecological WWT system. Several studies have examined the efficiency of municipal wastewater treatment using mixed cultures of *Chlorella* and bacteria (Aditya et al. 2022, Mujtaba et al. 2015, Otondo et al. 2018). The combination of *Chlorella vulgaris* and *Azospirillum brasilense*, has shown to enhance N and P removal from municipal wastewater as compared to microalgae alone. (De Bashan et al. 2004). The co-immobilization was found to be efficient in removing up to 100% ammonium, 15% nitrate, and 36% phosphorus within 6 days from varied wastewater sources, in contrast to 75% ammonium, 6% nitrate, and 19% P removal by the microalgae alone. Microalgae can also promote bacterial growth in algal-bacteria co-culture treatment processes through their exudates, which can be assimilated or digested by bacteria as a source of carbon (Fouilland 2012). The release of dissolved organic carbon (DOC) by microalgae enhance bacterial populations significantly in microalgal-bacterial co-cultures (Hulatt & Thomas 2010). Removal rates, especially for organic chemicals, can be increased when microalgae and bacterial growth are combined (Muñoz & Guieysse 2006).Su et al. (2012) co-cultivated algae and bacteria from activated sludge and removed more than 90% of N and P. Solovchenko et al (2016) asserted that bacteria can mineralize organic P to make it bioavailable for microalgae uptake in wastewater. This approach can increase P recovery, productivity, and have positive environmental and economic impacts.

2.10.4.2. Zooplankton Grazers and Pathogens

Internal species competition, zooplankton grazers, infections, and other biotic factors, along with environmental and operational factors, can affect algal growth and production in a fully mixed system. Competition for resources among species is particularly noticeable in such a system. Zooplankton, including ciliates, rotifers, cladocerans, copepods, and ostracods, feeds on microalgae, and its presence as a pollutant in HRAPs can negatively impact wastewater treatment if the system is ran in a monoculture (Acién et al. 2012). Effective wastewater treatment through algal ponds requires careful grazer management to prevent zooplankton from consuming the algal biomass too quickly (Gupta & Bux 2019). Studies have presented that creating optimal conditions for micro-algal cultivation can be crucial in protecting dominant species, but the requirements for this vary depending on the micro-algae species and cultivation technique used (Acién et al. 2012, Gupta & Bux 2019). Allowing a natural species to acclimatize to the expected or subsequent processing conditions in the future may be appropriate.

2.11. Algae Growth Dynamics

Algae growth in batch cultures experiences five different phases:

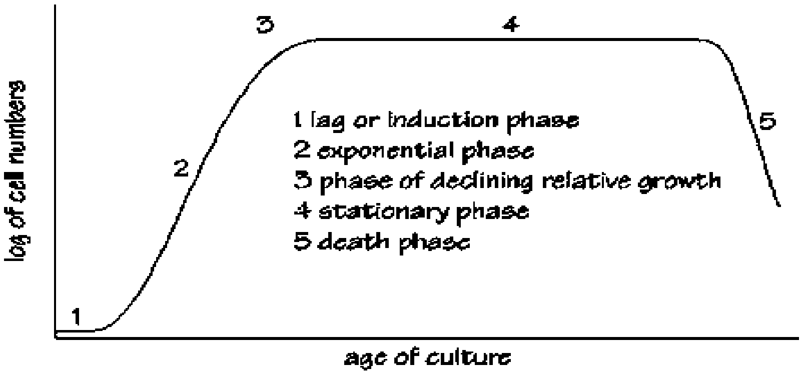

Figure 2. Growth stages of micro-algae cultures (Lavens & Sorgeloos 1996).

1. Lag (Induction) phase – A slow increase in cell density as the cell adapts to the environment.
2. Exponential phase – Rapid increase in cell density (biomass) due to optimal conditions.
3. Declining Relative Growth phase - One or more physical or chemical factors limit cell growth and cell division.
4. Stationary phase - Cell density stabilizes due to equilibrium between cell growth and limiting factors.
5. Death (Crash) phase - reduction in cell density, leading to culture collapse. (Lavens & Sorgeloos 1996, Fekete 2019)

2.12. Microalgae Harvesting

Harvesting is a crucial step in algal wastewater treatment systems with the aim to separate algae cells from the liquid medium in which it was cultivated since a significant portion of the nutrients ends up as biomass. However, microalgae are typically small and have slow settling rates, making it difficult to achieve efficient harvesting, which leads to high costs in the cultivating process (de la Noüe et al. 1992, Larsdotter 2006). This process is estimated to account for 20-30% of the total production costs cultivated on a large scale. As a result, it is essential to select most efficient but less expensive method (Lima et al. 2020, Gutiérrez et al 2015). These methods may, however, be employed in combinations or alone, to concentrate algal suspensions then manufacture final products. Harvesting methods, including sedimentation, filtration, centrifugation, coagulation and flocculation, flotation, and auto flocculation and bioflocculation have also been discussed.

2.12.1. Sedimentation

Gravity sedimentation is a widely used method for microalgae harvesting in wastewater treatment due to its simple method and low cost. But it is time-consuming and inefficient due to the low sedimentation rates of most microalgae, which are adapted to stay suspended in the medium. The suspension is caused by subtle difference in density of the algal cells and the liquid medium, and the natural inclination of algal cells to remain close to the surface of the culture medium to compete for light (Al-Jabri et al. 2020). Although sedimentation can be useful for cells with higher densities (Oilgae Report 2010), microalgae are typically flocculated to increase their particle size and density for harvesting. Stokes' Law has qualitatively described the process of gravity sedimentation of non-flocculated particles (Al-Jabri et al. 2020). However, this mechanism is not applicable to flocculated particles due to the complex nature of their structure and the significant amount of water they contain. As a result, the diameter, shape, and density of the floc become undefined, and the settling mechanism becomes complicated (Shelef et al. 1984). Microalgal cells have a negative surface charge, which prevents them from attaching to each other. However, as the culture density increases, the cells utilize soluble carbonate and the pH of the growth media increases, neutralizing the surface charge. Inorganic compounds can then precipitate at elevated pH levels, leading to microalgal cell coprecipitation. The addition of a chemical flocculant, such as NaOH, can speed up the process and the settling velocity (Kwietniewska et al. 2012, Shelef et al. 1984).

2.12.2. Coagulation and Flocculation

The process of coagulation-flocculation results in the formation of bigger clumps of algal cells, which can be filtered or settled down more quickly, making them easier to harvest. Coagulation involves the addition of a chemical coagulant(s) in order to neutralize or destabilize algal cells in suspension whereas flocculation involves the aggregation of destabilised cells and the precipitation products formed by one or more coagulants into larger particles or flocs. Microalgae cells, with a diameter of less than 15µm and a slightly more denser than water, remain suspended in the culturing medium and do not settle easily under gravity. Moreover, the cell wall of microalgae cells have ionized functional groups, causing a negative charge on the cell surface (Gerardo 2015). A cationic coagulant is used to neutralize the negative surface charge of microalgal cells to induce the spontaneous formation of cell aggregates or flocs (Chatsungnoen & Chisti 2016). The efficiency of flocculation relies on various factors such as the type of flocculants used, pH, salinity, microalgal species, their charge densities, cell concentrations, growth phase, and more (Zhu et al. 2018).

2.12.3. Autoflocculation and Bioflocculation

Autoflocculation refers to the spontaneous aggregation of cells caused by an increase in pH as a result of photosynthetic CO_2 consumption in the growing medium, which is accompanied by the precipitation of inorganic precipitates, mainly calcium phosphate, which leads to flocculation (Barros et al. 2015). Bioflocculation, on the other hand, involves the aggregation of algal species (or other organisms such as bacteria) that possess the ability to autoflocculate with those that lack this ability (Kwietniewska et al. 2012). Both techniques are cost-effective, require low-energy, non-toxic to microalgae, and do not require the use of flocculants. However, It has been observed that the addition of NaOH, a low-cost product, can simulate the process. According to Barros et al. (2015), Horiuchi et al. (2003) achieved a biomass recovery efficiency of over 90% by flocculating *Dunaliella tertiolecta* with NaOH in a short time at pH levels ranging from 8.6 to 10.5. Similarly, Vandamme et al. (2012) recovered 98% biomass in 30 minutes by raising the pH to 10.8 using NaOH, KOH and $Ca(OH)_2$ whereas 98% was recovered at pH of 9.7 using $Mg(OH)_2$. Despite their advantages, these methods are not preferred for the pre-concentration of microalgae on an industrial scale, as they are not reliable for controlled flocculation and can alter cell composition.

2.13. Flocculants for microalgae harvesting

Flocculation is a technique that borrows from sewage treatment to remove small suspended solids from colloidal solutions. Algae and cyanobacteria particles repel each other due to their surface charge, which prevents fast sedimentation. Additionally, these cells have a similar specific gravity to the solution. By using flocculants, the surface charge is eliminated, and the particles stick together to form flocs. Six different flocculants have been discussed for their potential to speed up the harvesting process.

2.13.1. Sodium Hydroxide, NaOH

Sodium hydroxide, also called lye or caustic soda, is a highly alkaline compound that is widely used in various manufacturing processes, such as pharmaceutical production (aspirin), soaps, detergents, and textiles. It is naturally extracted from seawater or produced industrially through the electrolysis of sodium chloride (NaCl), known as the Chlorine-alkali process. Sodium hydroxide is an integral ingredient in household cleaning and disinfecting products, cosmetics, and personal care products (Toedt et al. 2005). It is also used in the food and beverage production to adjust pH levels and act as a stabilizer. Drinking water may contain sodium hydroxide due to sodium (Na^+) and hydroxide ions (OH^-) present in many natural soils, groundwater, plants, and animal tissues. In wastewater treatment, conventional metal salts like ferric chloride and alum are used during the coagulation/flocculation stage, which may decrease the pH level. Ferric chloride coagulation works best in a lower pH range of 4-5. However, if biological treatment follows coagulation/flocculation, the pH range may not be suitable for efficient bacterial biodegradation. To neutralize the pH level and facilitate the biodegradation process, NaOH is frequently added. It is a cost-effective commodity that can be produced from sodium chloride, commonly known table salt, and can be a good source of chemical flocculant in terms of operation cost and environmental impact.

2.13.2. Potassium Hydroxide, KOH

Potassium Hydroxide, or caustic potash, is a strong base used available in the form of pellets, flakes, or powders, and is used in various industrial, commercial, and laboratory applications. Although it shares some properties with NaOH, they are fundamentally different. When it reacts with water, it releases heat, making it highly exothermic, similar to NaOH (Carson 2002). Its uses include soap and biodiesel production, textile manufacturing, battery production, paint production, and for cleaners. Potassium Hydroxide is typically produced by electrolyzing potassium chloride, which is a costly compound. As a result, it is generally more expensive than sodium hydroxide. KOH is utilised as an electrolyte in alkaline batteries, and sometimes to control pH levels in water treatment, unlike NaOH, which is frequently used in water treatment.

2.13.3. Calcium Hydroxide, $Ca(OH)_2$

Calcium hydroxide, also called slaked lime or hydrated lime, is a soft white powder that is widely used as a raw material in the chemical industry. It is formed by mixing calcium oxide (CaO) with water or by reacting sodium hydroxide (NaOH) with calcium chloride ($CaCl_2$) in an aqueous double displacement reaction. This compound is a main ingredient in cement production and can be found in soil or waterways where it reduces the impact of acid rain by reacting with acids to create water and salt. Calcium hydroxide is mainly used for pH adjustment, but it can also function as a coagulant aid and softener. It bonds with other particles, particularly phosphorus compounds, increasing the size and weight of flocs, which then settle out of the water more quickly. When combined with magnesium hydroxide (Mg $(OH)_2$, it can be used to flocculate algal suspensions at pH levels of 11.0 and 11.5 (Semarjian & Ayoub 2003). Hydrated lime is much cheaper and safer to handle than sodium hydroxide, making it a more readily available and cost-effective option for pH control in water treatment (Folkman & Wachs 1973). Liang et al. (2022) asserted that $Ca(OH)_2$ reached a flocculation efficiency of 96.7% and

97.08% of *C. vulgaris* at pH of 12 and synergistic action of chitosan and $Ca(OH)_2$ at pH of 8.97 respectively.

2.13.4. Ferrous Sulphate, FeSO4

Ferrous Sulphate, chemically known as iron (II) sulphate, is a blue-green chemical that exists as yellow, brown, or bluish-green crystals, and is sometimes referred to as green vitriol. It is used in medicine, ink and dye manufacturing, and agriculture. Ferrous sulphate can be found as common minerals such as $FeSO_4·7H2O$ (Melanterite, blue-green) and $FeSO_4·4H2O$ (Rozenite, white, a possible dehydration product of melanterite). It is an effective coagulant for industrial and sanitary wastewater treatment due to its high efficiency, effectiveness in clarification, and usefulness as a sludge dewatering agent. Coagulation treatment removes suspended solids and colour from water (Carson 2002). Ferrous sulphate has been proven to be effective in chlorite removal, coagulation for both drinking water and wastewater, heavy metal removal, odour control, and phosphorus removal. Moreover, it is relatively less expensive compared to other chemicals such as caustic soda, hydrated lime, and sulphate of potash.

2.13.5. Potassium Sulphate, K_2SO4

Potassium sulphate, or sulphate of potash, is a water-soluble salt primarily utilized as a fertilizer in agriculture to provide plants with one of their main nutrients, potassium (K). It is obtained by carefully washing natural K-containing minerals such as kainite and schöenite, which are mined, with water and salt solution to extract by-products and produce K_2SO_4 (Stewart 1985). Alternatively, it can be synthesized by reacting sulphuric acid (H_2SO_4) with potassium hydroxide (KOH). Compared to other chemicals like calcium carbonate, ferrous sulphate, caustic soda, and hydrated lime, it is relatively more expensive, but cheaper than caustic potash. However, the actual cost depends on various factors, including purity, quantity purchased, location, and market conditions. Potassium sulphate is not commonly used in water treatment since it lacks the ability to promote particle aggregation or precipitation.

2.13.6. Calcium Carbonate, $CaCO_3$

Calcium carbonate is a ubiquitous substance that occurs naturally in rocks as the minerals calcite and aragonite. Limestone, a sedimentary rock primarily made up of calcite, is the most well-known example of it. It is also a major component of various biological structures, such as eggs, snail shells, seashells, and pearls. In agriculture, calcium carbonate is used as an active ingredient in lime, which is created when carbonate ions (CO_3^{2-}) react with calcium ions (Ca^{2+}) found in hard water to form limescale. Calcium carbonate is found all over the world, with many natural and man-made structures made of limestone. When in contact with water containing CO_2, it is barely soluble and converts into calcium acid carbonate. Upon heating, calcium carbonate decomposes and releases carbon dioxide and calcium oxide. This substance is critical to the construction industry, both as a building material (e.g., marble), and as a component of cement. It is also used in paper production, as a dietary calcium supplement, and as an additive in pesticides and poultry feed. Calcium carbonate can form by reacting calcium hydroxide with dissolved carbon dioxide in water and precipitating out of the solution at a pH range of 9.1-9.5. Through sweep coagulation, it can entrap suspended and colloidal particles. However, it is not commonly used in water treatment as it does not have the ability to promote the aggregation or precipitation of particles.

2.14. Microalgae and the Circular economy

The bioeconomy emphasizes sustainable production of goods and services using new biological resources, methods, and principles, benefiting all economic sectors. Adopting a circular bioeconomy approach enables the maximization of resource value and minimization of waste through reuse and recycling (Nagarajana et al. 2020). This approach also allows for the recovery and reuse of nutrient-rich wastewater, specifically nitrogen and phosphorus, which can be utilized by microalgae for growth and biomass production (Nagarajana et al. 2020).

2.14.1 Biofertilizer

Microalgal biomass derived from wastewater treatment can be used as a nutrient source in agriculture, enhancing germination index, nutrient absorption, and biomass accumulation. (Navarro-López et al. 2020, Nayak et al. 2019). Microalgal biomass has biostimulant potential due to its association with vitamins, minerals, peptides, and amino acids, as well as the ability to produce plant growth-promoting hormones such as auxin and cytokinin (Stirk et al. 2013). Wuang et al. (2016) examined the use of *Spirulina platensis* biomass from aquaculture wastewater as a biofertilizer for leafy vegetables. The effects varied between plant species, with Bayam Red and Arugula producing the greatest results. Nayak et al. (2019) also investigated the use of Scenedesmus sp. biomass residual from lipid extraction as a rice crop biofertilizer. The biofertilizer was found to increase the availability of nitrogen, phosphorous, and potassium in the soil. In contrast, the addition of chemical fertiliser increased grain output, plant height, number of tillers, and plant dry weight.

2.14.2 Biofuel

Microalgae are valuable contributors to the circular bioeconomy due to their ability to produce high-quality products, including proteins, lipids, and colorants from biomass generated by the WWT nutrient removal process (Wollmann et al. 2019). Recent research has shown that microalgal-based wastewater nutrient removal is a promising alternative for cost-effective liquid biofuel production and renewable energy (Rusell et al. 2022, Schneider et al. 2013) to meet the world’s energy demands. Lipids produced from microalgae biomass are converted to biofuel through transesterification, a process that involves dewatering, but results in high operational costs due to the energy required. However, unlike non-renewable oil, biofuel is a renewable and sustainable source of fuel that can be used in vehicles. Biodiesel produced from microalgae lipids can reduce CO_2 emissions by up to 80%. The remaining biomass can be used to produce biomethane, which can generate sufficient electricity to power the biodiesel facility. Surplus energy can also be captured and traded to offset the cost of biodiesel production (Banu et al. 2020). Unfortunately, despite its ecological benefits, biofuel cannot currently compete economically with conventional fossil fuels. Further technological development is needed to scale up production capacity (Russell et al. 2022) and reduce production costs to make microalgae biomass a viable source for biofuel production in the foreseeable future (Zhang et al. 2014).

2.15 Perspectives and Ideas for the future

Considering the current limitation and non-renewable nature of phosphorus, exploring alternative but sustainable means of phosphorus sources is essential. Nutrient recovery from municipal wastewater could play a vital role in supplementing the demand for nutrients, especially phosphorus, and ensuring a more sustainable way of human life. The MBWWT process has been studied extensively for its viability and potential in treating various types of wastewater sources. However, most of these studies have been conducted at the laboratory scale, and the performance of the process may differ significantly when scaled up to pilot or greater stages. Surprisingly, there have been very few economic evaluations conducted. Therefore, future studies are needed to determine the economic viability of the MBWWT process when treating actual wastewater under typical operating settings.

To enhance the economic viability of the MBWWT process, it is crucial to explore the potential for reducing labour costs. This suggests that more automated designs should be integrated into the design and operation of MBWWT to replace human labour. Additionally, the materials used to build the system should prioritize low cost and longevity. Improved design and operation could also decrease the energy required for aeration, mixing, and liquid transportation, as well as for microalgae harvesting and dewatering. The current state of algal production in Europe is relatively unknown, despite its potential to be a leading sector within the EU bioeconomy. Thus, it is essential to obtain robust and extensive information on the European algae sector to drive knowledge-based decisions and policies that promote socioeconomic growth and environmental sustainability.

3.0. MATERIALS AND METHOD

3.1. Sampling Location

Gödöllő is a small town in the Pest County of Hungary and about 34km away from the capital, Budapest with a population less than 40,000 inhabitants. The town has a wastewater treatment facility responsible for treating all the domestic wastewater generated by the people of Gódóllő and its environs. The treatment plant has the capacity to treat 6,000m^3 wastewater in a day with up to 10 trucks unloading per day. The treatment process involves pre-treatment, primary, secondary, and tertiary treatment. The treatment facility has a parallel pair of settling tank and aeration tank to ensure that the treatment process can continue in the situation that one pair of the facilities stops working.

3.2. Water Sampling

Grab samples of wastewater were collected from the municipal wastewater treatment facility. Sterile polyethylene bottles of 1.5L were each filled with pre-treated municipal wastewater collected from the primary clarification/settling tank. After primary sedimentation, there is a very low solid content thus we just examined the water soluble contaminants after the physical treatment phase. Samples were then transported immediately to the laboratory.

Figure 3: Images of sampling site

3.3. Analytical methods

The physicochemical parameters of the wastewater were conducted at the ProfiKomp Research Laboratory, Gódóllő. The wastewater samples were filtered through 0.45μm cellulose filter paper in order to eliminate suspended solids, organic matter, and microorganisms.

3.4. The algae strain

The algae species, *Chlorella vulgaris* belonging to the true green algae (Chlorophyta) tribe. This algae species was selected due to its resilience to environmental changes, ease of handling for experimentation, and capacity to withstand breeding at high levels of carbon dioxide. The algae strain used, H1955 *Chlorella vulgaris* BEIJERINCK, was isolated at Culture Collection of Algae of Charles University in Prague (CAUP). This authenticated strain was originally isolated by Beijinck from a eutrophic lake near Delft in 1889.

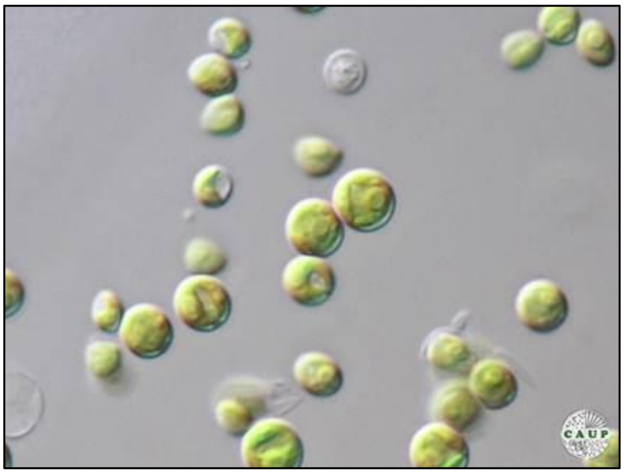

Figure 4: Chlorella vulgaris cells under microscope

3.5. Chemicals and Materials

The algae broth, used as nutrient solution for algae, was purchased from Scharlau (Hungary). All the six chemical flocculants (NaOH, KOH, $Ca(OH)_2$, $FeSO_4$, K_2SO_4, $CaCO_3$) were purchased from ES Lab Magyarország Kft., formerly, Scharlab Magyarorszag Kft (Debrecen, Hungary). All chemicals were reactive grade and used as received.

3.6. Microalgae Cultivation

Chlorella vulgaris obtained from Culture Collection of Algae at the Charles University (Staré Město, Prague, Czech Republic) was used for the experiment. The microalgal culture was cultivated at room temperature (20–25 °C) in a 500mL sterilized Erlenmeyer flask under axenic conditions in a phytotron. The flasks were tightly covered with cork stopper to prevent contamination and to allow gas exchange. Respiratory tubes, which contain respiratory bubblers were connected to the flask to provide aeration to the culture. The cultures were placed in a chamber illuminated by four Osram biolux l36w/965 cool-white fluorescent lamps at intensities of 2082lux (2 lamps above) and 2665lux (2 lamps below) in a 12/12h light/dark cycle. Retention time was set at 7 days, but analyses were conducted every 24 hours to monitor the growth, metabolic activity, and nutrient uptake of the microalgae and to determine the optimal hydraulic retention time. The table (2.0) below shows the treatment and the volume ratios of the wastewater and algae culture measured after 1 and 7 days. All treatments were conducted in quadruplicates.

Table 2: Treatment Detail

Comp (ml)	Control day1	Algae 1 day1	Algae 2 day1	Control day7	Algae 1 day7	Algae 2 day7
WW	200	200	200	200	200	200
Algae stock	0	30	100	0	30	100
DW	100	70	0	100	70	0

Figure 5: Algae growth in 500mL flasks supplied with air and illuminated with fluorescent lightning

3.7. Microalgae Growth rate

The rate of biomass growth is the change in biomass over a specific time period. The biomass growth rate was assessed by measuring the optical density (OD) of the culture medium every 24 hours, using the Beer-Lambert laws of optical density and the Hach Lange DR 6000 spectrophotometer at 682nm (OD_{680}), as described by Hardesty and Attili (2010). The daily pH and EC were measured using WTW InoLab pH 7310 pH meter and WTW InoLab Cond 7110 meter respectively after spectrophotometric measurements.

3.8. Centrifugation

Samples were centrifuged at the Department of Agro-environmental Studies Research Laboratory at the Hungarian University of Agriculture and Life Sciences, Budapest. The liquid phase was separated from the solid phase by centrifugation (5,000 rpm; 15 minutes) until supernatant liquid was clear. The supernatant liquid was decanted, and the pellets were discarded.

3.9. Nutrients analysis

The Hach-Lange (Germany) spectrophotometric system with kits including Hach Lange DR 6000 Spectrophotometer UV-VIS (provides peak performance for both routine laboratory tasks and demanding photometric applications) ; Hach Lange Thermostat LT 200 - for standard and special digestions were used to determine the standardized procedure of NH_4^+- N, NO_3^-- N and NO_2^--N (LCK 138 Laton Total Nitrogen cuvette test 1-16 mg/L TNb) and PO_4^{-3}-P (LCK 349 Laton Total Phosphate cuvette test 0.05-1.5 mg/L PO4-P) in the supernatant before and after the cultivation period. Briefly, the determination of TN, using cuvette tests, is based on the oxidation of nitrogen compounds to nitrate ions with potassium persulfate with subsequent reaction with colorimetric sulphanilamide and N-(1-naphthyl)ethylenediamine dihydrochloride to form a pink coloured compound. The determination of total PO_4^{-3}-P in a wastewater sample, using cuvette tests, is based on the reaction between phosphate ions and molybdate ions, as well as subsequent reduction by ascorbic acid to form a blue-coloured phosphomolybdate complex (LCK 349; Hach-Lange, Germany).

4.0. Harvesting Test

The Sedimentation (gravity) method with the use of flocculants to quicken the settling process was used. The analyses of harvesting were evaluated through jars tests at room temperature (25 °C) according to Rodríguez-Núñez et al. (2012) and Acosta-Ferreira et al. (2020). The dosages of sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide $Ca(OH)_2$, ferrous sulphate ($FeSO_4$), potassium sulphate (K_2SO_4) and calcium carbonate ($CaCO_3$) investigated were 0.001g/L, 0.01g/L and 1.0 g/L. The chemicals were all evaluated as primary flocculants. Specifically, 100mL of microalgae cultures and 200mL distilled water were poured into beakers of 400mL followed by the addition of each chemical at each concentration. Mixing was started and adjusted to a quick mix on the 2mag magnetic motion stirrer at 350rpm for 2mins followed by resting for 0, 5 and 60 mins (settling time). The conditions were similar for all treatments. The absorbances were determined by UV/VIS spectrophotometry (Hach Lange, DR600) at 550nm. Images were taken with Fujifilm X30 12 MP Digital Camera to have a visual representation of the biomass concentration and quality of the harvested microalgae. The pH and EC were measured using WTW InoLab pH 7310 pH meter and WTW InoLab Cond 7110 meter respectively after each absorbance determination.

4.0.1.Turbidity

Turbidity principle was employed in the microalgae harvesting test (ISO 7027-1:2016). The effectiveness of the coagulants were determined by measuring the turbidity of the supernatant with time. However, the turbidity levels measured at 550nm wavelength using spectrophotometer were later expressed as a ratio. For this reason, their conversion to Formazin Turbidity Unit (FTU) by the calibration standards of formazine suspension was not necessary (ISO 2016).

Table 3: Calibration values used to determine the concentration from a previous study

Dry biomass weight (mg/L)	1932	1288	966	386	193	97	39	19	8
Absorbance @ 682nm	1.967	1.424	1.082	0.452	0.222	0.106	0.049	0.026	0.009

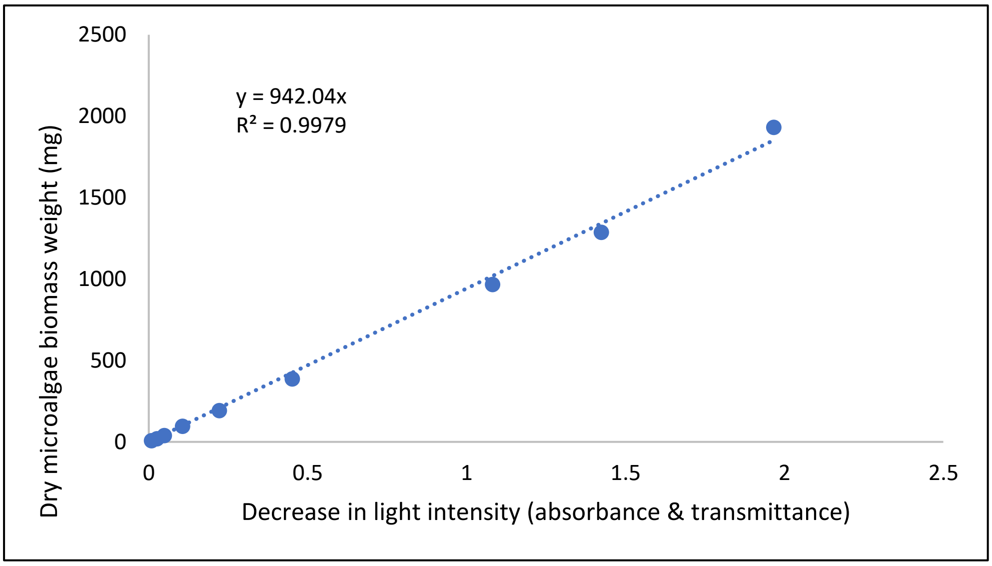

Figure 6: Relationship between Dry Biomass Weight (ml) and Absorbance of Chlorella vulgaris based on a previous study

The factor X represents the Absorbance (A) from formular Y = 942.04 * X to calculate dry biomass weight (Y).

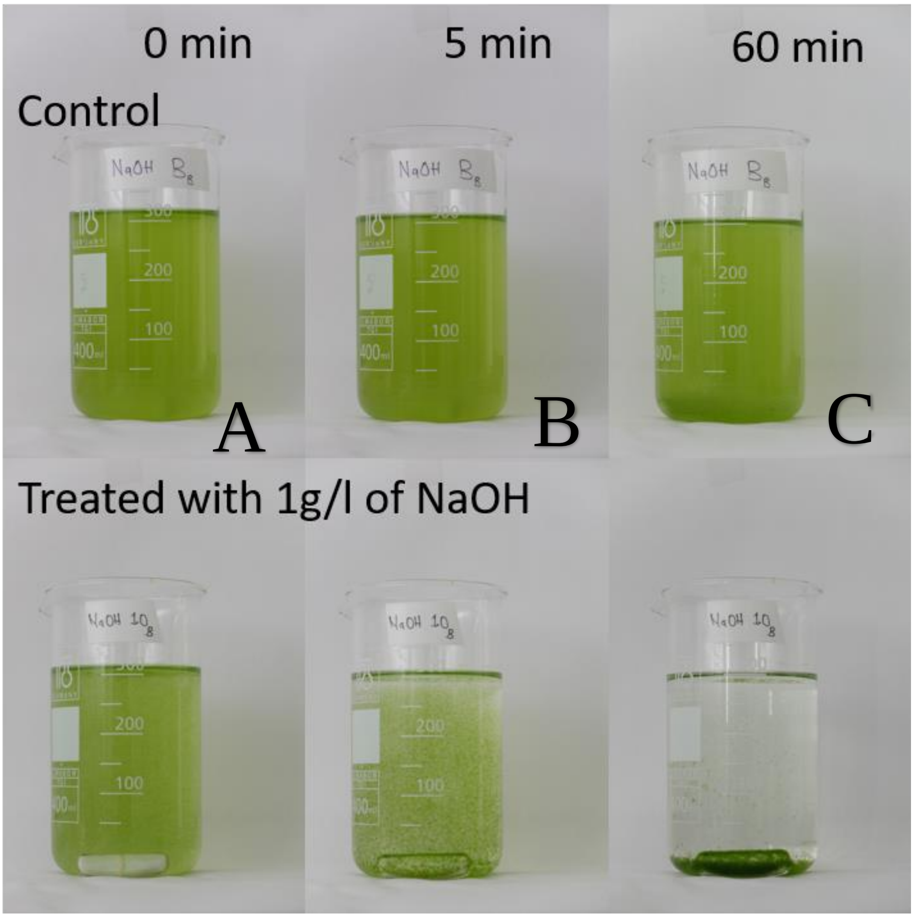

Figure 7: Harvesting Chlorella vulgaris by sedimentation with Na(OH)2 at 0mins (A), 5mins (B) and 60mins (C).

4.1. Statistical Analysis

The biomass growth data, along with the corresponding pH and EC, were subjected to ANOVA in GenStat Statistical Package 12.0 Edition. Mean separation was performed using the Fisher’s Protected least significant difference (PLSD) at a significance level of 95% to identify pairs of means that differed significantly. Nutrient analyses and microalgae harvesting data were analysed using Microsoft Excel software included in the Microsoft 365 Apps for enterprise produced by Microsoft Corporation.

4.0. RESULTS AND DISCUSSION

4.1. Growth of *Chlorella vulgaris* in pre-treated municipal wastewater at 25°C

Figure 8 presents the growth performance of *C. vulgaris* for Algae 1 (30mL of Algae stock) and Algae 2 (100mL of Algae stock) cultures. Both cultures displayed an increase in dry matter overtime, indicating a general growth trend in the respective culture media. However, the growth patterns of Algae 1(R^2=0.978) and Algae 2 (R^2=0.9826) were distinct. Algae 1 initially exhibited a slow growth rate, with dry matter weight from 73mg/L to 80mg/L and then to 172 mg/L after the first two days, and there was no significant difference ($p > 0.05$) between the two days. This suggests that Algae 1 was acclimatizing to the wastewater medium during the initial period (lag phase), followed by an exponential growth from day 3 to day 7, reaching a dry matter weight of 1140mg/L on day 7. The significant difference ($p < 0.05$) in growth rate during the lag and exponential phases of Algae 1 clearly indicates that the algae culture was responding positively to the wastewater medium, which contained a substantial amount of CO_2, nitrogen, and phosphorus. On the other hand, Algae 2 showed a faster growth rate than Algae 1, with a slow growth rate observed only on day 1, followed by rapid growth to reach a biomass weight of 1418mg/L on day 7. This indicates that Algae 2 only experienced a short lag phase before entering the exponential phase, which is consistent with its higher initial concentration of algae stock (100mL). However, a slowing down of the growth rate was observed on days 6 and 7, suggesting that the algae culture may have entered a stationary phase or that the nutrients in the wastewater medium were becoming depleted as described by Lavens & Sorgeloos (1996). This could suggest that the initial concentration of Algae stock can have a significant impact on the growth rate and biomass production of the algae culture.

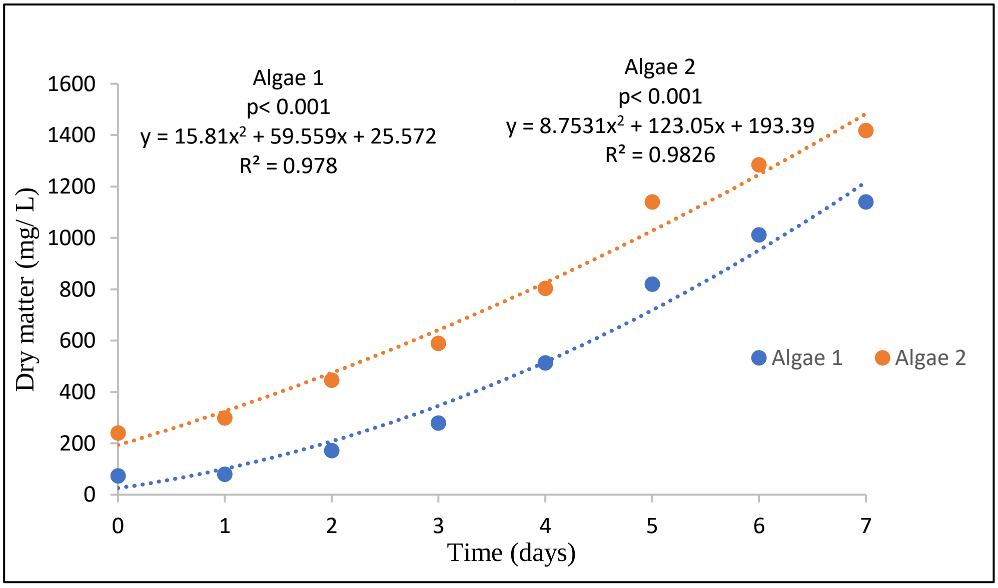

Figure 8: Growth of Chlorella vulgaris in pre-treated municipal wastewater for 7 days

4.2. Changes in pH of the media over time

Figure 9 illustrates the pH variations of the culture media containing C. vulgaris during a 7-day growth period. The control treatment (No Algae) (R^2=0.6428) exhibited a relatively constant pH throughout the growth period. Nevertheless, significant differences ($p < 0.05$) in pH were observed within the initial three days, followed by a relatively stable pH until day 7. The pH fluctuations during the first three days could be attributed to microbial activity in the culture media. It is plausible that the initial dissolved CO2 in the media was consumed by bacteria (both autotrophic and heterotrophic) and other microbes, or it diffused out of the media.

In contrast, Algae 1(R^2=0.9024) and Algae 2 (R^2=0.8667) treatments demonstrated a more dynamic change in pH over the growth period. However, there was no significant difference ($p > 0.05$) in the pH changes of Algae 1 and Algae 2 over time as both treatments showed an increasing trend. The pH changes observed correspond to the growth rates of the two treatments as depicted in Figure 8. Nevertheless, significant differences ($p < 0.05$) in pH were observed within Algae 1 and Algae 2 with increasing time. The fluctuating pH levels in the algae treatments can be attributed to the photosynthetic activity of C. vulgaris. During photosynthesis, algae consume CO_2 and release O_2, leading to an increase in pH. This observation is consistent with the findings of Mousavi et al. (2009) and Sayadi et al. (2016). This is an indication that pH changes during *C. vulgaris* cultivation are strongly influenced by the presence of algae and microbial activity.

Table 4: Averages of pH changes in culture media over time

	pH Averages		
Days	**Control**	**Algae 1**	**Algae 2**
0	7.9	7.9	8.0
1	8.8	9.0	9.0
2	8.5	8.7	8.7
3	8.8	9.5	9.5
4	8.8	9.5	9.5
5	8.8	9.8	9.5
6	8.8	9.9	9.9
7	8.8	9.7	9.8

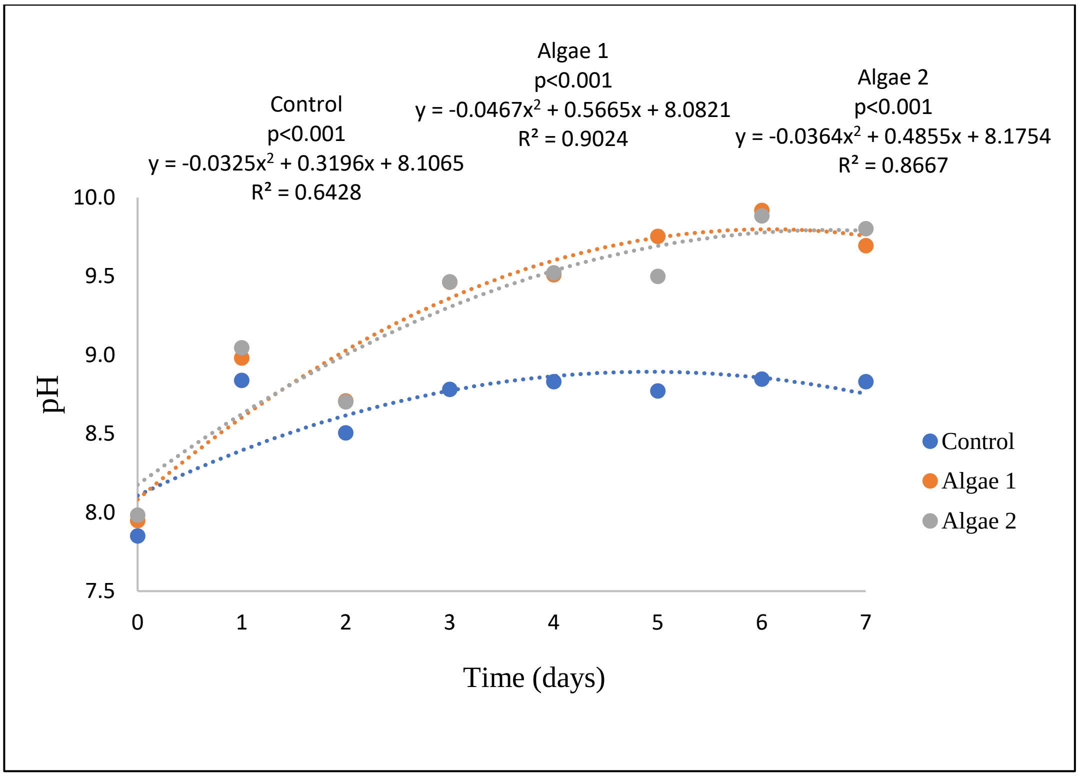

Figure 9: Changes in pH of the culture media over time(The pH presented is the average of the four replicate samples)

4.3. Changes in Electrical Conductivity of the media over time

During the growing period of two algal cultures, Algae 1 (R^2=0.9612) and Algae 2(R^2=0.9066), a significant decrease ($p < 0.05$) in electrical conductivity (EC) values was observed. This phenomenon was attributed to the consumption of dissolved salts and ions by the *C. vulgaris*. Interestingly, Algae 1, which had a lower biomass (30mL of algae stock), consistently showed lower EC values compared to Algae 2, despite the fact that both cultures were derived from the same stock of *C. vulgaris*. These differences could be due to physiological and metabolic variations of *C. vulgaris*.

The trend in pH values for the Control (R^2=0.6076) treatment was relatively stable from day 2 of the experiment, as depicted in Figure 9. Similarly, EC values for the control treatment remained relatively stable, with only a significant decrease in dissolved salts, nutrients, and ions observed after the first day of growth, followed by no significant changes over time ($p > 0.05$). However, bacteria and other microbes were not able to consume large amount of dissolved nutrients and salts in the growing media in the absence of *C.vulgaris*. This is reflected in the difference in the magnitude of the conductivity between the control treatment and the two algae cultures. The results propose that *C. vulgaris* has a significant impact on the quality of water by removing nutrients and dissolved salts. This further highlights the importance of algae in nutrient removal and the potential for *C. vulgaris* to remediate municipal wastewater (Oswald 1963, Wang et al. 2010, Pooja et al. 2022).

Table 5:Averages of Electrical Conductivity Changes in culture media over time

Averages of Electrical Conductivity (μS/cm)			
Days	**Control**	**Algae 1**	**Algae 2**
0	1312	1332	1379
1	1261	1256	1274
2	1281	1238	1250
3	1267	1149	1167
4	1259	1111	1123
5	1268	1018	1075
6	1262	1017	1058
7	1266	1028	1076

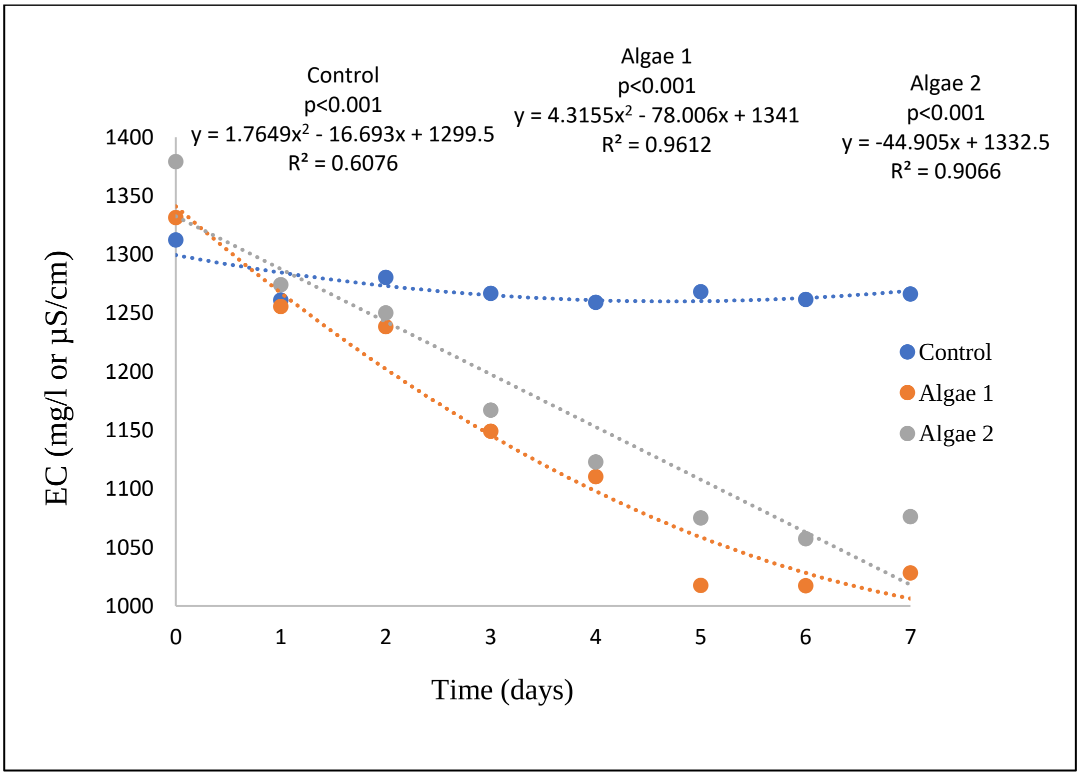

Figure 10: Changes in the Electrical Conductivity (mg/L) of the culture media over time (The EC presented is the average of the four replicate samples)

4.4. Nitrogen Removal by *C. vulgaris* in Pre-treated Municipal Wastewater

The study investigated the changes in nitrogen content in culture media after the cultivation of Chlorella vulgaris over a 7-day period. The results were presented in Figure 11 as concentration and Figure 12 as percentages of day 0 values. The Control treatment showed a decrease in mean nitrogen content from 36 mg/L at day 0 to 29 mg/L at day 1 and 22 mg/L at day 7, indicating a reduction in nitrogen concentration over time. This decrease in nitrogen content could be attributed to the normal growth and metabolism of microorganisms, including bacteria, which convert nitrogen compounds to nitrogen gas. The Algae 1 and 2 treatments maintained relatively constant mean nitrogen content between day 0 and day 1, with values of 38 mg/L and 31 mg/L for Algae 1 and 43 mg/L and 33 mg/L for Algae 2, respectively. However, by day 7, both treatments showed a significant decrease in nitrogen content, with values of 4 mg/L and 6 mg/L for Algae 1 and Algae 2, respectively. This suggests that *C. vulgaris* utilizes nitrogen as a nutrient source for growth and reduces the nitrogen content in the culture media compared to the Control treatment.

Algae 1 (30mL of algae stock) displayed a notable decrease in nitrogen content compared to Algae 2 (100mL of algae stock), with a removal efficiency of approximately 88% compared to 87% for Algae 2 on day 7, despite having a lower proportion of *C. vulgaris*. This difference in removal efficiency could be associated with the growth patterns and nutrient absorption of the two algae cultures as reflected in the EC values shown in Figure 10. Additionally, both algae cultures exhibited the ability to maintain a relatively stable nitrogen content between day 0 and day 1, indicating successful acclimation to the culture conditions during the initial stage of growth (lag phase). This further indicates that the algae cultures were able to adapt to the wastewater composition and utilize nitrogen as a nutrient source for growth, source for growth, which resulted in a subsequent decrease in nitrogen content over time. This finding is consistent with the conclusions drawn in previous studies by Larsdotter (2006) and Jia and Yuan (2016), who similarly observed a reduction in nitrogen content over time as a result of algae growth in wastewater.

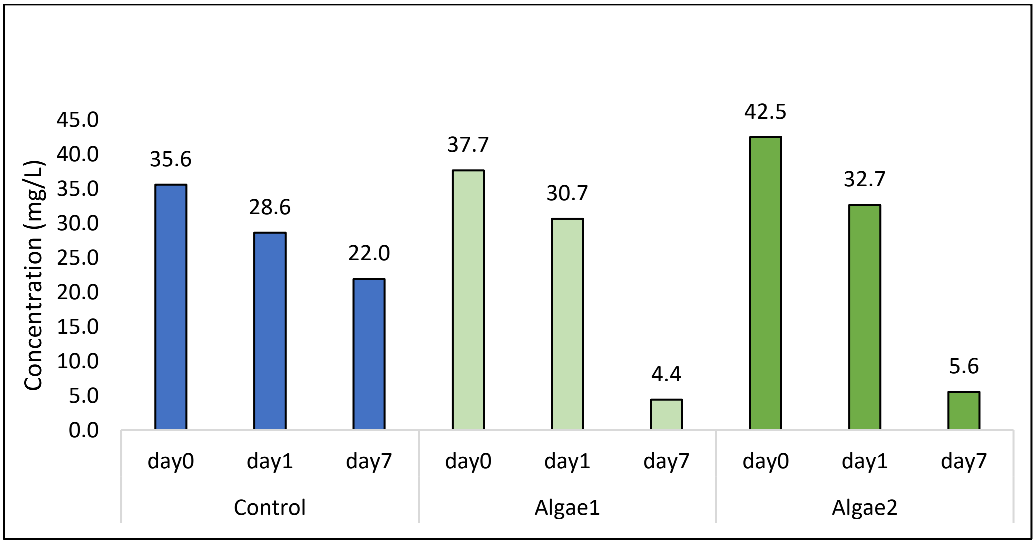

Figure 11: Changes in dissolved N content of the samples expressed in the concentration values (The N content presented is the average of the four replicate samples)

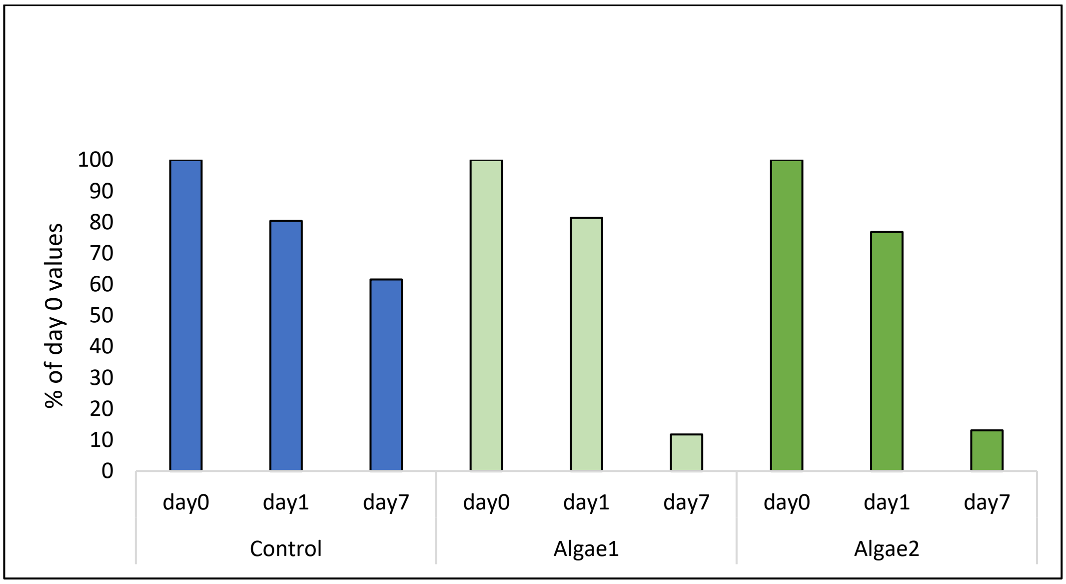

Figure 12: Changes in dissolved N content of the samples expressed as % of the day 0 values

4.5. Phosphorus Removal by *C.vulgaris* in Pre-treated Municipal Wastewater

The changes in phosphorus content of the culture media were analyzed after seven days of Chlorella vulgaris culture and are presented in Figure 13 as concentrations and in Figure 14 as percentages of the day 0 values. The trend observed in Figure 11 and Figure 12 was similarly demonstrated in the phosphorus content of the culture media. Algae treatments 1 and 2 significantly reduced the phosphorus content to 0.1 mg/L, representing a 95% reduction on day 7. In contrast, the control treatment exhibited a minor reduction in phosphorus content, from 2.7 mg/L at day 0 to 2.1 mg/L at day 7, representing a 23% reduction in phosphorus content after 7 days whereas, both algae cultures were effective in reducing the phosphorus content in the culture media over the 7-day period. The substantial difference observed in phosphorus content in algal cultures compared to the control treatment may be credited to the metabolic activity of the *Chlorella vulgaris*, which is known to be highly efficient in breaking down, assimilating and uptake of phosphorus from pre-treated municipal wastewater. (Mostert & Grobbelaar 1987, Solovchenko et al. 2016).

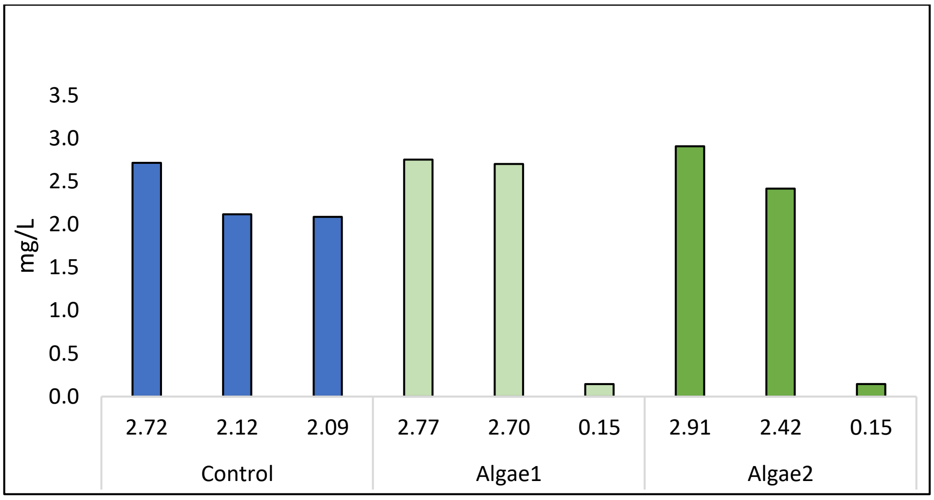

Figure 13: Changes in dissolved P content of the sapmles expressed in the concentration values

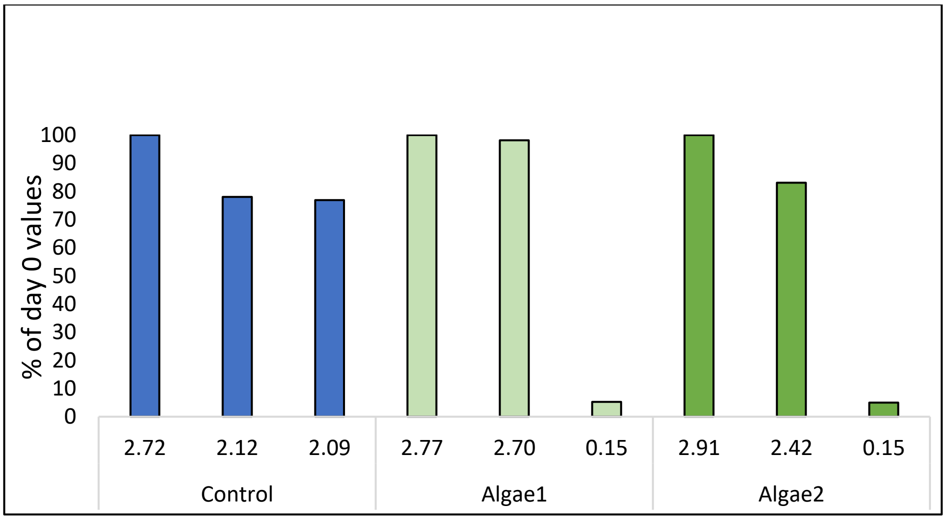

Figure 14: Changes in dissolved P content of the samples expressed as % of the day 0 values

4.6. Effectiveness of Flocculants in Microalgae Sedimentation Over Time

4.6.1. Sodium Hydroxide, NaOH

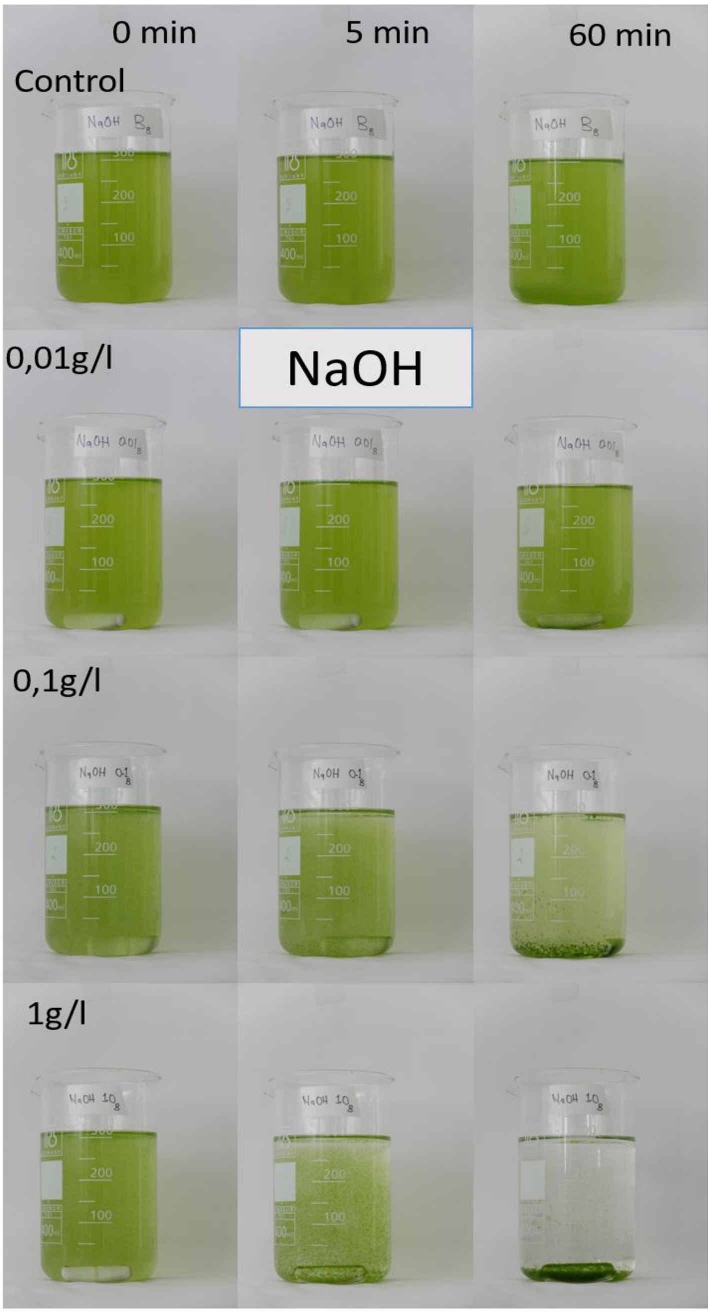

Figure 15:A pictorial view of enhanced algae sedimentation with NaOH

The results in Figure 16 shows the effectiveness of different concentrations of NaOH as a flocculant in harvesting microalgae through sedimentation. The control treatment showed a slow settling of the biomass over time This is consistent with previous studies that have found that some microalgal species, such as C. vulgaris, have a slow settling nature due to their small size and low density (Al-Jabri et al. 2020). Therefore, the use of a flocculant is necessary to speed up the process (Kwietniewska et al. 2022). Surprisingly, the lowest concentration of NaOH tested (0.01g/L) was not effective in improving the settling process, as it resulted in even less biomass settling than the control treatment after 5 and 60 minutes. This suggests that the concentration was too low to observe a significant settling of the biomass. The 0.1g/L concentration was more effective, with a final percentage of 51% after 60 minutes, while the 1.0g/L concentration was the most effective, with a final settling percentage of only 27% after 60 minutes. The higher concentrations of NaOH resulted in more effective sedimentation of *C. vulgaris*, with the 1.0g/L treatment being the most effective. It is noteworthy that the increase in the rate of sedimentation is most prominent in the 0.1g/L and 1.0g/L treatments. Therefore, selecting the appropriate concentration of flocculant is crucial to balance the trade-off between effectiveness and cost. In this regard, NaOH has shown to be an effective method for harvesting microalgae. However, its effectiveness also depends on the concentration of flocculant and settling time used.

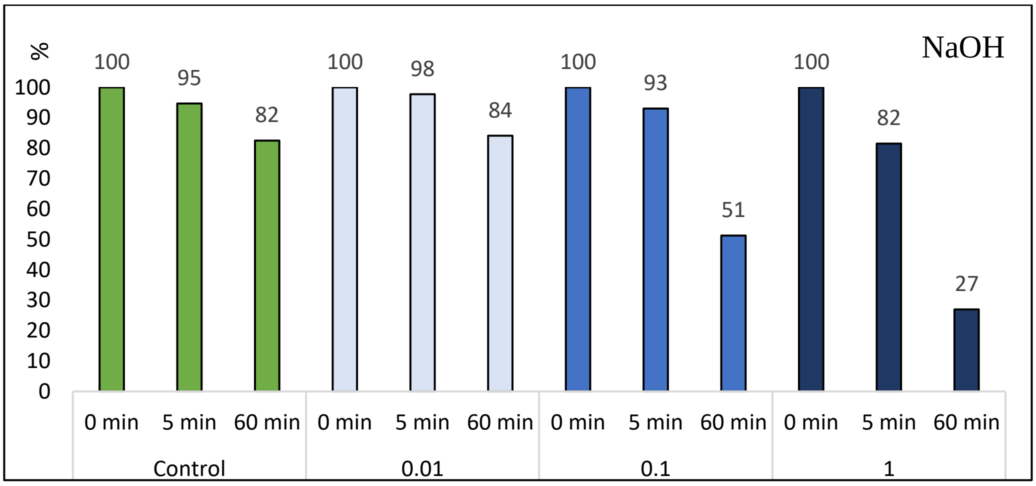

Figure 16: Effectiveness of NaOH in microalgae sedimentation over time

4.6.2. Potassium Hydroxide, KOH

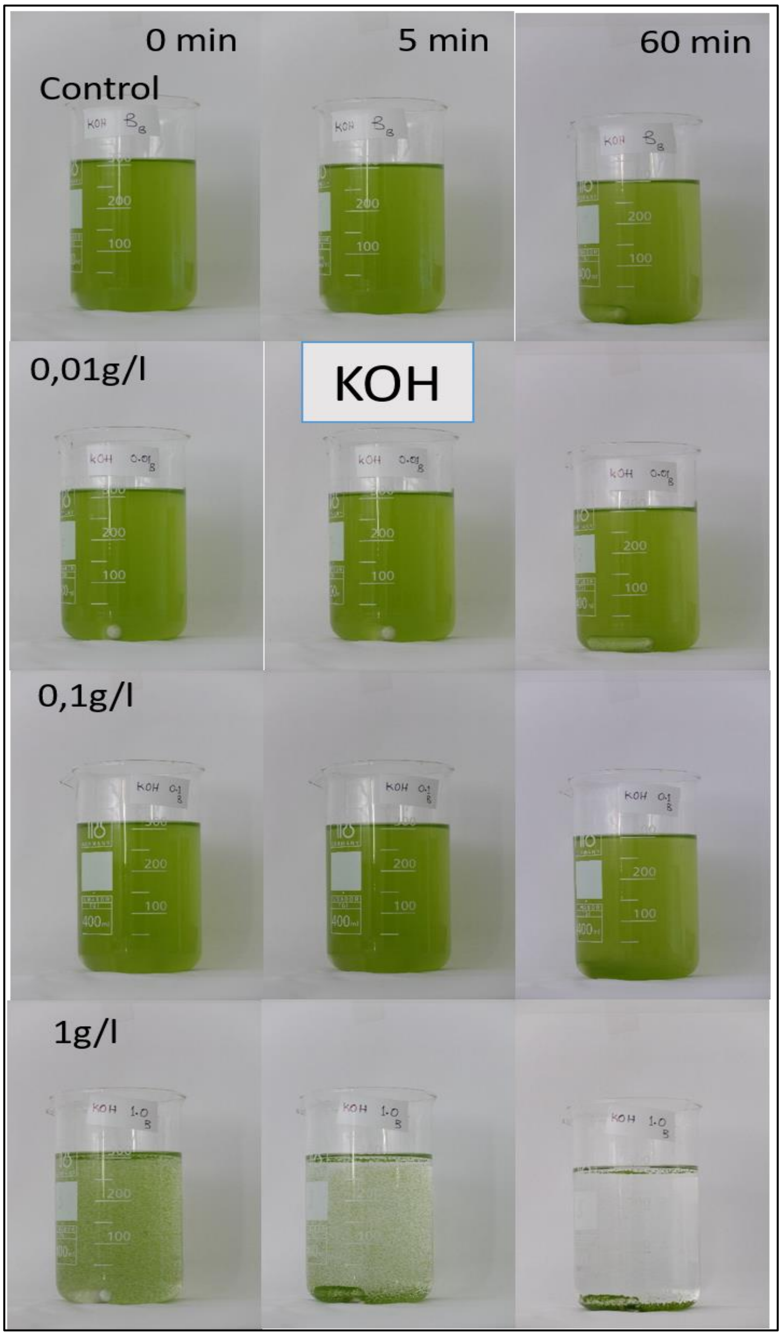

Figure 17: A pictorial view of enhanced algae sedimentation with KOH

From Figure 18, it is apparent that the control treatment had a settling rate that was relatively similar to that of Figure 16. This can be attributed to the minute size and relative density of the *C. vulgaris*, which makes it difficult for them to settle quickly. The negative surface charge on the cell wall of *C. vulgaris* could also contribute to the slow settling rate, as it causes repulsion between the cells, preventing them from aggregating and settling (Chatsungnoen & Chisti 2016). Additionally, algal cells tend to remain close to the surface of the culture media to compete for light (Al-Jabri et al. 2020). Notably, the 0.1g/L concentration displayed a higher turbidity than the Control and 0.01g/L after 60 minutes. However, the 1.0g/L concentration was the most effective, with a final settling percentage of only 13% after 60 minutes. The effectiveness of KOH in settling *C. vulgaris* is dependent on its concentration, and a higher concentration could potentially result in a better settling rate. However, there is a trade-off between effectiveness and cost, as higher concentrations of flocculant are also more expensive.

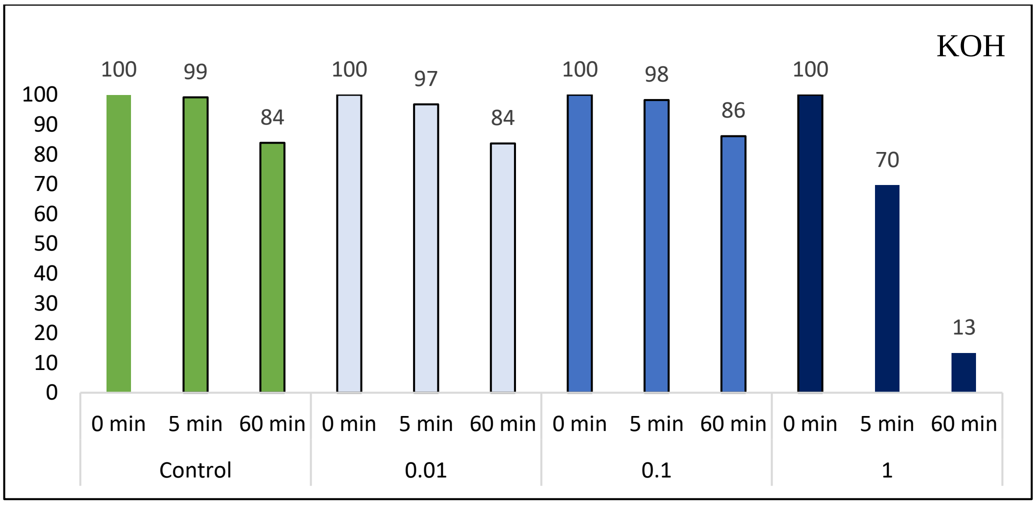

Figure 18: Effectiveness of KOH in microalgae sedimentation over time

4.6.3. Calcium Hydroxide, $Ca(OH)_2$

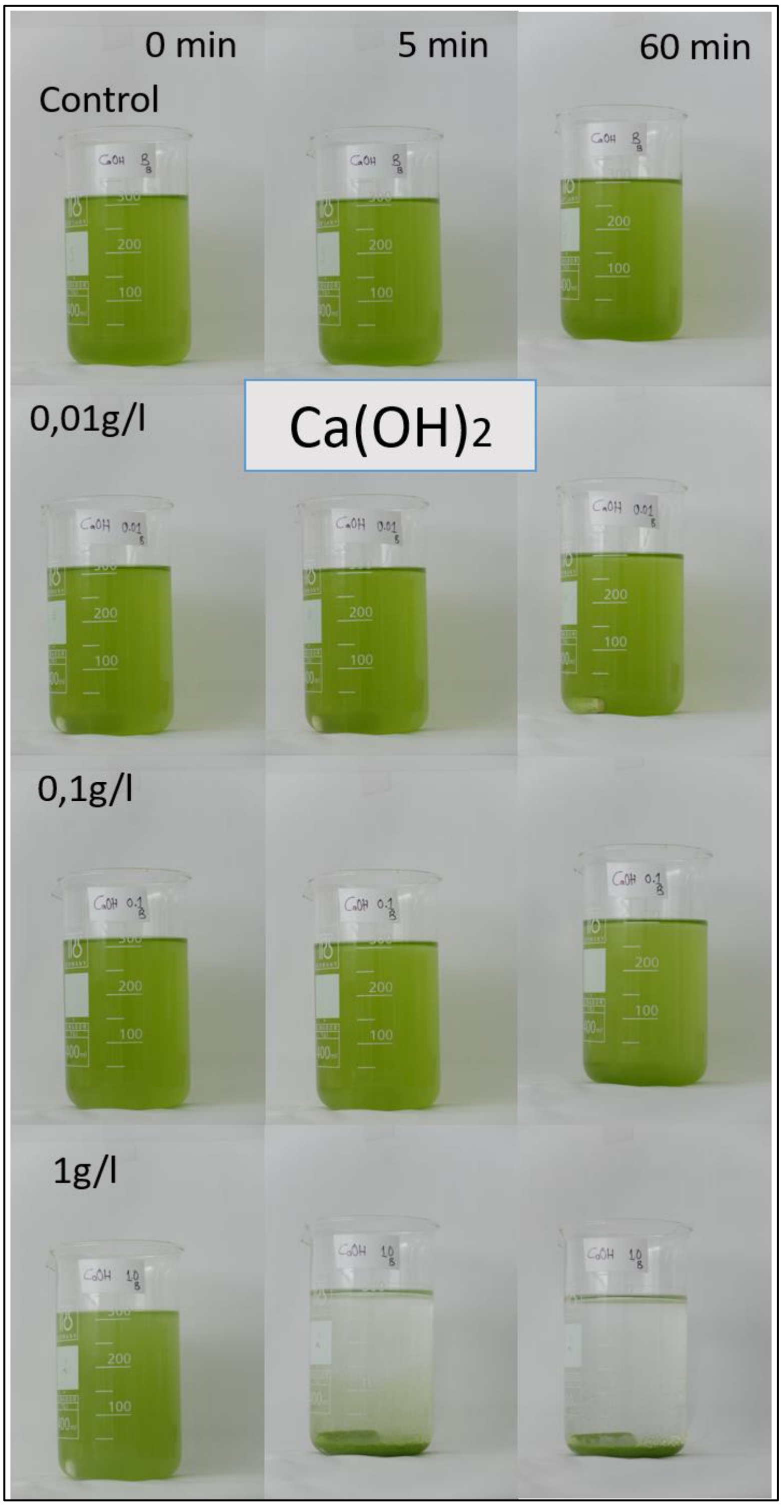

Figure 19: A pictorial view of enhanced algae sedimentation with $Ca(OH)_2$

Figure 20. also revealed that there was no significant difference between the settling rate of 0.01g/L concentration and the Control. This finding supports the explanations provided under Figure 16 and Figure 18. The settling rate increased with increasing concentration, with the 1.0g/L treatment demonstrating the most effective settling rate. However, the surprising observation was that the settling efficiency at 1.0g/L concentration was higher in 5 minutes than in 60 minutes.

One possible explanation for this outcome is that the addition of $Ca(OH)_2$ raised the pH level, leading to a more negative surface charge on the cells. This negative charge can prevent the cells from aggregating and settling, and this idea is consistent with the findings of Vandamme et al. (2012), who asserted that microalgae cells become more negatively charged at high pH values, which prevents the cells from aggregating settling at high pH changes. This effect is due to the presence of calcium and magnesium salts in the medium, rather than charge neutralization. Also, it could be that the initial rapid formation of flocs due to the fast neutralization of the negative charge on the surface of the algae cells by $Ca(OH)_2$, causing them to aggregate and settle more quickly. However, the flocs may become increasingly large and denser over time settling at or near the surface of the media (personal observed experience).

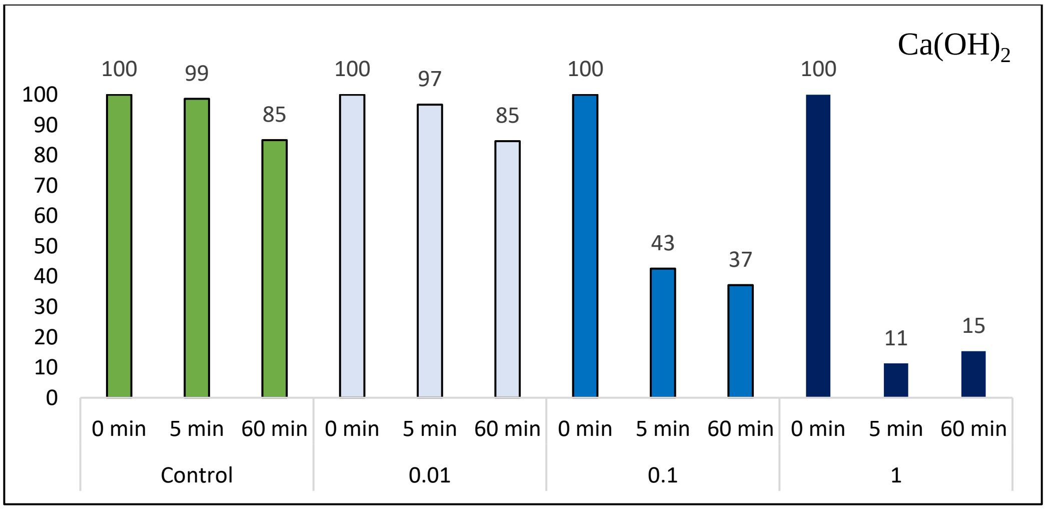

Figure 20: Effectiveness of $Ca(OH)_2$ in microalgae sedimentation over time

4.6.4. Potassium Sulphate, K_2SO_4

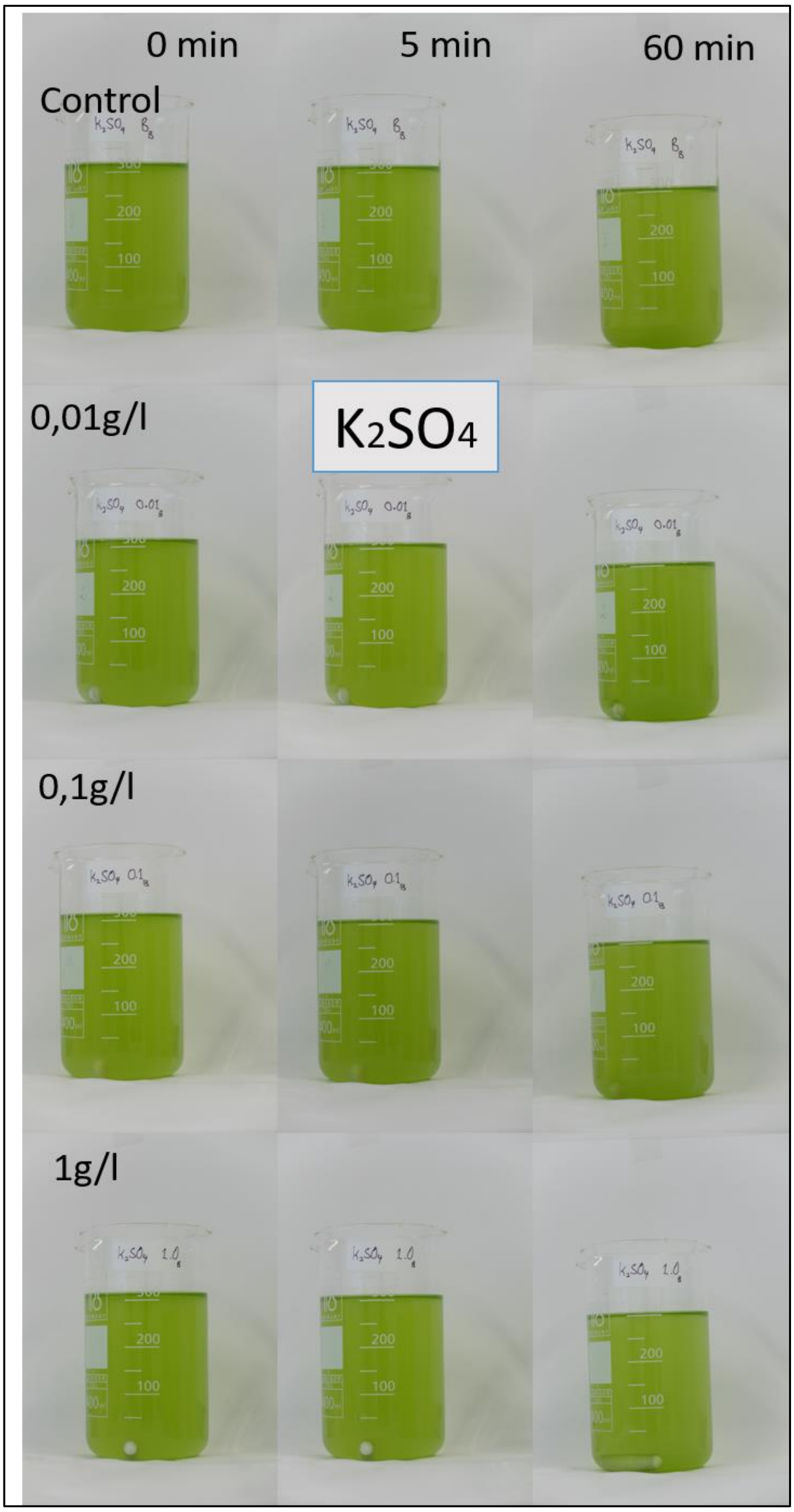

Figure 21: A pictorial view of enhanced algae sedimentation with K_2SO_4

The settling rate of the Control and various concentrations of K_2SO_4 were compared as shown in Figure 22. the settling rate of the Control was found to be similar to all concentrations of K_2SO_4 tested. These results suggest that increasing the dosage of K_2SO_4 may not result in improved settling efficiency. Hence, K_2SO_4 could be considered a poor flocculant for the sedimentation of algal biomass.

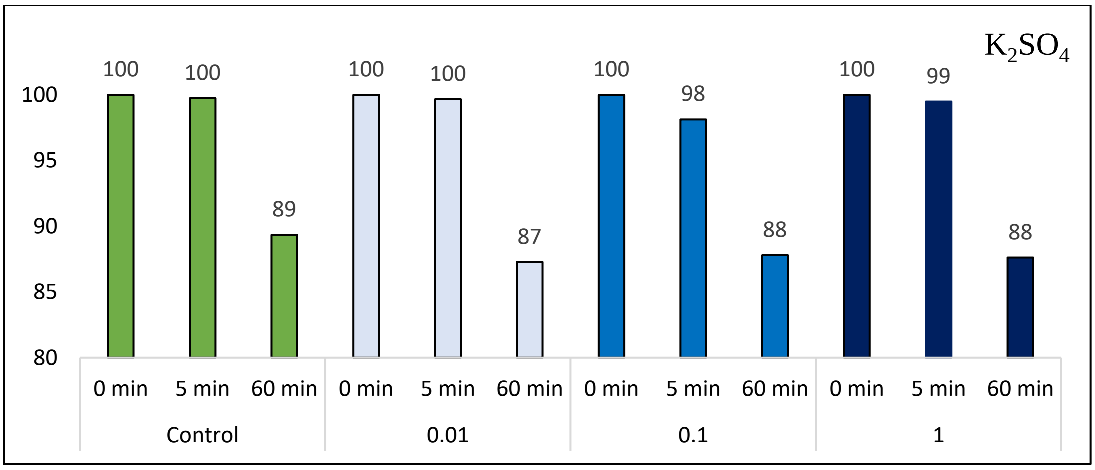

Figure 22: Effectiveness of K_2SO_4 in microalgae sedimentation over time

4.6.5. Ferrous Sulphate, $FeSO_4$

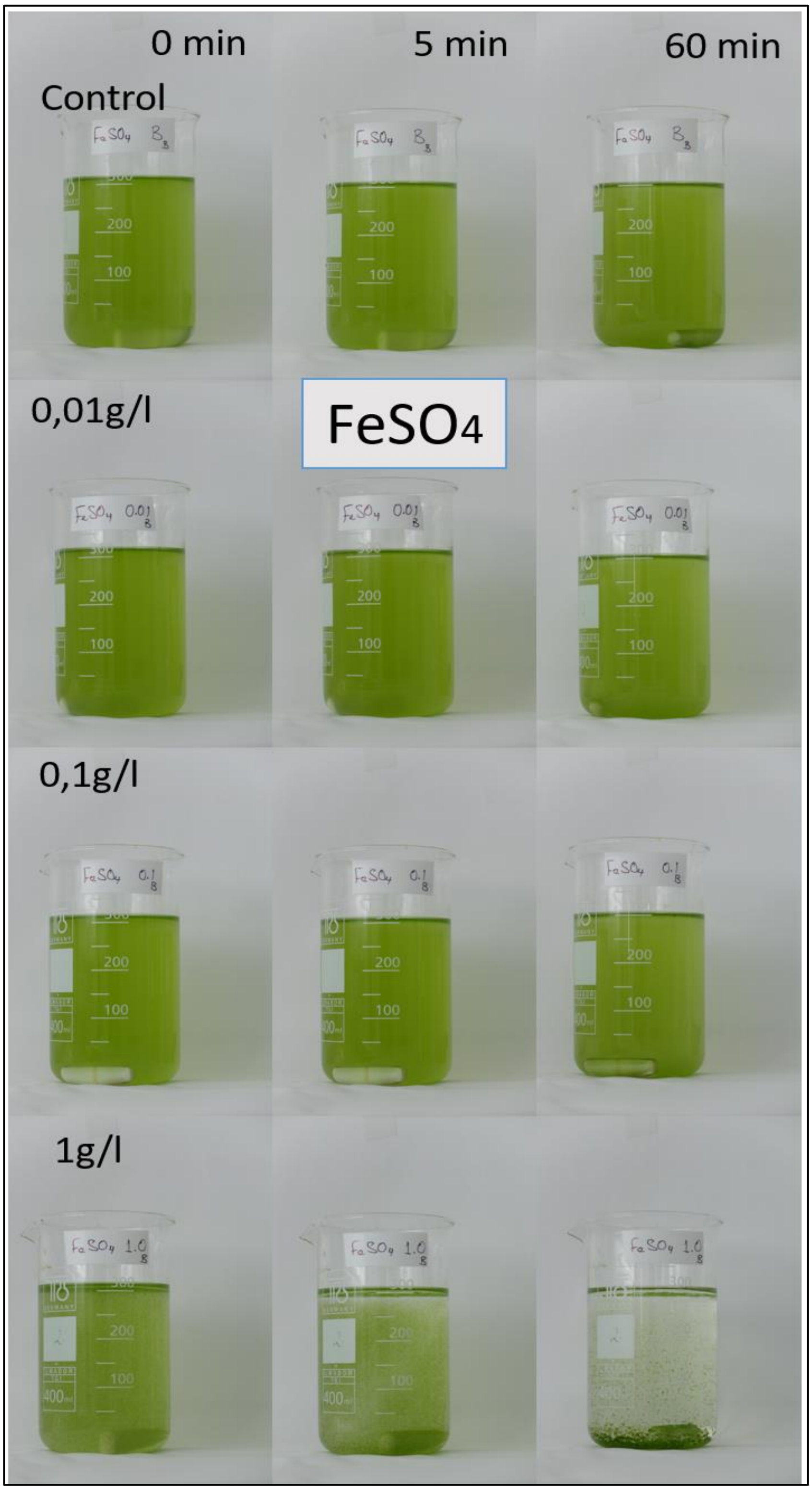

Figure 23: A pictorial view of enhanced algae sedimentation with $FeSO_4$

Observations made from potassium sulphate (Figure 22) were similar in the test of ferrous sulphate ($FeSO_4$) as shown in Figure 24 in that, ferrous sulphate did not significantly accelerate the aggregation and settling rate of algal biomass at concentrations of 0.01g/L and 0.1g/L compared to the Control over time. The low concentrations of 0.01g/L and 0.1g/L were found to be insufficient to effectively promote neutralization and aggregation of the algal biomass when compared to the Control after 5 and 60 minutes. However, the use of a higher concentration of 1.0g/L resulted in a profound settling rate of algal cells in suspension, with 27% of cells remaining unsettled after 60 minutes. The results suggest that a high concentration of ferrous sulphate can initially promote rapid sedimentation, similar to the effects observed with NaOH, KOH, and $Ca(OH)_2$. However, over time, the high concentration of $FeSO_4$ may also lead to cell aggregation and reduced settling of the algal biomass.

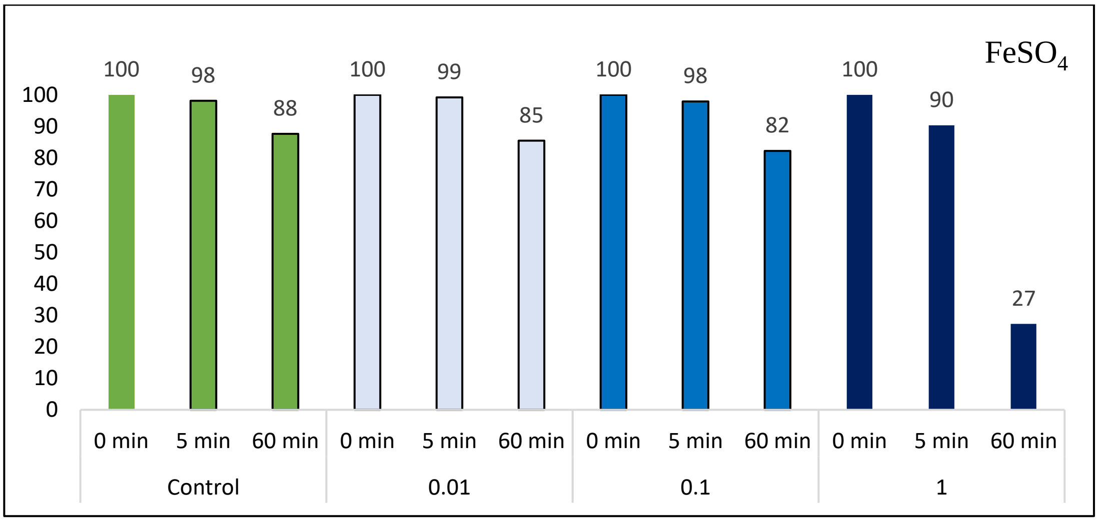

Figure 24: Effectiveness of $FeSO_4$ in microalgae sedimentation over time

4.6.6. Calcium Carbonate, $CaCO_3$

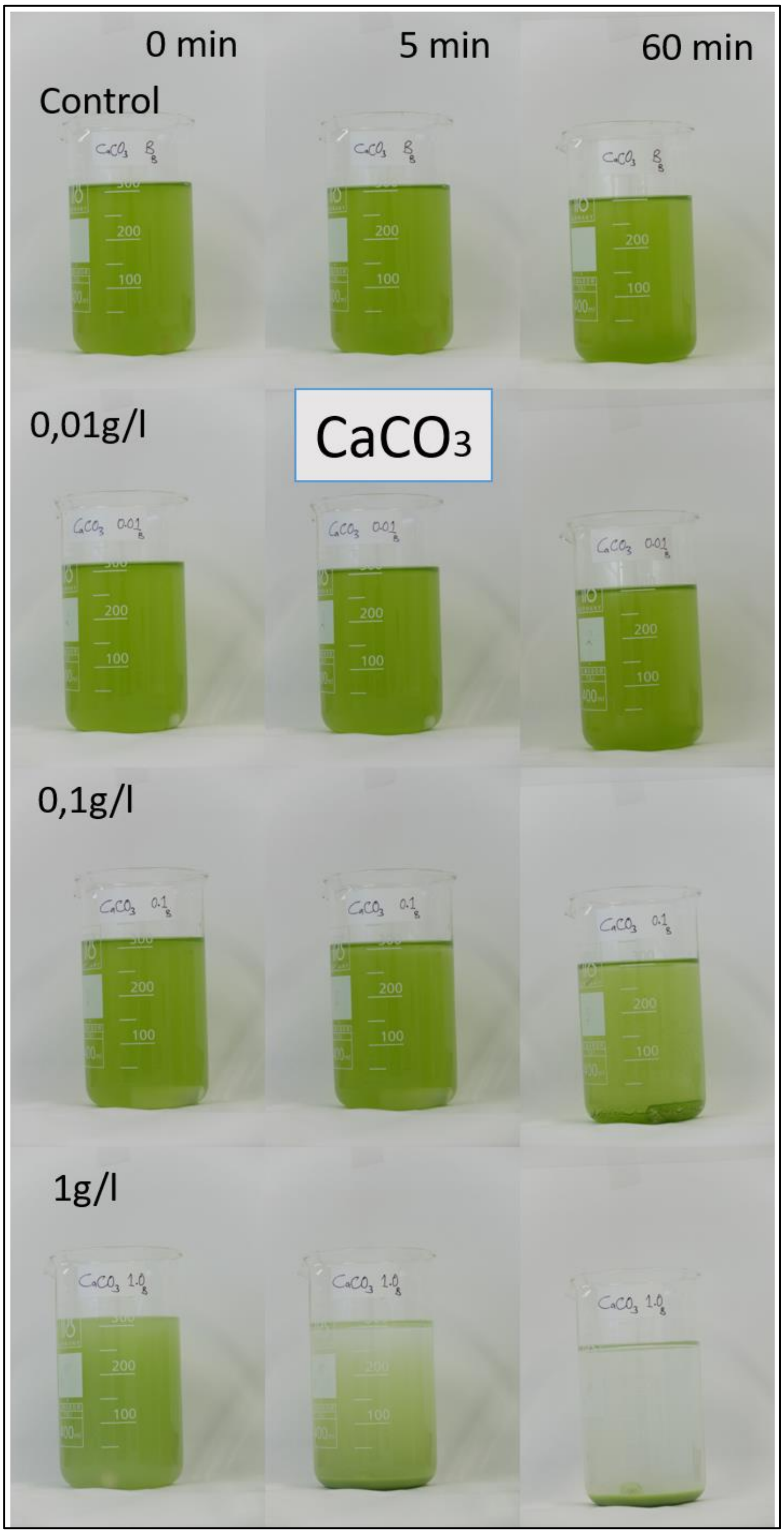

Figure 25: A pictorial view of enhanced algae sedimentation with $CaCO_3$

Figure 26 illustrates the results of the effect of calcium carbonate-induced aggregation and sedimentation on the turbidity of the media containing microalgal cells. Results showed that there was no significant reduction in turbidity with the use of calcium carbonate-induced aggregation and settling compared to the Control. The settling rates of the Control and the 0.01g/L and 0.1g/L concentrations of calcium carbonate were similar.

However, when the concentration of calcium carbonate was increased to 1.0g/L, there was nearly a 50% increase in aggregation and settling after 60 minutes. These findings also propose that an increase in the concentration of calcium carbonate over time could enhance the sedimentation of microalgal cells and reduce the turbidity of the suspension.

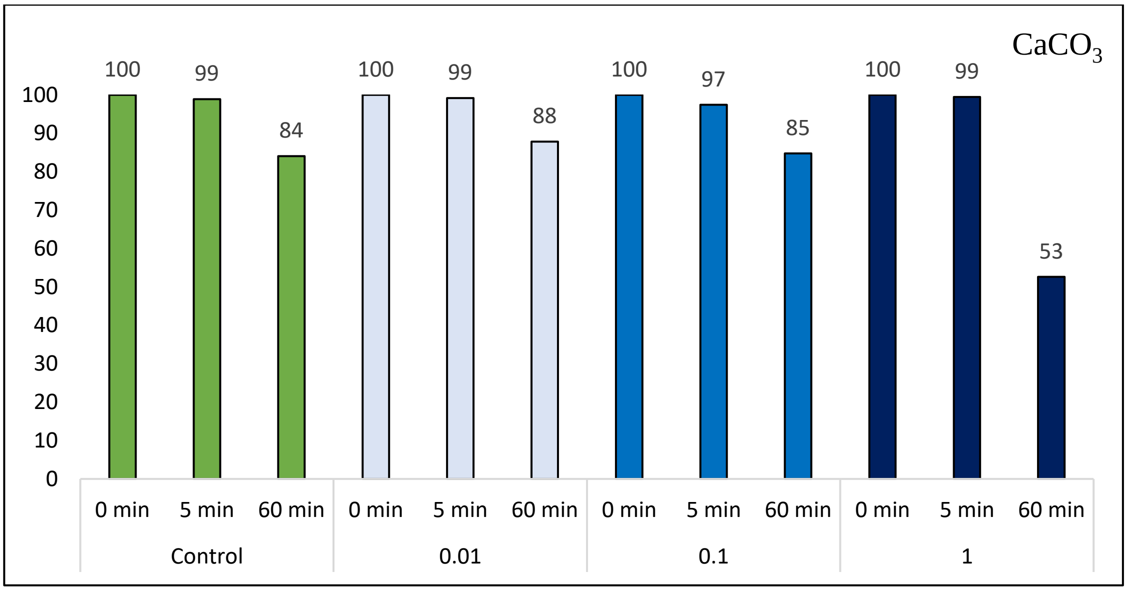

Figure 26: Effectiveness of CaCO3 in microalgae sedimentation over time

4.7. Comparison of sedimentation effectiveness

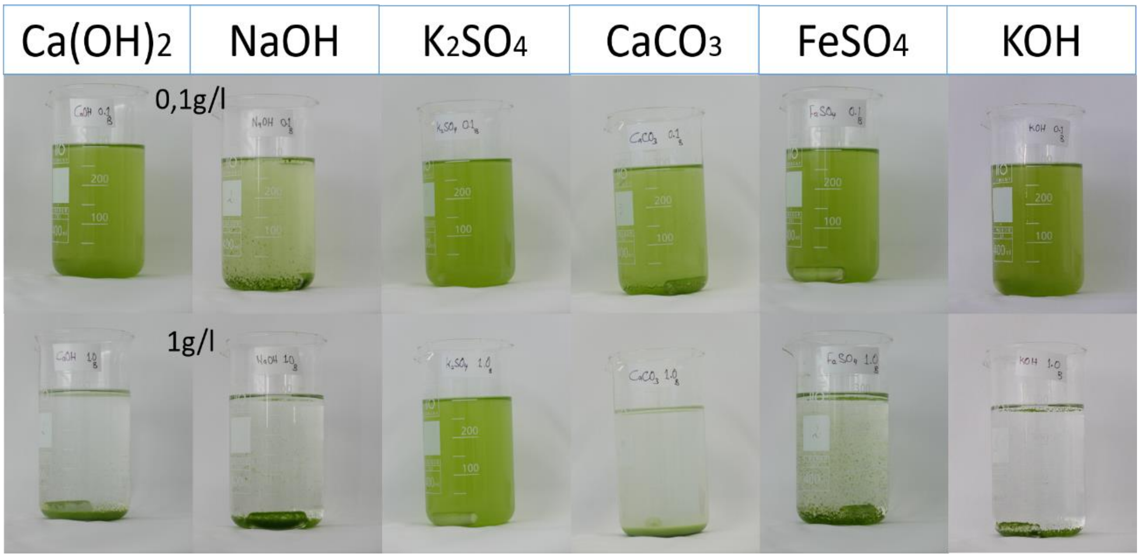

Figure 27: Comparison of flocculants enhanced algae sedimentation in 0.1g/L & 1.0g/L concentrations after 60 minutes

Figure 28 presents a comparison of the settling rates and dosages of all the flocculants. After 5 minutes of sedimentation, the control treatments did not show any significant differences. NaOH had the highest settling rate, which was 5% faster than the other flocculants, whereas K_2SO_4 remained in suspension even after 5 minutes. After 60 minutes, all the coagulants settled approximately 10% more, with no noticeable differences among them. NaOH had the fastest settling rate, which was 18% faster than the other flocculants, while K_2SO_4 had the slowest settling rate, which was 11% slower than the other flocculants. However, the differences in settling rates were relatively small and may not be significant in practical applications.

The dosages of K_2SO_4 in algae media showed the least ability to destabilize and aggregate algal cells, regardless of the dosage and time. $FeSO_4$ showed a similar sedimentation rate to K_2SO_4 at first but demonstrated a significant improvement with the highest dosage after 60 minutes. $CaCO_3$ also showed a comparable trend to $FeSO_4$, but with a lower settling velocity at the highest dosage in 60 minutes. Since sulphate ions are negatively charged, they can increase the negative charges of cells, and the effectiveness of destabilizing and aggregating cells may be influenced by the type of cation bound to the sulphate ion. This may explain why $FeSO_4$ had better flocculating ability than K_2SO_4.

Generally, the hydroxide-based (OH^-) flocculants were more effective than the sulphates (SO_4^{2}) and carbonates (CO_3^{2-}) flocculants due to their high alkalinity and greater propensity to neutralize algal cells and cause them to form larger aggregates that can settle faster due to their mass. In 0.01g/L concentration, KOH and $Ca(OH)_2$ showed a faster settling rate, but $Ca(OH)_2$

displayed an even faster settling rate and lower turbidity by 57% and 89%, respectively, at 0.1g/L and 1.0g/L concentrations after 5 minutes of settling time. $Ca(OH)_2$ also showed a similar faster settling rate by 63% and 85% at 0.1g/L and 1.0g/L, respectively, after 60 minutes. The enhanced sedimentation with NaOH also showed that its effectiveness depended on the amount of dosage with time. However, $Ca(OH)_2$ demonstrated a better settling rate, which may be attributed to calcium being a divalent ion, unlike sodium and potassium, which are monovalent ions. This agrees with the findings of Malik (2018) and Okoro et al. (2019) who asserted the effectiveness of charge neutralization increases with a higher cation charge.

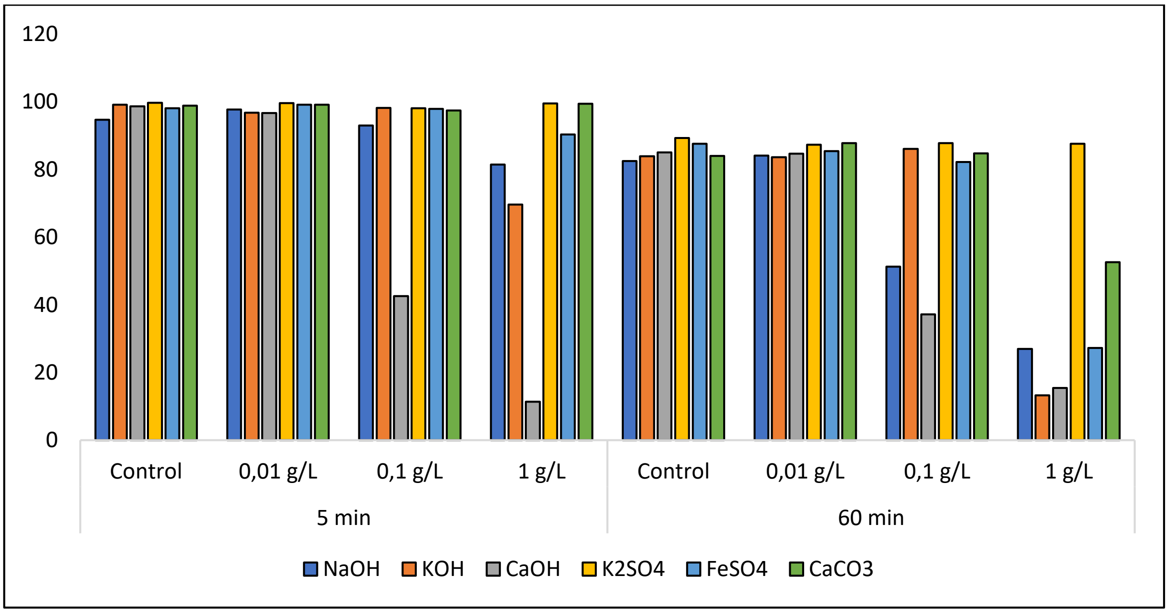

Figure 28: Comparison of sedimentation effectiveness

4.8. Changes in pH during sedimentation process

The pH affects the charge and solubility of the particles and the efficiency of the flocculant. The effect of different flocculants on the pH was investigated, and the results are shown in Table 6. As expected, hydroxide-based flocculants, such as $Ca(OH)_2$, increased the pH due to their strong alkaline nature, and the effect was more pronounced at higher dosages. Calcium carbonate also increased the pH steadily with increasing dosage, as it reacts with water to form bicarbonate and hydroxide ions. On the other hand, potassium sulfate demonstrated a cycling change in pH at a decreasing rate, probably due to the formation of acidic and basic species in solution. Iron-based $FeSO_4$, caused a decreasing pH as the dosage increased, likely due to the hydrolysis of Fe^{3+} ions and the formation of H^+ ions. The observed increase in pH with hydroxide-based flocculants suggests that a higher pH may improve sedimentation and settling rate by reducing the repulsive forces between the particles and enhancing their aggregation.

Table 6: Changes in pH during sedimentation process

	Control	**0,01 g/l**	**0,1 g/l**	**1 g/l**
NaOH	5,6	8,8	11,3	12,4
KOH	4,4	7,3	9,9	11,9
$Ca(OH)_2$	4,1	7,3	10,7	12,3
K_2SO_4	4,9	5,0	4,9	5,0
$FeSO_4$	5,7	5,1	5,0	4,3
$CaCO_3$	5,9	7,4	8,9	8,7

In all comparisons, $Ca(OH)_2$ could be considered the most effective flocculant for improving sedimentation in biomass harvesting, particularly at higher concentrations, as it demonstrated a better reduction in turbidity in both short (5 mins) and long (60 mins) times. However, the choice of flocculant depends on various factors, such as effectiveness, availability, location, cost, and safety. $Ca(OH)_2$ is relatively cheap compared to the other alkalis, costing only \$0.10/kg compared to \$0.36/kg NaOH and \$3.78/kg KOH, and is also safer to handle than Na+ and K+ (Rodrigues et al. 2016). Although $CaCO_3$ and $FeSO_4$ are relatively cheaper than $Ca(OH)_2$, the former displayed demonstrated no comparable settling rate to $Ca(OH)_2$.

5.0. CONCLUSION

This study aimed to investigate the potential of using microalgae for the removal of nitrogen and phosphorus from pre-treated municipal wastewater. The results demonstrate that pre-treated municipal wastewater contains high levels of N and P, which provide sufficient nutrients for the growth and removal of nutrients by microalgae.The study found that the initial concentration of the algae culture had a significant impact on its growth rate and biomass production. The growth of microalgae increased the pH of the culture media, which can be attributed to their photosynthetic activity that utilizes carbon dioxide (CO_2) and releases oxygen, leading to an increase in the pH of the culture media. The electrical conductivity (EC) values also decreased significantly in both algae cultures, indicating high consumption of nutrients, salts, and ions for growth and metabolism.

The algae culture (*C. vulgaris*) displayed a good nitrogen removal efficiency of approximately 88% after seven days of culture period, whereas the control treatment showed a steady decrease in nitrogen content over time. Similarly, *C. vulgaris* demonstrated a high phosphorus utilization efficiency by removing 95% of total phosphorus after the culturing period. These results suggest that the use of microalgae for municipal wastewater treatment can significantly enhance nutrient recovery.

Regarding microalgae harvesting, a comparison between cell destabilization and aggregation of flocculants revealed that hydroxide-based flocculants were more effective than sulphates and carbonates. Among them, $Ca(OH)_2$ was found to be the most effective and relatively cheaper flocculant for microalgae harvesting by sedimentation, particularly at higher concentrations. $Ca(OH)_2$ was able to reduce turbidity in both short and long time periods, making it an ideal choice for microalgae harvesting.

ABSTRACT OF THESIS

NUTRIENT REMOVAL BY MICROALGAE FROM PRE-TREATED MUNICIPAL WASTEWATER

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Conventional wastewater treatment methods are constrained by high energy consumption, operation costs, and poor nutrient recovery. Phosphorus is an essential element for all living organisms after nitrogen, but it is a finite and a non-renewable resource, largely derived from phosphate rock. With P reserves dwindling and population growth increasing, it is imperative to find sustainable ways to recover and recycle phosphorus. Microalgae-based wastewater treatment provides a promising solution to recover essential nutrients while mitigating eutrophication caused by excessive nutrient levels in surface waters. This study evaluates the efficiency of *Chlorella vulgaris* in utilizing nitrogen and phosphorus nutrients for growth and biomass production in municipal wastewater. The algae was cultured in pre-treated municipal wastewater for 7 days using different dosages. The effectiveness of chemical flocculants in microalgal biomass harvesting were also investigated. Sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide ($Ca(OH_2)$), ferrous sulphate ($FeSO_4$), potassium sulphate (K_2SO_4) and calcium carbonate ($CaCO_3$) were employed in different dosages to harvest microalgal biomass by sedimentation over time. Results showed that *C. vulgaris* grew without any inhibitory effect in the wastewater samples and efficiently removed N and P at 88% and 95% respectively. This growth was also detected by an observed increase in pH, and the nutrient removal was proven by a measured decrease in electrical conductivity values. Surprisingly, lower dosages of *Chlorella vulgaris* displayed a higher increase in biomass density compared to higher dosages despite both ending at similar biomass density levels. Our investigation on algae flocculation indicated that calcium hydroxide was the most effective flocculant after both 5 and 60 minutes. However, all low dosages of the flocculants in our modelling experiments were found to be insufficient to improve the normal settling rate of algal biomass. The application of $Ca(OH)_2$ as a flocculant appears to be a promising option due to its safe use and relatively low cost.

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APPENDICES

Appendix I: Measured values of Total Nitrogen and phosphorus content after 1 and 7 days.

	DAY 1						DAY 7					
	value	dilution X	TN (mg/l)	value	dilution	TP (mg/l)	value	dilution	TN (mg/l)	value	dilution	TP (mg/l)
A1	5.58	4	22.32	0.15	4	0.61						
A2	5.20	4	20.80	0.19	4	0.78						
A3	4.75	4	19.00	0.09	4	0.36						
WW1	18.30	3	54.90	1.31	3	3.93						
WW2	18.20	3	54.60	1.31	3	3.93						
WW3	12.70	4	50.80	1.09	4	4.36						
BD1-1	8.97	3	26.91	0.82	3	2.45	24.00	1	24	2.14	1	2.14
BD1-2	8.75	3	26.25	0.63	3	1.90	23.30	1	23.3	2.05	1	2.05
BD1-3	30.40	1	30.40	2.04	1	2.04	23.10	1	23.1	2.08	1	2.08
BD1-4	31.00	1	31.00	2.08	1	2.08	17.40	1	17.4	2.09	1	2.09
A1D1-1	15.40	2	30.80	1.38	2	2.76	4.74	1	4.74	0.12	1	0.118
A1D1-2	16.60	2	33.20	1.30	2	2.60	4.39	1	4.39	0.13	1	0.128
A1D1-3	17.00	2	34.00	1.47	2	2.94	4.15	1	4.15	0.14	1	0.136
A1D1-4	8.24	3	24.72	0.84	3	2.51	4.44	1	4.44	0.20	1	0.198
A2D1-1	17.20	2	34.40	1.17	2	2.34	5.66	1	5.66	0.13	1	0.13
A2D1-2	16.00	2	32.00	1.22	2	2.44	5.67	1	5.67	0.15	1	0.15
A2D1-3	17.70	2	35.40	1.24	2	2.48	5.56	1	5.56	0.17	1	0.17
A2D1-4	9.64	3	28.92	0.80	3	2.41	5.34	1	5.34	0.13	1	0.132

Value was measured in mg/L

Appendix II: Measured and calculated concentration of Total Nitrogen content after 1 and 7 days

	rep1	**rep2**	**rep3**	**rep4**	**Average**
BD day1	26.91	26.25	30.40	31.00	**28.6**
BD day7	24.00	23.30	23.10	17.40	**22.0**
A1 day1	30.80	33.20	34.00	24.72	**30.7**
A1 day7	4.74	4.39	4.15	4.44	**4.4**
A2 day1	34.40	32.00	35.40	28.92	**32.7**
A2 day7	5.66	5.67	5.56	5.34	**5.6**
Algae stock	22.32	20.80	19.00		**20.7**
WW	54.90	54.60	50.80		**53.4**

Appendix III: Measured & calculated concentration of Total Phosphorus content after 1& 7 days

	rep1	rep2	rep3	rep4	Average
BD day1	2.45	1.90	2.04	2.08	**2.1**
BD day7	2.14	2.05	2.08	2.09	**2.1**
A1 day1	2.76	2.60	2.94	2.51	**2.7**
A1 day7	0.12	0.13	0.14	0.20	**0.1**
A2 day1	2.34	2.44	2.48	2.41	**2.4**
A2 day7	0.13	0.15	0.17	0.13	**0.1**
Algae stock	0.61	0.78	0.36		**0.6**
WW	3.93	3.93	4.36		**4.1**

Appendix IV: Calculated concentrations of Total Nitrogen and Phosphorus for day 0

	N	P
BD day 0	35.62	2.72
A1 day 0	37.69	2.77
A2 day 0	42.52	2.91

Appendix V: Final concentrations of total nitrogen and phosphorus

	BD day0	BD day1	BD day7	A1 day0	A1 day1	A1 day7	A2 day0	A2 day1	A2 day7
N	35.6	28.6	22.0	37.7	30.7	4.4	42.5	32.7	5.6
P	2.7	2.1	2.1	2.8	2.7	0.1	2.9	2.4	0.1

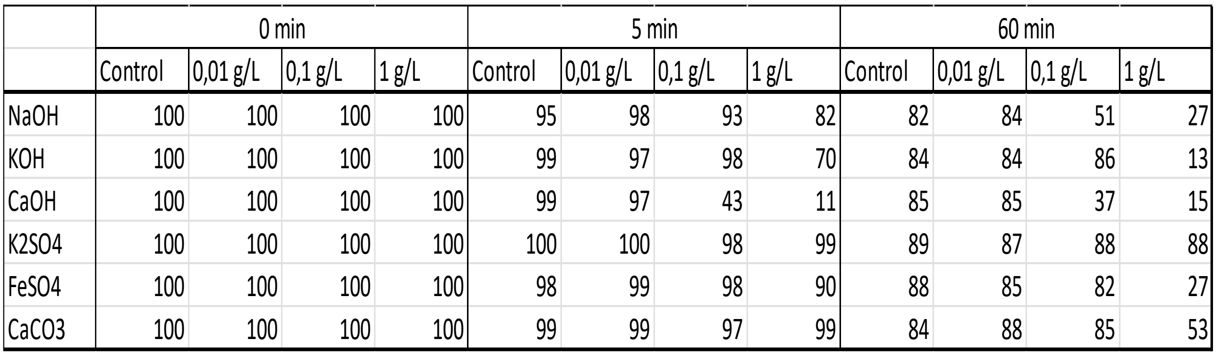
Appendix VI: Measured pH values of the culture during the 7-day culture period

Control	**rep1**	**rep2**	**rep3**	**rep4**	**Average**
Day 0	7.79	7.81	7.88	7.92	**7.9**
Day 1	8.82	8.81	8.87	8.86	**8.8**
Day 2	8.54	8.53	8.50	8.46	**8.5**
Day 3	8.79	8.78	8.82	8.75	**8.8**
Day 4	8.84	8.84	8.82	8.82	**8.8**
Day 5	8.79	8.78	8.79	8.74	**8.8**
Day 6	8.84	8.84	8.85	8.86	**8.8**
Day 7	8.85	8.84	8.81	8.83	**8.8**
Algae 1	**rep1**	**rep2**	**rep3**	**rep4**	**Average**
Day 0	7.90	7.95	7.97	7.97	**7.9**
Day 1	8.95	8.99	8.99	8.99	**9.0**
Day 2	8.74	8.53	8.82	8.75	**8.7**
Day 3	9.50	9.47	9.26	9.63	**9.5**
Day 4	9.34	9.53	9.61	9.56	**9.5**
Day 5	9.53	9.76	9.84	9.89	**9.8**
Day 6	9.53	9.77	9.91	10.46	**9.9**
Day 7	9.23	9.88	9.12	10.55	**9.7**
Algae 2	**rep1**	**rep2**	**rep3**	**rep4**	**Average**
Day 0	8.00	7.99	7.94	8.00	**8.0**
Day 1	9.00	9.12	9.00	9.06	**9.0**
Day 2	8.58	8.99	8.64	8.60	**8.7**
Day 3	9.74	9.69	9.23	9.19	**9.5**
Day 4	9.37	9.49	9.72	9.51	**9.5**
Day 5	9.62	9.32	9.33	9.73	**9.5**
Day 6	9.72	10.23	9.96	9.62	**9.9**
Day 7	9.16	9.62	10.89	9.53	**9.8**

Appendix VII: Measured EC (mg/L) values of the culture during the 7-day culture period

Control	**rep1**	**rep2**	**rep3**	**rep4**	**Average**
Day 0	1318	1319	1310	1302	**1312**
Day 1	1272	1272	1250	1250	**1261**
Day 2	1295	1289	1271	1267	**1281**
Day 3	1285	1280	1254	1248	**1267**
Day 4	1276	1262	1250	1249	**1259**
Day 5	1284	1270	1259	1259	**1268**
Day 6	1285	1258	1248	1255	**1262**
Day 7	1286	1263	1255	1261	**1266**
Algae 1	**rep1**	**rep2**	**rep3**	**rep4**	**Average**
Day 0	1333	1327	1333	1333	**1332**
Day 1	1260	1254	1254	1254	**1256**
Day 2	1228	1243	1241	1241	**1238**
Day 3	1142	1151	1176	1128	**1149**
Day 4	1113	1116	1111	1102	**1111**
Day 5	1039	1018	1012	1001	**1018**
Day 6	1045	1026	1014	984	**1017**
Day 7	1069	1027	1043	974	**1028**
Algae 2	**rep1**	**rep2**	**rep3**	**rep4**	**Average**
Day 0	1376	1376	1381	1384	**1379**
Day 1	1284	1260	1274	1278	**1274**
Day 2	1261	1227	1257	1255	**1250**
Day 3	1147	1140	1188	1194	**1167**
Day 4	1146	1129	1087	1130	**1123**
Day 5	1070	1101	1074	1056	**1075**
Day 6	1070	1026	1067	1067	**1058**
Day 7	1088	1030	1110	1077	**1076**

Appendix VIII: Measured concentration of algae remained in suspension with time expressed as percentages.



	0 min				5 min				60 min			
	Control	0,01 g/L	0,1 g/L	1 g/L	Control	0,01 g/L	0,1 g/L	1 g/L	Control	0,01 g/L	0,1 g/L	1 g/L
NaOH	100	100	100	100	95	98	93	82	82	84	51	27
KOH	100	100	100	100	99	97	98	70	84	84	86	13
CaOH	100	100	100	100	99	97	43	11	85	85	37	15
K2SO4	100	100	100	100	100	100	98	99	89	87	88	88
FeSO4	100	100	100	100	98	99	98	90	88	85	82	27
CaCO3	100	100	100	100	99	99	97	99	84	88	85	53

DECLARATION

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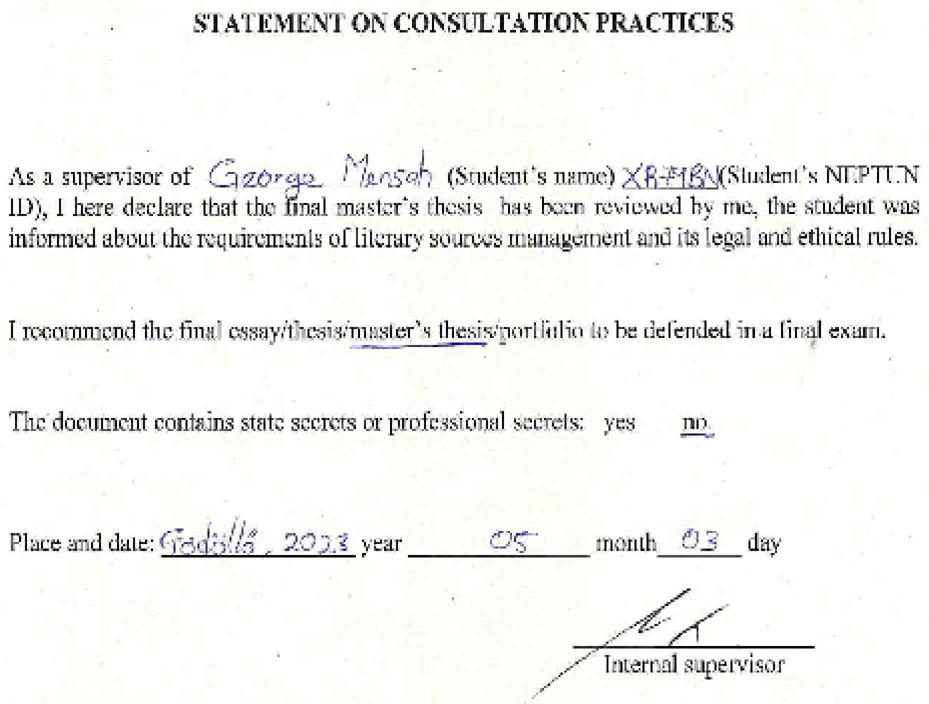

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