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Nutrient Removal by Algal Polyculture in Dairy Farm Effluent

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Abstract

Nutrient pollution from dairy farm effluent, particularly nitrogen (N) and phosphorus (P), significantly contributes to environmental degradation and eutrophication, necessitating effective bioremediation strategies. This study explores the efficacy of algae polyculture E3 in remediating dairy farm wastewater, focusing on nutrient removal and algal growth. Conducted over a 28-day period, the research compares the bioremediation capabilities of algae polyculture in autoclaved and non-autoclaved effluent samples. Findings reveal that algae polyculture demonstrates superior bioremediation performance in non-autoclaved conditions, with growth rates ranging from 8.07 to 10.13 mg L^{-1} d^{-1}. The algal polyculture achieved significant nutrient removal, eliminating 90.9% of ammonium (NH_4^+) and 99.1% of phosphate (PO_4^{3-}), highlighting its potential in mitigating nutrient pollution. The study underscores the importance of operational conditions, as non-autoclaved environments fostered more robust algal growth and nutrient uptake. Further, the research involved the optimisation of the model data, leading to improved accuracy in simulating the biological removal processes. The optimised model better reflects real-world bioremediation dynamics, facilitating more reliable predictions of algal performance and nutrient cycling. Additionally, the study identifies a 10-day hydraulic retention time (HRT) as optimal for balancing algal biomass growth with nutrient removal, addressing common challenges such as algal washout and inefficiency in nutrient uptake. In conclusion, the findings advocate for the application of algae polyculture E3 in the bioremediation of dairy farm effluent, proposing a sustainable approach to addressing the critical issue of nutrient pollution. By optimizing conditions for algal growth and nutrient removal, this research contributes to the development of more effective environmental management practices.

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List of Abbreviations

Abbreviation	
BOD	Biochemical Oxygen Demand
C	Carbon
CO_2	Carbon dioxide
COD	Chemical Oxygen Demand
DO	Dissolved oxygen
DW	Dry weight
HRAPs	High-rate algal pond systems
HRTs	Hydraulic retention time
N	Nitrogen
NH_3	Ammonia
NH_4^+	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate
O_2	Oxygen
P	Phosphorus
PBR	Photobioreactor
PO_4^{3-}	Phosphate
TKN	Total Kjeldahl Nitrogen
TN	Total nitrogen
TP	Total phosphorus
WSP	Waste stabilization ponds
WW	Wet weight

Chapter 1: Introduction

1.1 Background

The production of wastewater often arise from continuous population growth and increase in agriculture and economic activities, which pollute the natural water sources in both developing and developed countries (Corcoran, 2010). Approximately 380 trillion L y^{-1} of wastewater is produced globally as reported by Qadir et al. (2020), with Asia as the biggest producers of wastewater with roughly 159 trillion L y^{-1}, followed by Europe and North America with estimated 68 trillion L y^{-1} and 67 trillion L y^{-1} of wastewater generated, respectively. Moreover, about 80% of wastewater is released into water bodies without proper treatment, according to reports from UN-Water (2017). Likewise, over 40% of the earth are expected to suffer the water crisis by 2030, indicating the severe economic and social challenges in the approaching years (Goswami et al., 2021; Li et al., 2019).

New Zealand's economy is strongly reliant on the dairy industry, whereby the production of milk has reached over 50% in the Waikato region (Selvarajah et al., 1994). New Zealand’s dairy farm system has generated massive amount of nutrient rich wastewater, which derived from animal shelters, milking sheds, holding yards, standoff pads as well as feed pads. According to Dairynz (n.d.), the farm dairy effluent produced by dairy cows on average reaches approximately $25 worth of nutrients per annum. This equates to roughly $10,000 worth of nutrients per year for a dairy herd of 400 cows.

The primary step towards representing commitment to environmental protection and sustainability can be achieved by adequate waste and wastewater management of the dairy industry (Cruz et al., 2023). The land application of farm dairy effluent as the principal approach of farm dairy effluent management have been implemented by many countries, including New Zealand. Beside land application, the farm dairy effluent is treated via oxidation ponds (Longhurst et al., 2000). Despite the fact that the land application of farm dairy effluent offers numerous advantages, i.e. recycle of valuable nutrients, enhancement of soil structure and increase in pasture yield (Che et al., 2023; Choi, 2016), government bodies as well as environmental organizations have recently emphasized the multiple negative effects, which includes increase in the emissions of greenhouse gas and excessive nutrient loss to freshwater bodies, e.g. lakes, rivers and groundwater (National Institute of Water and Atmospheric Research, 2014).

Pollution of nutrients, particularly nitrogen (N) and phosphorus (P), on the natural ecosystems caused by the waste and wastewater from the dairy industry has become a peak concern as this often results in environmental degradation and eutrophication (Mohsenpour et al., 2021; Sand-Jensen, 2013). The TN and TP threshold concentrations at which eutrophication occurs in water range from 0.21 to 1.2 mg L^{-1} and 0.01 to 0.1 mg L^{-1}, respectively, as evaluated by Chambers et al. (2012).

Reducing the N and P levels in wastewater effluent represents an aspect that many developed and developing countries devote on focusing to enhance ecological status of their water supplies (UN-Water, 2017). Multiple mitigation approaches have been developed for removing N and P in dairy farm effluent such as constructed wetlands, woodchip bioreactors and riparian management (Milne, 2022). However, few studies in the country focus on the utilization of algae polyculture in dairy farm effluent. In recent years, several studies have documented the potential of algae polyculture in various applications such as the removal of nutrients and the production of biomass, particularly in wastewater (Gonçalves et al., 2017; Rawat et al., 2011). The algae polyculture is known to withstand the environmental changes as well as invasion by other species (Lage et al., 2021; Nalley et al., 2014). They exist either naturally in the environment or artificially engineered for a particular purpose, for instance, combining microbes which do not always coincide (Novoveská et al., 2016). It has been observed that the combination of microbes with different metabolic activities and different environment conditions requirement enhance the effectiveness of biological wastewater treatment system for nutrient removal, which indicates that the application of polyculture is therefore beneficial (Gonçalves et al., 2017; Johnson & Admassu, 2013).

Furthermore, the utilization of dairy effluent as the medium for algae polyculture cultivation could be advantageous since N and P are the two main nutrients required for the growth of the algae polyculture and provision of additional oxygen by algae polyculture into the system (Podkuiko et al., 2020). Other than N and P, various species are capable to take up other pollutants, e.g., heavy metals, toxic chemicals (Mohd Udaiyappan et al., 2017; Priya et al., 2022; Rahman et al., 2020).

To mitigate the nutrient pollution as well as to reduce the environmental impact of dairying while simultaneously improving the dairy farm system, the application of algae polyculture in dairy effluent would be a promising approach to improve farm runoff.

1.2 Problem Statement

In 2013, (Parliamentary Commissioner for the Environment) reported the definite correlative between the expansion of dairy farming and deterioration of water quality. Multiple pollutants have been identified as a severe threat to the ground water of Waikato. This includes contamination of N and P in dairy effluent. The significant loss N and P from the farm dairy effluent to watercourses mainly occurs on poorly drained soils (Mercer et al., 2011).

1.2.1 High nitrogen level in dairy farm effluent

More than 90% of the streams in New Zealand possess either high or moderate N levels particularly in intensively farmed catchments (Waikato Regional Council, n.d.-a). Furthermore, the intensive dairy activity in New Zealand has a high risk of nitrate (NO_3^-) leaching as reported by (Selvarajah et al., 1994). Due to the high N content, almost 40% of the relatively shallow ground water in Hamilton Basin is unsafe for drinking (Waikato Regional Council, n.d.-b). A considerable volume of effluent produced from the dairy farm comprises urine and faeces from cows that have been diluted with wash-down water (Che et al., 2023). Over a third of N from cow urine flows into the soil and eventually, into ground water (Waikato Regional Council, n.d.-c).

1.2.2 High phosphorus level in dairy farm effluent

The increased productive potential of land application in dairy farming has caused the increase supply of P fertilizers into dairy catchments, which later leads to higher stored P level as well as higher risks of P loss in runoff (Larned et al., 2018). Although most studies show P concentration in intensively farmed catchments do not surpass the guideline range to the extent observed for N, research on P is still relatively less in number especially in the region of Waikato (Mercer et al., 2011). Moreover, about 50 percent of the P content in Waikato river is derived from the dairy farming (Waikato Regional Council, n.d.-c).

1.3 Research Objectives

This research study aims to investigate the efficacy of algae polyculture in the bioremediation of dairy farm wastewater, focusing on the removal of nutrients. The specific objectives include:

1. To examine the operational parameters, such as temperature, pH, dissolved oxygen (DO), and turbidity, in dairy farm effluent over the cultivation period.
2. To quantify the nutrient removal efficiency and the nutrient consumption rate of ammonium (NH_4^+), NO_3^-, nitrite (NO_2^-), and phosphate (PO_4^{3-}) by the algal polyculture.
3. To examine changes in carbon levels using absorbance values at 215 nm and 245 nm, alongside protein concentration at 280 nm.
4. To determine the growth rate of the algal polyculture over 28-day cultivation period.
5. To identify algal species in the polyculture using a light microscope based on their unique characteristics.
6. To simulate and compare the model data and farm system model for optimisation of bioremediation by algal polyculture.

1.4 Thesis Structure

A literature review of algal polycultures and dairy farm effluent are presented in Chapter 2. This includes the advantages and roles of algal polycultures in nutrient removal, their mechanisms in nutrients removal and factors influencing their growth. The treatment of dairy farm effluent and other wastewaters with algal polycultures are presented in tables. The dairy farm effluent characteristics and their impacts on the environment are also described in this chapter.

Chapter 3 outlines the step-by-step experimental methodologies used to investigate the performance of nutrient removal by algal polyculture and their growth, impact of conditions on the process, while ensuring the study's goals are clearly addressed.

All experimental results are illustrated and discussed in Chapter 4 and Chapter 5, respectively.

Conclusions obtained from this research are presented in Chapter 6, along with recommendations for future work.

Chapter 2: Literature Review

2.1 Overview

This chapter presents a comprehensive review of the literature on algal polycultures, focusing on their application and significance in wastewater treatment. Section 2.2 and 2.3 delve into the general perspective on algae and microalgae, highlighting their classification and morphology and the importance of microalgae in wastewater treatment, emphasizing their role in sustainable nutrient and water management, respectively. The following sections (2.4 to 2.6) explore the algal polycultures and their advantages over monocultures, their functions in nutrients removal as well as applications in different wastewater. Section 2.7 covers the limitations and research need of algal polyculture. Various factors influencing algal growth within polycultures, such as temperature, pH, salinity, nutrient supply, and light availability are examined in Section 2.8, followed by the mechanisms involved in nutrient removal by algal polycultures, such as absorption and adsorption, which are essential for the effective wastewater treatment are discussed in Section 2.9. The different phases of microalgae growth in batch cultivation system are briefly described in Section 2.10. Impacts on water quality by excessive nutrients including the eutrophication are also studied in Section 2.11. The subsequent sections (2.12-2.13) focus on the importance and characteristics of dairy effluent and how it impacts the environment. In Section 2.14, the application of algal polyculture in dairy effluent is explored, highlighting the objectives and advantages over other types of wastewater treatment. Sections 2.15 addresses the various operating conditions such as typical effluent concentrations, flows, loading rates, algal concentrations, and others to achieve effective algal remediation. Finally, Section 2.16 provides some examples of algal remediation set ups such as shallow ponds, algal turf scrubber, raceways, and rotating drum scrubbers. Through this chapter, a comprehensive understanding of how algal polycultures contribute to environmental management, particularly in wastewater treatment, is presented.

2.2 Algae and Microalgae – General Perspective

Algae, a diverse group of aquatic plants that do not possess typical leaves, branches, or roots and their cell walls are composed of cellulose (Priyadharshini et al., 2021). There are two types of algae: macroalgae and microalgae. According to Liu et al. (2020), macroalgae are multicelled species that can reach several meters of size and can be seen by human naked eye, while microalgae are a distinct range of prokaryotic and eukaryotic cyanobacteria that are microscopic and invisible to the human eye, with maximum size of 0.2–100 µm. Lee (1989) (as cited in Umamaheswari and Shanthakumar (2016)) divided algae into a group of four: the first group was designated for prokaryotic algae, while the remaining three were categorized as eukaryotic algae and were determined by analyzing the chloroplast (Figure 2.1). This classification leads to the formation of important taxonomic groups, such as *Chlorophyta* (green algae), *Rhodophyta* (red algae), *Bacillariophyta* (diatoms), and *Chrysophyta* (golden algae). Because of its unique physical traits, *Cyanophyceae* which was previously linked to eukaryotes has been reclassified as cyanobacteria (Stengel et al., 2011).

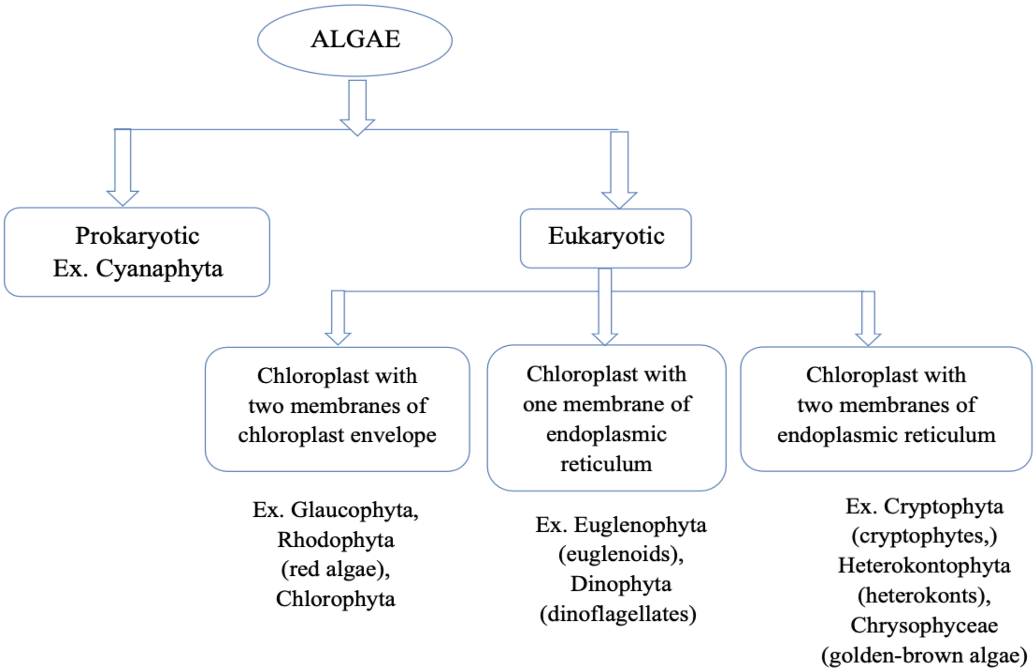


Figure 2.1: Classification of algae (Umamaheswari & Shanthakumar, 2016).

The cellular components of microalgae include nuclei, mitochondria, vacuoles, plasma membranes and chloroplast—for photosynthesis. The algal cells serve as crucial to life as they contribute the backbone of the food chain and over 50% of the entire photosynthetic activity (S. Rani et al., 2021). Their simple multicellular or unicellular structures allow them to grow in insensitive environment. Commonly found in shallow water, microalgae also grow on soil or any types of land surfaces (Umamaheswari & Shanthakumar, 2016). In addition, microalgae can be identified as autotrophic, heterotrophic and mixotrophic. Most of microalgae are autotrophic, where they require light to perform photosynthesis, carbon dioxide (CO_2), and other nutrients to grow. While heterotrophic microalgae depend on the external supply of nutrients and organic compounds to survive, mixotrophic are capable to conduct photosynthesis using organic nutrients (Mata et al., 2010).

2.3 Importance of Microalgae in Wastewater

Water is limited resource which is not easily accessible and implies a significant cost. As most algae can grow in brackish and saline waters where resources are available, wastewater is of specific interest due to its global distribution and the likelihood that it will continue to grow in tandem with the population growth, economic development as well as urbanization (Audu et al., 2018). According to Hariz et al. (2019), microalgae are potent bioremediation agents that effectively break down and eliminate contaminants from wastewater streams. Microalgae have shown to be a sustainable and environmentally friendly alternative to traditional biological treatment processes that are still widespread today, which often contribute to secondary pollution (Figure 2.2). For instance, C and N in conventional treatment methods disperse into the atmosphere as CO_2 and N gas, respectively (Abu Hasan et al., 2023). Microalgae assimilate CO_2, NH_3 and PO_4^{3-} for metabolic function and cell development (Falkowski & Raven, 2013). Consequently, this reduces the pace of the greenhouse gas emissions as most of the C and N is taken up by the microalgae rather than being transformed into toxic gases (Mohsenpour et al., 2021). Not only an efficient and affordable means for CO_2 biofixation, but microalgae treatment in wastewater is also a renewable source for biomass (Almomani et al., 2019). Furthermore, because of their transformation ability, microalgae have been widely recognized in a variety of settings, including freshwater and marine habitats (Abu Jayyab & Al-Zuhair, 2020). *Chlorella*, *Nitzschia*, and *Scenedesmus* are the most tolerant species that are found in wastewater systems (Muñoz & Guieysse, 2006).

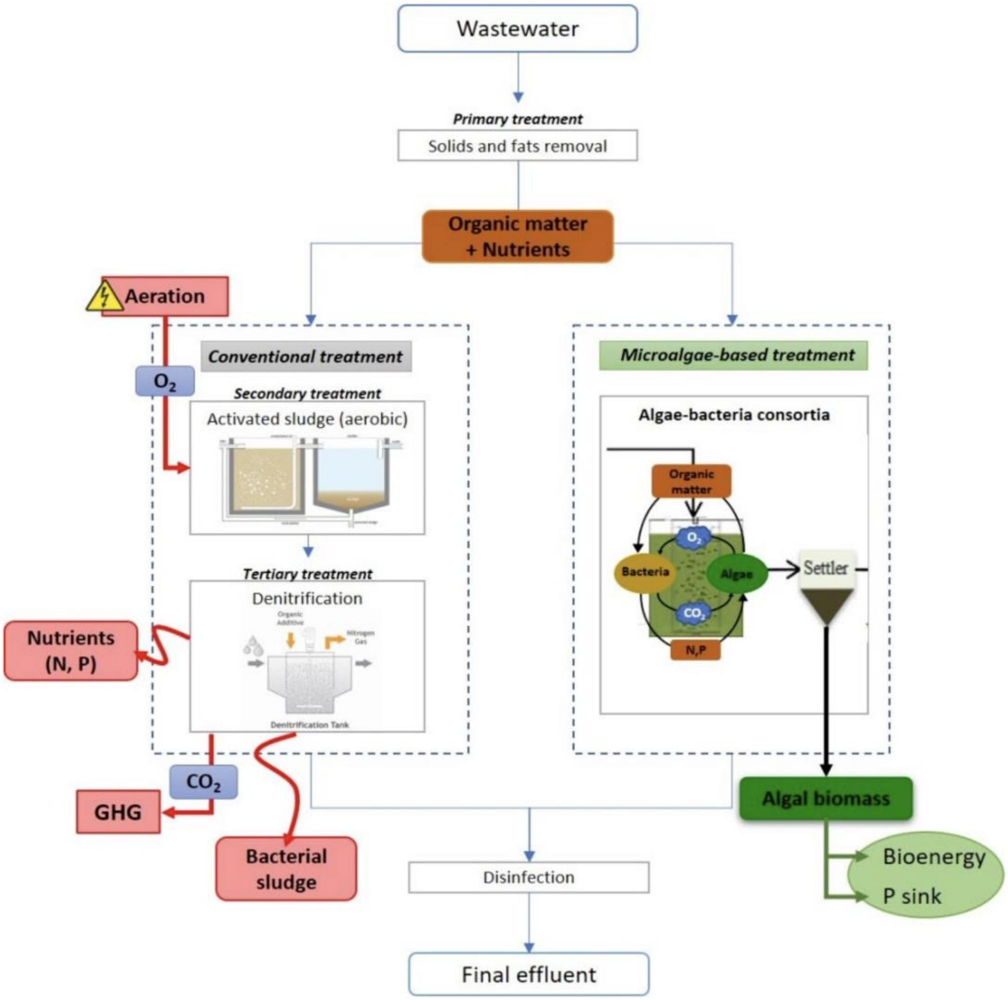


Figure 2.2: Flowchart of conventional wastewater treatment vs. by wastewater treatment-based microalgae (Ferreira et al., 2019; Morais et al., 2021).

2.4 Algal Polyculture

The species composition of algae in wastewater studied in the past has varied, ranging from strictly controlled monocultures to assembled polycultures to fully uncontrolled natural polycultures. (Chinnasamy et al., 2014; Thomas et al., 2019). Algal polycultures comprising of entirely photosynthetic algae that are prokaryotic and/or eukaryotic form biological consortia to enhance their own health and resource foraging opportunities (Mugnai et al., 2023). They can either be found naturally in the environment or are artificially engineered, i.e., combination of microbes which do not co-occur, for a specific purpose (Jagmann & Philipp, 2014).

2.4.1 Monoculture vs polyculture

While algae have been effectively used to remove nutrients from various wastewaters, maintaining an algal monoculture is relatively challenging. Wild algal strains, pathogens and grazers often contaminate monocultures through aerial colonization, which makes it difficult to cultivate monocultures especially in outdoor settings (Carney et al., 2016; Vallina et al., 2014). A number of layers of defense have been set up by the agriculture industry against invaders such as pesticides, herbicides, etc. to increase the monoculture crops yields (Newby et al., 2016). Additionally, algal crops are mostly vulnerable in aquatic systems with invading host of invertebrates and other microbes. Such interaction of concern includes competition of nutrients and allelochemicals with non-target algal strains which later depletes the production rates as well as the quality of produced biomass (Leão et al., 2012; Schmidtke et al., 2010). Invasion by algal pathogens or predation by grazers is another concern that could harm the monocultures in the span of days, reducing the production stability (Lage et al., 2021; Letcher et al., 2013). For example, open ponds that are contaminated by grazers and crop specific pathogens have lost about 30% of annual production according to (Richardson et al., 2014). Furthermore, these interactions may have an impact on the temporal consistency of the biomass production and lipid content of an algae, thus altering the stability of the algal culture (Newby et al., 2016).

In contrast to algal monoculture, algal polyculture may develop a robust system, especially in complex degradation processes as long as the algal species diversity is maintained, and sufficient nutrient are supplied (Priyadharshini et al., 2021). The algal polycultures of diversified multispecies can withstand the environmental fluctuations which result from temporal and spatial heterogeneity in resources (e.g., nutrient availability and light) (Johnson & Admassu, 2013), by forming several niches for species with various optimal nutrient and light requirement. In addition, the invasion by other species is often very selective for a particular strain or group of algae, whereby when the algal polyculture become affected by such stresses, other algal species in the assemblage can multiply instantly to take over the new niche. Numerous studies have demonstrated that species diversity in algal polyculture promote ecosystem stability and productivity, i.e., higher diversity can result in increased biomass and lipid production (Chinnasamy et al., 2014; Stockenreiter & Litchman, 2019). Several advantages and limitations of applying algal polyculture in wastewater treatment processes have been documented in numerous studies (Table 2.1).

Table 2.1: Advantages and challenges of cultivating algal polyculture in wastewater.

Advantages	Challenges
1. Able to withstand changes in the environment and invasion of other species, hence creating a robust system (Johnson & Admassu, 2013)	1. Difficulties in maintaining the polyculture in longer processes (Newby et al., 2016)
2. Able to remove multiple nutrients simultaneously hence maintaining a balanced nutrient cycle in the ecosystem (Gonçalves et al., 2017)	2. Challenges in selecting the ideal operating conditions for different species (González-Fernández et al., 2011)
3. Cooperative interaction within the polyculture substantially enhances removal efficiencies (Priyadharshini et al., 2021)	3. Increase operational costs as additional resources may be required to maintain the polyculture (Gonçalves et al., 2017)
4. Promote ecosystem stability through competition and minimization of a single species dominance (Schmidtke et al., 2010)	
5. Able to increase productivity by creating different ecological niches for different species (Chinnasamy et al., 2014)	

2.5 The Role of Algal Polyculture in Nutrients Removal

The last two decades have seen a great deal of research on the application of the algal polyculture in wastewater treatment due to their ability to remove nutrients while generating valuable biomass. In addition, wastewater treated by algal polyculture is recognized for its energy saving and less complexity as they only require a single treatment step for the removal of N and P (Gouveia et al., 2016; Sturm & Lamer, 2011). Cultivating algae polyculture may lead to both competitive and cooperative relationships (Priyadharshini et al., 2021). Cultivation of the algae polyculture may lead to the production of allelochemicals, which are secondary metabolites that have an adverse effect on the co-cultivated algae. For example, the growth of co-cultivated *Pseudokirchneriella subcapitata* was inhibited by the fatty acids chlorellin, which was secreted by C. *vulgaris* in an experiment carried out by Fergola et al. (2007). Conversely, through the exchange of metabolites, the algae polyculture has the potential to form cooperative interactions that will contribute to an overall improvement in biomass productivities and, consequently, efficiencies in the removal of nutrients (Bacellar Mendes & Vermelho, 2013).

The relationship between the diversified multispecies in algae polyculture particularly in wastewater treatment processes offer a number of benefits that include: (1) develop resistance to predators and contaminants through allelochemical excretion; (2) increase the efficiency of overall nutrient uptake, given that adequate nutrients are supplied; (3) introduce a settleable system by combination of single cell algae and other flocculating microbes, thereby eliminating the needs for harvest; (4) ensures the viability of the remediation process, whereby the loss of algae can be compensated by others in the polyculture (Renuka et al., 2013).

2.6 Application of Algal Polyculture for Nutrient Removal

The application of native and artificial algae polycultures in different type of wastewaters for the removal of nutrients has been documented in several research as shown in Table 2.2. Molinuevo-Salces et al. (2019) referred that algal polyculture that grows in the wastewater have a higher pollution tolerance level. For example, removal efficiencies between 96.6-99.8% of NO3-N and PO4-P were achieved by native algae polyculture from a carpet mill industry wastewater (Chinnasamy et al., 2010). Likewise, an experiment conducted by Koreivienė et al. (2014) showed high total nitrogen (TN) and total phosphorus (TP) removal efficiencies, with 88.6–96.4% and 99.7–99.9%, respectively, by non-native algae polyculture composed of *Chlorella* sp. and *Scenedesmus* sp. Furthermore, the removal rates of TN and TP usually range from 22-100% and 20-98% of the initial TN and TP in the effluent, respectively (Molinuevo-Salces et al., 2019).

Table 2.2: List of algal polyculture cultivation in wastewater for nutrient removal.

Algae species	**Type of wastewater**	**Nutrient Removal**	**Reference**
Scenedesmus sp. *Chlorococcum* sp. *Chlorella* sp. *Phaeodactylum tricornutum*	Simulated municipal wastewater	>80% TP	(Johnson & Admassu, 2013)
Spirulina platensis Mixed algal culture	Municipal wastewater	97.2% COD 99.6% TIN 99.41% TP	(Almomani et al., 2019)
Chlorella sp. *Calothrix* sp. *Lyngbya* sp. *Ulothrix* sp.	Primary treated sewage water	97.8 PO_4-P 90% NO_3-N	(Renuka et al., 2013)
Chlorella sp. *Arthrospira platensis* *Raphidocelis subcapitata*	Municipal wastewater + CO_2	97.94% $NO_2 + NO_3$ 83.43% PO_4^{3-}	(Podkuiko et al., 2020)
15 Native algal polyculture	Carpet mill wastewater^{-}	99.7–99.8% NO_3-N 98.8–99.1% PO_4-P	(Chinnasamy et al., 2010)
Desmodesmus communis Native algal polyculture	Domestic wastewater	~100% NH_3 ~100% PO_4^{3-}	(Samorì et al., 2013)

Tetraselmis sp. Native algal polyculture	Tannery wastewater	14.26-51.02% COD 99.90% NH_3-N 87.8-91.75% PO_4-P	(Pena et al., 2019)
Chlorella sp. *Scenedesmus* sp. *Chroococcus* sp. *Oscillatoria* sp. *Melosira* sp.	Wastewater treatment plant effluent	>99% PO_4^{3-} 61-79% NO_3-N	(Hu et al., 2017)
Chlorella sp. *Scenedesmus* sp. *Nannochloropsis* sp.	Saline municipal wastewater	100% NO_3-N 100% PO_4-P	(Fallahi et al., 2020)

2.7 Practical Limitations of Algal Polyculture and Research Needs

While cultivating algal polyculture have many advantages, they also pose several limitations especially in the real conditions. For example, algal polyculture do not grow as effectively under defined and stable conditions as a selected monoculture, hence the cultivation of the polyculture may require additional equipment such as a heater, artificial lighting, etc. to obtain favorable conditions (Johnson & Admassu, 2013). Supplementary steps such as pretreatments to regulate pH of the wastewater may be required, which increases the cost of the entire operation (Gonçalves et al., 2017). Moreover, the characteristic of the wastewater is crucial to an effective treatment, whereby the performance of polyculture-based systems may operate poorly due to presence of toxic compounds or a lack of essential nutrients (Mugnai et al., 2023). Inadequate selection of the different species in the polyculture may lead to problems to adjust the performance of the algal polyculture in continuous treatment of different pollutants as the efficiency varies according to the nutrients to be removed (Gupta et al., 2019; Nagarajan et al., 2020).

For these reasons, several improvements were described as the following: (i) further understanding on the impacts of the environmental conditions on the polyculture; (ii) operating the extensive outdoor experiments; (iii) comprehending of the interactions between the multispecies of the polyculture; and (iv) implementing of correct mathematical models expressing the behaviour of the polyculture to determine the process design (Bordel et al., 2009; Fouilland, 2012). Additionally, reintroducing or eliminating of algae in the engineering of polyculture are required and comprehensive monitoring of the tasks established within the polyculture. Functional genomics, metabolic profiling, and combinatorial biochemical approaches can be applied for the development of the algal polyculture exhibiting high pollutants removal capability (Gonçalves et al., 2017).

2.8 Factors Influencing Algal Polyculture Growth

The growth of algal polyculture can be affected by multiple factors, i.e., biotic, and abiotic. Examples of abiotic factors are temperature, pH, salinity, light, DO level, qualitative and quantitative profiles of nutrients, as well as the presence of toxic compounds. In contrast, biotic factors include the presence of pathogens, e.g., bacteria, fungi, and viruses, as well as the competition between algae species. Moreover, light exposure and the availability of CO_2 are often controlled by operational parameters such as gas transfer, mixing, hydraulic residence time and harvesting rates, which have an impact on the algal polyculture growth (Gonçalves et al., 2017).

2.8.1 Temperature

Temperature is another factor that has a significant impact on the algal polyculture growth. The optimum temperature as well as the upper and lower temperature limits, which results in the maximum growth of the algal polyculture are used to characterize the algae species (Eppley, 1977). Often, the general trend shows the higher the optimum temperature, the higher will be the maximum specific growth rate. Barati et al. (2018) observed that the optimum temperature required for microalgae growth was in the ranges of 18–24 °C. However, above the optimum range might promote oxidative stress and reduce the photosynthetic activity of the polyculture (Posadas et al., 2017). Lower temperatures may also restrict the growth of polyculture (Xin et al., 2011). Therefore, algal polyculture should ideally be kept at temperatures that are similar to what was observed in the environments from where they were collected (Gonçalves et al., 2017). In addition, temperature tends to shift slightly as other factors (e.g., salinity) are varied, but they are nevertheless very useful values.

2.8.2 pH

Most algae species grow between pH 7 to 9 (Kumar et al., 2010; Mohsenpour et al., 2021). Algal polycultures must be kept within an ideal pH range to prevent culture loss due to excessive pH values since the pH of the culture medium is what causes physiological changes in the algae polyculture. The pH in the culture medium can be linked to the supplied CO_2 on account of the chemical equilibrium that have been formed (Kumar et al., 2010). The pH in the culture decreases as CO_2 level in gaseous input stream increases. In order to prevent the algal polyculture cells from being damaged by a drastic drop of pH, the amount of CO_2 supplied to the algal polyculture needs to be carefully controlled (Mohsenpour et al., 2021). Hence, daily monitoring of pH in the culture medium is crucial to obtain optimal pH range for the growth of algal polyculture.

2.8.3 Salinity

Growth responses to salinity have been investigated in numerous algae species, whereby the optimal salinity levels vary depending on the type of algae (Nagarajan et al., 2020). Similar to other factors, changes in the salinity of the culture medium can negatively impact the growth and composition of the algae polyculture because of the following: (i) osmotic stress; (ii) changes in the permeability of membrane to ions; and (iii) ion (salt) stress (Glass, 1983). The two primary contributors influencing the changes in the salinity of the culture medium are rainfall and evaporation loss. García-González et al. (2003) have discussed extensively about the detrimental effects of these changes on the algae growth. The addition of salt or freshwater can be added to regulate and maintain the salinity levels in the culture medium (Mata et al., 2010).

2.8.4 Nutrient supply

Inorganic C is considered as the primary nutrient needed for the growth of autotrophic algae. N and P are also necessary for the algae growth, which are required to produce nucleic acids and proteins. The growth rates as well as the biomass productivities are decreased when their availability is at limiting concentrations. The common N form required by majority of the algae species are in the form of urea, NH_4-N and NH_3-N. Since not all phosphorus compounds are bioavailable for the algae polyculture, this nutrient should be provided in great quantities as soluble PO_4^{3-} (Kumar et al., 2010). Aside from these nutrients, referred to as macronutrients, trace elements – particularly metals, e.g., Mg, Ca, Zn, are still required for cell metabolism and enzymatic activity of the algal polyculture (Priya et al., 2022). Furthermore, vitamin supplementation has been reported to enhance the growth of various algae (Kumar et al., 2010).

2.8.5 Light

Algal polyculture that are photoautotrophic rely on light for growth considering light is the energy source that converts CO_2 into organic C. Photosynthetic activity is proportional to light irradiance, i.e., the algal polyculture growth increases when the light irradiance increases until optimum value is reached. The optimum light intensity required for microalgae growth as observed by Barati et al. (2018) was in the ranges of 80–200 µmol $m^{-2}s^{-1}$. Moreover, according to Acién Fernández et al. (2018), photosynthetic activity reaches saturation at comparatively low irradiances of between 100 and 200 $\mu E/m^2$ day. Nevertheless, depending on the species of the algae polyculture as well as other culturing conditions, high light irradiance may damage the photosynthetic receptor system, inhibiting photosynthesis and the growth of algal polyculture (Park et al., 2011).

2.8.6 Operational factors

Selection of a suitable method of operation is reliant upon the size and the cultivation system. An equal distribution of nutrients and light among the algal cells can be obtained by mixing, which is one of the significant growth parameters in algal polyculture. This provides turbulence and improves the gas exchange between the air and culture medium as well as prevents the occurrence of stagnant zones (Kumar et al., 2010). The air bubbling into the culture media provides CO_2 for photosynthesis and removes the produced oxygen. Vigorous mixing, however, may cause cell rupture and shear stress, which are detrimental to the growth of algal polyculture (Molinuevo-Salces et al., 2019). Additionally, not only the mixing of algal polycultures is important in preventing the settling of algae, but also preclude thermal stratification (Eriksen, 2008; Kumar et al., 2010).

2.9 Mechanisms Involved in Nutrients Removal by Algal Polyculture

Understanding the mechanisms of algal nutrient uptake is crucial to optimise the removal of nutrients from wastewater streams by different species of algae. Metabolic processes of the algal cell can be broadly classified through elemental composition. For algae to thrive, the supply of trace elements as well as ionic components (potassium, sodium, iron, calcium, magnesium) are required in addition to the basic elements such as C, N, P, and sulfur. Both N and P are often highlighted as they can be considerably increased by the anthropogenic waste, which leads to nutrient runoff that can promote eutrophication (Cai et al., 2013). The mechanisms involved in removal of C, N and P by algae are summarized and depicted in Table 2.3 and Figure 2.3, respectively.

Table 2.3: Mechanisms of carbon, nitrogen and phosphorus removal by microalgae (Gonçalves et al., 2017).

Nutrients	Mechanisms	Cell incorporation
Carbon		
CO_2	Calvin cycle	Diffusion or active transport
Organic carbon	Respiration metabolism	Diffusion or active transport
Nitrogen		
N_2	Fixed into NH_3, then converted into amino acids	-
NO_3^- ,NO_2^-	Reduced into NH_4^+, then converted into amino acids	Active transport
NH_4^+	Direct conversion into amino acids or stripping	Active transport
Phosphorus		
PO_4^{3-}	Phosphorylation or chemical precipitation	Active transport

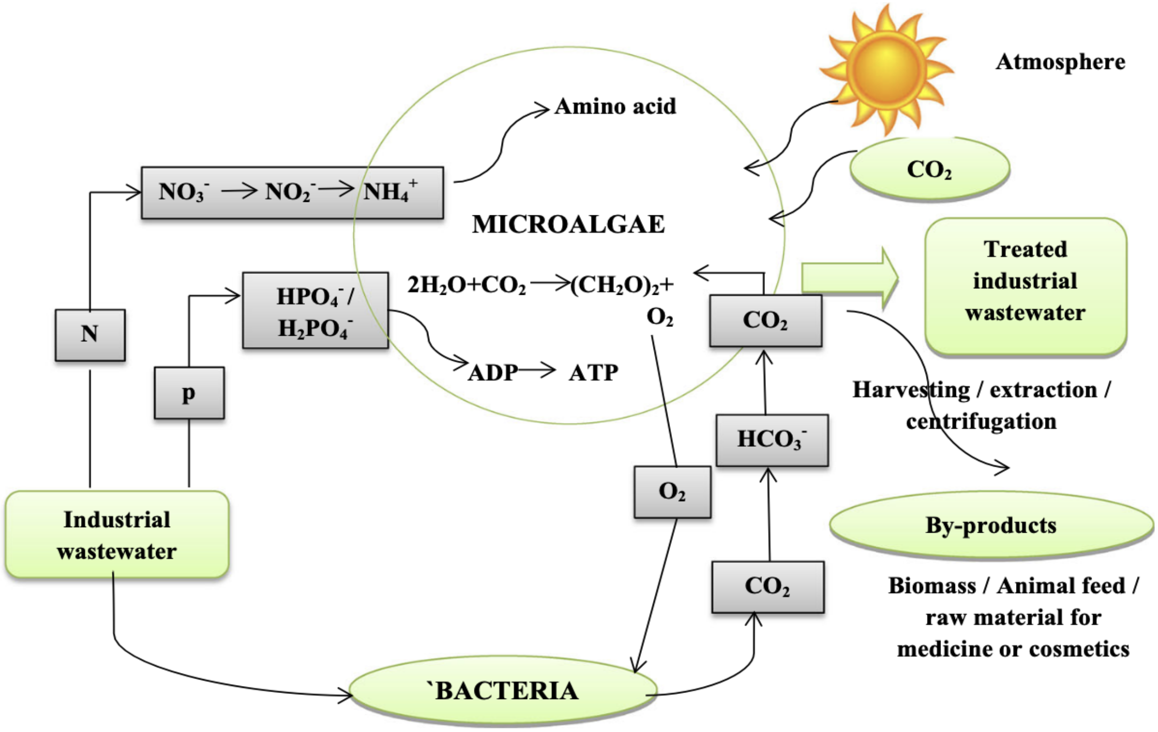


Figure 2.3: Nutrient uptake mechanism of microalgae in wastewater (Umamaheswari & Shanthakumar, 2016).

2.9.1 Carbon

Carbon, in the form of CO_2 from the atmosphere or industrial gas emissions can be fixed by autotrophic algae via photosynthesis. Furthermore, algae are capable to take up soluble carbonate as a source of CO_2. The uptake of CO_2 at low pH (pH 5 to 7) occurs through diffusion. At pH values greater than 7, bicarbonate (HCO^{-}_3) facilitates the active transport of the C source into algal cells through carboanhydrase activity (Cai et al., 2013; Gonçalves et al., 2017; Sayre, 2010). Once within the cells, HCO^{-}_3 is converted into CO^{-}_2 that is fixed by rubisco (ribulose biphosphate carboxylase oxygenase) to generate two 3-phosphoglycerate molecules (Sayre, 2010). While most of algae are autotrophic, heterotrophic algae use organic C such as acetate, ethanol, glucose, and glycerol as C source and mixotrophic algae use an organic C source in addition to CO^{-}_2 (Cai et al., 2013).

2.9.2 Nitrogen

Nitrogen, mainly as molecular N (N_2) constitutes roughly 78% of the Earth's atmosphere. The conversion of N into numerous chemical forms along the atmosphere, terrestrial as well as aquatic ecosystems is known as the N cycle (Gruber, 2008). N cycle is apparently the most fascinating and complex among each of biogeochemical cycles that occur in water. Fixation, ammonification, nitrification as well as denitrification are all important processes in the N cycle.

Algae play a significant role in N fixation as well as assimilation. An abundant amount of N in the form of NH_4^+, NO_2^- and NO_3^- as well as organic sources (e.g., urea) can be found in dairy effluent. N fixation is predominantly performed by prokaryotic algae (cyanobacteria) as they are incapable of utilizing N_2 and converting it into ammonia (NH_3) (Eq. (1)), which later can either be released to the environment or incorporated into proteins or amino acids (Cai et al., 2013; Gruber, 2008).

$$\mathrm{N_2 + 8H^+ + 8e^- + 16ATP \xrightarrow{\text{Nitrogenase}} 2NH_3 + H_2 + 16ADP + \ 16P_i} \qquad (1)$$

The fixed N such as NH_4^+, NO_2^- and NO_3^- are consecutively assimilated by all eukaryotic algae through the active transport across their cell membrane (Gupta et al., 2019). Between 2 to 10 tons of N ha^{-1} $year^{-1}$ are assimilated by algae when the irradiation is low, while 5 to 25 tons of N ha^{-1} $year^{-1}$ in high irradiation condition as examined by (Acién Fernández et al., 2018). The two-step process of NO_2^- and NO_3^- reduction to NH_4^+ are catalyzed by the enzymes nitrate reductase (in cytosol) and nitrite reductase (in chloroplast), respectively (Gonçalves et al., 2017). In the first step, two electrons are transferred using nicotinamide adenine dinucleotide phosphate (NADPH) in the reduction of NO_3^- into NO_2^- catalyzed by nitrate reductase enzyme (Eq. (2)). Subsequently, in the reduction of NO_2^- into NH_4^+ by nitrite reductase, 6 electrons are transferred using ferredoxin as the only electron donor (Eq. (3)) (Becker, 1994).

$$\mathrm{NO_3{}^- + 2H^+ + 2e^- \xrightarrow{\text{Nitrate reductase}} NO_2{}^- + \ H_2O} \qquad (2)$$

$$\mathrm{NO_2{}^- + 8H^+ + 6e^- \xrightarrow{\text{Nitrite reductase}} NH_4{}^+ + \ 2H_2O} \qquad (3)$$

Incorporated actively into the cells of algae, the resulting NH_4^+ is ultimately converted into amino acids through glutamine synthetase-glutamate synthase pathway (Gupta et al., 2019). The production of glutamine from glutamate and adenosine triphosphate (ATP) is catalyzed by the enzyme glutamine synthase (Eq. (4)).

$$Glutamate + \mathrm{NH_4}^+ + \mathrm{ATP} \xrightarrow{Glutamine\ systhase} Glutamine + ADP + \mathrm{P_i} \tag{4}$$

As no redox reaction is involved in NH_4^+ assimilation, NH_4^+ is assumed to be the ideal and economically favored nitrogen form for algae (Becker, 1994). NH_4^+ tends to be favored by algae over NO_3^- as NO_3^- consumption does not happen until NH_4^+ is almost entirely consumed according to multiple studies, which also indicates that high NH_4^+ in effluents induce the rapid growth of algae. According to (Nagarajan et al., 2020), algae have the capacity to remove 100% of NH_4^+ in contrast to other nitrogen form such as NO_3^-, NO_2^- and TN. Excessive NH_4^+ level can be repressive, whereby different algae species can only tolerate NH_4^+ from 25 to 1000 mmol NH_4^+ L^1 (Becker, 1994; Collos & Berges, 2002). On the other hand, known for its thermodynamically stable as well as highly oxidized properties than other form of inorganic nitrogen, NO_3^- are often present in aquatic environments and can also be essential for algae as it activates the enzyme nitrate reductase (Abu Hasan et al., 2023).

Not only is NH_4^+ are removed by direct assimilation, but also through a process known as ammonia stripping, which high temperature and pH are applied to remove NH_4^+ though volatilization of NH_4^+ in large amount (Cai et al., 2013).

2.9.3 Phosphorus

P is another significant nutrient for the cell growth and metabolism of algae due to its presence in energy molecules, polysaccharides, lipids as well as nucleotides (Abu Hasan et al., 2023). Mono-hydrogen phosphate (HPO_4^{2-}), di-hydrogen phosphate ($H_2PO_4^-$), organic phosphate and polyphosphate are the most abundant forms of P present in wastewater (Gupta et al., 2019). The assimilation of organic and inorganic P by algae takes place mainly after mineralization by phosphatase enzyme and formation of orthophosphate (Markou et al., 2014). HPO_4^{2-} and $H_2PO_4^-$, the most readily forms of inorganic P, enter the algal cells via active transport through symporter channel using the driving force of hydrogen ions or sodium ions across the plasma membrane (Gonçalves et al., 2017; Mohsenpour et al., 2021). Later, these phosphates incorporate into organic components such as phospholipids, protein and nucleic acids by the process of phosphorylation (Gupta et al., 2019). This process includes a substrate level phosphorylation, oxidative phosphorylation, and photophosphorylation, which involves the synthesis of ATP from adenosine diphosphate (ADP), along with an energy input coming acquired through oxidation of respiratory substrates, mitochondrial electron transport chain and light energy transformation, respectively (Cai et al., 2013; Gonçalves et al., 2017). The general equation of the reaction is shown below (Eq. (5)).

$$\mathrm{ADP} + \mathrm{P_i} \xrightarrow{\text{Energy}} \mathrm{ATP} \tag{5}$$

Furthermore, wastewater with higher level of PO_4^{3-} cause the excess PO_4^{3-} to be stored as acid-insoluble polyphosphate granules in algae cells, is yet another approach of P removal from wastewater and the mechanism is termed as ‘luxury uptake’ (Abu Hasan et al., 2023; Mohsenpour et al., 2021). Later, the stored PO_4^{3-} are taken up by algae when the external supply of P is restricted (Umamaheswari & Shanthakumar, 2016). In addition, it was reported that several algae species are able to utilize P in organic ester (Cai et al., 2013).

Similar to N removal, P are removed by external factors such as pH and DO. High DO and pH values (pH >8.0) will result in P precipitation from the medium as PO_4^{3-} do not exist in gaseous state (Cai et al., 2013).

2.9.4 Other nutrients

Micronutrients, e.g., iron and silicon, may have an impact on the abundance of phytoplankton communities although N and P are the main nutrients of concern in eutrophication. For example, silicon is present in cell walls of most algae which is an essential nutrient for their growth and production (Grobbelaar, 2004). Nevertheless, these micronutrients only become toxic to algal species at high concentrations. Additionally, some of them can alter and reduce the availability of other essential elements by precipitation (Hecky & Kilham, 1988). On the other hand, it has been shown that certain algal strains have a higher tolerance of heavy metals and that heavy metals must be in proper chemical form for algae to absorb them (Eppley, 1977; Mehta & Gaur, 2005). Moreover, a marked growth response in algae has been observed in several enrichment experiments to the addition of micronutrients (Eppley, 1977).

2.10 Algal Growth Rate

Like the growth of other microbes, the six distinct phases of microalgae growth in batch cultivation system are as follows: (1) lag phase, (2) exponential phase, (3) linear phase, (4) declining phase, (5) stationary phase, as well as (6) death phase as illustrated in Figure 2.4 (Grobbelaar, 2004; Lee et al., 2015; Málek & Ricica, 1966; Mata et al., 2010). Growth is delayed in the lag phase by the presence of dead cells or the internal adaptation to new surroundings. In the exponential phase, the cells grow and constantly divide at an exponential rate, whereby nutrients and light intensity do not restrict their growth. Subsequently, microalgae biomass builds up at a constant rate during the linear growth phase until nutrients become the limiting factors. As the light becomes a limiting factor, the dividing cells start to slow down. Declining phase occurs when the cell division rate decreases, followed by the stationary phase, when the growth rate drops to zero as the nutrients run out in the culture media and the storage C products and lipids accumulate. The death phase, also known as the crash phase, is characterized by a drastic decline in the concentration of microalgae cells because of contamination, overheating, pH disruption, and nutritional depletion.

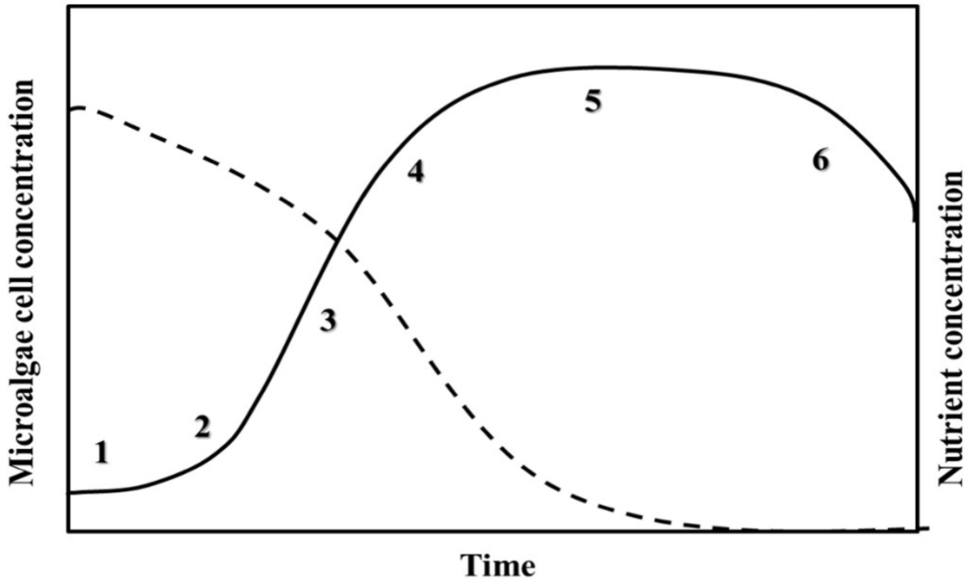


Figure 2.4: Representation of algae growth rate in batch culture (solid line) and nutrients concentration (dashed line) (Grobbelaar, 2004; Lee et al., 2015; Mata et al., 2010).

Growth kinetic models are crucial particularly in process model simulating microalgae cultivation systems as they provide a better understanding of the algal growth and their optimisation of cultivation conditions. As reviewed by Lee et al. (2015), existing microalgae growth models were categorized into three groups which are expressed as a function of: (1) a single substrate factor (CO_2, N and P); (2) a light factor; and (3) multiple factors including both substrate and environment. Several researchers have employed the Monod model to explain the relationship between a single nutrient concentration and the growth of microalgae due to its straightforward formula (Aslan & Kapdan, 2006; Hsueh et al., 2009; Xin et al., 2010). For example, the growth of *Chlorella vulgaris* was restricted when N and P concentrations were below 31.5 mg N L^{-1} and 10.5 mg P L^{-1}, respectively as observed by (Aslan & Kapdan, 2006) using Monod model.

Up to this day, the study of existing models particularly in the cultivation of algal polyculture composed of different species are still limited. This is due to complexity of polyculture system and unable to estimate the biomass concentration and specific growth rate. On the other hand, the utilization of polyculture growth rate from native species makes it possible to predict the microalgae production in the polyculture system D. S. Rani et al. (2021).

2.11 How Nutrients Affect Water Quality

According to Parliamentary Commissioner for the Environment (2013), nutrients can affect the water quality in two ways. The first impact is NO_3^- and NH_3 toxicity. Excess amount of N, especially NO_3^-, can harm animals and humans consuming the water, as well as kill vulnerable organisms (Bhandari et al., 2023). As the NO_3^- interferes the blood carrying oxygen in the body, a disease called 'blue baby syndrome' are common in bottle-fed infants. However, this illness is easily curable once it is identified (Farley, 2012). In addition, excess amount of NH_3 or NO_3^- particularly in dairy shed or raw sewage effluent appears to be highly toxic to aquatic life. The significantly more prevalent and widespread impact of nutrient pollution is the excessive growth of unwanted plants (e.g., slimes, choking weeds and algae), which occurs when N concentrations are low. These unwanted plants cause depletion of oxygen level in water, deteriorating areas used for fishing and swimming, often to the point of suffocating fish and other aquatic organisms.

Many of these plants are indigenous and component of natural stream ecosystems, which can be classified into four groups: (1) Periphyton, often forms thick slime layers on submerged logs and stones and are visible in shallow water throughout the summer; (2) Macrophytes, larger plants usually grow in deeper water, rooted in the sediment and grow upward toward the sun; (3) Phytoplankton, tiny plants that float on water and multiply rapidly covering enormous water surfaces with bright green algal blooms; (4) Cyanobacteria, typically known as blue-green algae although they are not actually algae and can vary in color, including red or dark brown. Moreover, they are considerably more likely to produce toxic blooms. In lakes, they usually float while in rivers, they are part of the periphyton (Figure 2.5). Excess of these plants will alter the composition of the basis of the food chain in water bodies, which later affect other species such as fish and waterbirds.

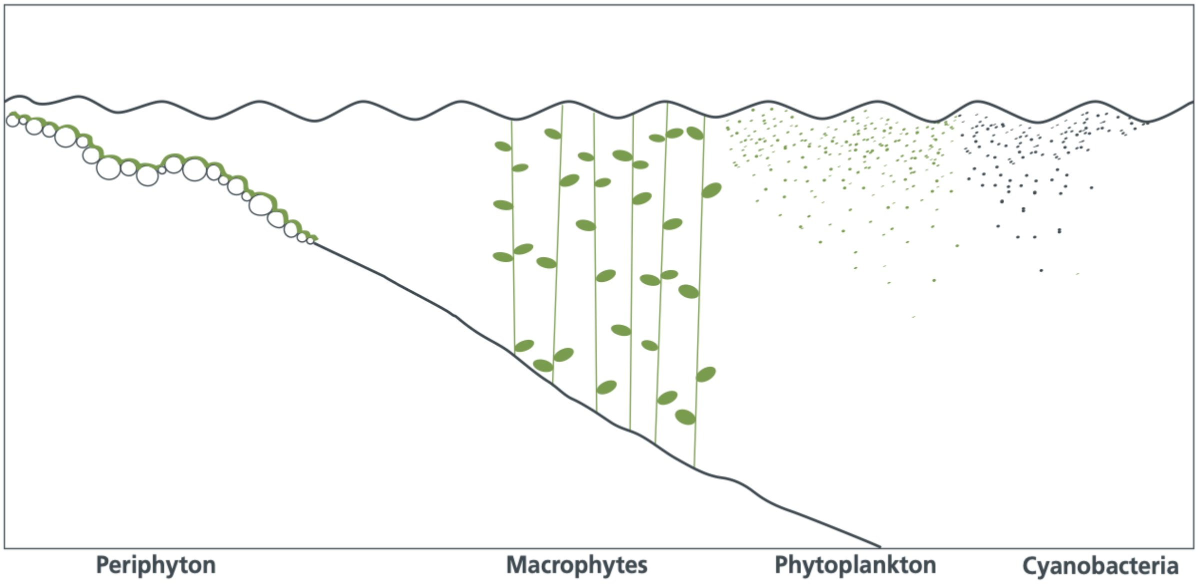


Figure 2.5: Native plants found in natural stream from impact of nutrient pollution (Parliamentary Commissioner for the Environment, 2013).

2.11.1 Eutrophication

The degradation of the water quality caused by harmful algal blooms (cyanobacteria) due to excessive nutrients such as N and P, known as eutrophication, is emerging as a severe environmental challenge in nutrient-rich water bodies, which needs immediate action to prevent further disparaging impacts. These harmful algal blooms are toxic to not only aquatic life, but also human beings and are hindering the water consumption for both recreational and commercial purposes (Bhandari et al., 2023). According to International Food Policy Research Institute (IFPRI) and VEOLIA (2015), excess N and P through 2050 are predicted to expose one-sixth and one-fourth of the estimated world population to poor water quality, respectively. Hence, causing nutrient pollution as one of the most serious water quality threats. Moreover, both N and P loadings have escalated, mainly from agricultural fertilizers (Steffen et al., 2015).

As a result of nutrient enrichment, a global trend of increased algal bloom risks was reported, by which several lakes across the globe have been entirely covered with immense cyanobacterial blooms, especially in the developing areas such as Asia and Africa (Suresh et al., 2023). Areas with rapidly rising population, high food demand and inadequate sanitation infrastructure are mostly susceptible to the harmful effects of algal blooms (Suresh et al., 2023). For example, the severe algal blooms in one of the China's freshwater lakes, Lake Taihu, left nearly two million of the population without access to drinking water for at least a couple of weeks (Qin et al., 2010; Wang et al., 2019). Similarly, of the five North American Great Lakes, Lake Erie, was the most severely affected by significant *microcystis* (blue-green algae) cyanobacterial blooms, which forced the city of Toledo, Ohio, to shutdown its water supply for a period of three days (Mohamed et al., 2019; Watson et al., 2016). In addition, the eutrophication in Lake Victoria of east Africa resulted local people to face higher risks of food and water safety as well as health-related issues, which have been rising steadily the last several decades (Kabenge et al., 2016; Njagi et al., 2022).

2.12 Dairy Effluent

Dairy companies represent one of the most important industries in the food sector and are one of the major water consumers as well as wastewater producers (Ma et al., 2023). The dairy industry in New Zealand has been exporting dairy products to over 140 markets worldwide for over the past 170 years, establishing a reputation as a reliable source of sustainable and safe dairy products. China, Australia, the United States, Japan and Malaysia are the top five dairy importers in 2019 (Dairy Companies Association of New Zealand, n.d.). The production of dairy wastewater has increased because of the immense growth of the dairy industries and a constant increase in the demand for milk and milk-based products (Gramegna et al., 2020). According to (Hena et al., 2015), the dairy farm produced nearly 182.5 million L effluent annually. Wastewater produced from dairy farm is mostly from cleaning and sterilization of the facilities, which consists of milk spillages, milking parlors and dairy wash water, soiled yards runoff, silage effluent and leachate from manure piles (Birwal et al., 2017; Kaur, 2021).

2.12.1 Characteristics of dairy effluent

Different types of wastewaters are distinct in their physical and chemical properties. In general, dairy farm effluent is characterized by high pH (4.5 to 9.4) and various compounds, such as fats, lactose, sucrose, proteins, TN, TP, and residues of detergents including other disinfectant chemicals (Gogoi et al., 2021; Goswami et al., 2021). Dairy wastewater is often white or yellowish brown, green in color with unpleasant odor and turbidity. Temperature of the effluent varies between 22-25 °C in summer and 17-18 °C in winter (Slavov, 2017). In addition, organic waste in the dairy effluent poses a severe environmental risk, owing to high levels of chemical oxygen demand (COD) and biological oxygen demand (BOD) with values varying from 1000 to 4500 mg L^{-1} and 500 to 3000 mg L^{-1}, respectively, and concerns associated with rapid putrefaction (Beevi & Sukumaran, 2014; Pilli et al., 2022). Along with these, dairy effluent also contains low amount of vitamins, organic acids, e.g. citric or lactic acid, and urea or uric acid (Brar et al., 2019). Nevertheless, the content of the effluents varies depending on the season along with the cattle farm (Labbé et al., 2017).

2.13 Effects of Dairy Effluent on the Environment

The main environmental problems related to the dairy industry include the pollution of water, land, air as well as biodiversity. As one of the most polluting industries in terms of both characteristics and volume of the effluent generated, it has been reported that wastes produced from the dairy industry are released into nearby water bodies or land without any prior treatment. This pollutes the ecosystem, which denote a serious threat towards human health and aquatic systems. As mentioned by Shete and Shinkar (2013), the production of dairy effluents is sporadic, and their flow rates vary greatly. Rapid decomposition and depletion of the DO in the receiving streams will instantly cause anaerobic conditions and release unpleasant odor due to nuisance conditions. Consequently, the receiving water turns into a breeding ground for flies and mosquitoes, which can spread diseases such as malaria, dengue fever and others. Furthermore, high dairy waste concentration often leads to the growth of unwanted and harmful microbes that deplete the oxygen level in the water and eventually causing the disappearance of fish, including flora and fauna.

Moreover, dairy farm effluent application to soils is a frequent process of waste treatment in dairy industry. Release of N, as NO_3^- form, into groundwater may happen at certain irrigation points, which commonly lead to NO_3^- pollution that are subsequently serve as sources of water for people and livestock. Under these circumstances, it is common practice to follow standard guidelines of drinking water. On the other hand, due to PO_4^{3-} are immobilized in soil with excellent retention capabilities, phosphorus often does not leach into groundwater.

CO_2, methane, and nitrous oxide from dairy effluent treatment systems contribute to significant greenhouse gases, which the effects of their emissions to the atmosphere will certainly have to be addressed in future generations (Shete & Shinkar, 2013). Moreover, high emission of particulate materials into the atmosphere may result in formation of dust, which could be corrosive to buildings and cause visual pollution. Numerous waste treatment plants have been reported to generate objectionable odors, hence need to be considered at the treatment site to lower the detrimental effects to the atmosphere.

2.14 Treatment of Dairy Effluent by Algal Polyculture

Specialized treatments of dairy effluent are essential to prevent eutrophication of receiving surface waters and groundwater as well as to lessen the impact of effluent addition to land, which the latter can result in soil structure degradation, waterlogging, salinization, chemical pollution and/or erosion (Labbé et al., 2017). Today, numerous processes have been established for the efficient treatment of dairy effluents, including algae cultivation (Goswami et al., 2021).

Birwal et al. (2017) specified the objectives of the dairy effluent treatment such as (1) lower the organic matter levels in the wastewater; (2) reduce or eliminate nutrients triggering pollution of receiving surface waters and groundwater; (3) eliminate or inactivate pathogenic microorganisms; and (4) prevent or mitigate environmental issues concerning the high biodegradable organic compounds, which has the potential to enhance the complexity of the treatment process.

Excess nutrient loads are the major limitation which occurs during the treatment process of dairy effluents. Besides, the high-cost multistep treatment process is mainly not economically feasible for dairy industries (Goswami et al., 2021). Additionally, preparing culture media could constitute for up to 35% of the cost of raw materials (Grima et al., 2003). Because dairy farm effluents are rich in nutrients, especially N and P, they can be employed as culture media to cultivate algal polyculture, hence, reducing the COD and nutrient load (Lu et al., 2023; Zou et al., 2021).

The utilization of algal cultivation in dairy effluent has been proposed and several advantages were mentioned by Beevi and Sukumaran (2014) which include: (1) a decrease in BOD and COD of the wastewater; (2) production of biomass that utilize N, organic C, and other minerals with no additional nutrients required; (3) aeration of the treated effluent; and (4) extraction of valuable products such as carbohydrates, lipids, and proteins for fuel, pharmaceutical and chemical industries. *Chlorella pyrenoidosa, Anabaena ambigua, Scenedesmus abundans, Chlorella vulgaris, Chlamydomonas polypyrenoideum* and *Acutodesmus dimorphus* are some of algal species which have been proved to be successfully cultivated in dairy wastewaters (Gramegna et al., 2020). Algal polyculture composed of a diverse species have been effectively applied to treat dairy effluent and numerous previous studies involving effective cultivated of algal polyculture for nutrients removal from dairy effluents along with their respective nutrient removal are listed in Table 2.4.

Table 2.4: Application of algal polyculture in different type of dairy wastewaters and respective nutrients removal efficiencies.

Algae species	**Type of wastewater**	**Operation conditions**	**Nutrient removal**	**Reference**
Microspora willeana, *Ulothrix zonata, Ulothrix aequalis, Rhizoclonium hieroglyphicum,* and *Oedogonium* sp.	Dairy manure wastewater	Semi batch operation Reactor type: Benthic algae growth chambers Cultivation volume: 229 L Cultivation time: 9 weeks Temperature: 22 °C Light: 40-140 µmol m^{-2} s^{-1} (16 h light)	60-62% TN 70-93% TP	(Wilkie & Mulbry, 2002)
Actinastrum, *Scenedesmus*, *Chlorella*, *Spirogyra, Nitzschia*, *Micractinium*, *Golenkinia*, *Chlorococcum*, *Closterium*, *Euglena,* and two unidentified species	Anaerobically treated dairy wastewater	Semi continuous operation Reactor type: Aquarium tanks Cultivation volume: 40 L Cultivation time: 15 days Temperature: up to 37 °C Light: Natural sunlight Aeration: 1.5 L min^{1}	96% NH_4-N >99% PO_4-P	(Woertz et al., 2009)
10 native algal polyculture	Dairy farm treated wastewater	Batch operation Reactor type: HRAP Cultivation volume: 600 L Cultivation time: 10 days Temperature: 27-32 °C Light: 80–120 mol µmol m^{-2} s^{-1} (12h:12h) Aeration: 5 L min^{-1}	98.8% COD 98.8% PO_4-P 99.4% NO_3-N ~100% NH_4-N	(Hena et al., 2015)
Chlorella sp., *Scenedesmus* spp., and *Chlorella zofingiensis*	Dairy wastewater	Batch operation Reactor type: tbcPBRs Cultivation volume: 400 mL Cultivation time: 7 days Temperature: 25 ± 1 °C Light: 150 ± 5 µ mol photons m^{-2} s^{-1} Aeration: 1 vvm	87.04-91.02% TN 91.16–95.96% TP 57.01–62.87% COD	(Qin et al., 2016)

n.d.	Brackish dairy manure effluent	Semi batch operation Reactor type: PBR Cultivation volume: 37 L Aeration: 1.5 L min^{1}	90% COD 90% NH_3-N 80% PO_4^{3-}	(Audu et al., 2018)
Chlorella variabilis, Parachlorella kessler, Thermosynechococcus elongatus, Chlamydomonas, Phaeodactylum tricornutum, Oscillatoriales, Synechocystis sp., *Microcystis aeruginosa, Nostocales, Naviculales, Stramenopiles*, and other members of *Chlorophyceae, Trebouxiophyceae, Chroococcales*	Dairy wastewater	Batch operation Reactor type: Aquarium tank Cultivation volume: 2 L Cultivation time: 14 days Temperature: 25 ± 2 °C Light: 6309 lux Aeration: mild aeration	87.2% NH_4-N 93% COD ~100% NO_3-N ~100% PO_4^{3-}	(Biswas et al., 2021)
Chlorella minutissima, Scenedesmus abundans, Nostoc muscorum, and *Spirulina* sp.	Dairy wastewater	Reactor type: Conical flask Cultivation volume: 300 mL Cultivation time: 12 days Temperature: 27 ± 1 °C Light: 10,000 lux (12h:12h) pH: 7.5 ± 0.3	75.16% BOD 61.37% COD 58.76% TN 84.48% TP	(Chandra et al., 2021)
Chlorella variabilis and *Scenedesmus obliquus*	Synthetic dairy wastewater	Batch operation Reactor type: Conical flask Cultivation volume: 200 mL Light: 40, 25, 10 μ mol photons m^{-2} s^{-1} Aeration:	70.19% PO_4^{3-} 86.22% NH_4-N 54.72% COD	(Loganathan et al., 2021)

Note: n.d. = no data

2.15 Operational Parameters for an Efficient Algal Remediation

The biomass plays a vital role for effective algal remediation, whereby maintaining stable operating parameters must be taken into account from both abiotic and biotic factors. Depending on the design of the system, certain systems offer greater control over the parameters to produce better effluent quality (Valchev & Ribarova, 2022). This section describes various operational parameters and their ideal ranges based on current research and best practices for algal remediation.

The performance of algal remediation, particularly, rates of nutrient removal, is significantly impacted by N and P loading at the inlet (Valchev & Ribarova, 2022). Different wastewater streams have been studied with remediation data showing a wide range of influent concentrations of NH_4^+ and P, from 3.3-309 mg L^{-1} and 0.04-770 mg L^{-1}, respectively (Whitton et al., 2015). Inadequate or low N level may prevent P from being absorbed by algal polyculture since N is required for protein synthesis for P assimilation. Thus, an ideal nutrient load is necessary for the algae-based wastewater treatment system to operate more effectively, and the adaptation of the employed algal species depends on the type of wastewater (S. Rani et al., 2021).

The flow rates of effluent, commonly measured in cubic meter per day (m^3 d^{-1}), are critical determinants of the performance for algal remediation, as they impact the retention time and subsequently, the exposure of algae to nutrients (Coughlan et al., 2022). Typical systems operate with flow rates that allow a residence time of 7 to 10 days, ensuring significant nutrient removal (Goswami et al., 2021).

Optimal algal concentrations depend on the system design and the specific algae species used. Generally, maintaining lower algal concentration, i.e. lower volumetric production rates, allows effective nutrient uptake while avoiding issues such as self-shading and longer light path (Novoveská et al., 2023). The common volumetric production rates in ponds range between 0.010–0.12 g L^{-1} d^{-1} (Chisti, 2007).

Light is the main energy source of the photoautotrophic or mixotrophic algae. Algae primarily absorbs light more effectively in the blue (~400 nm) and red (~600–700 nm) regions, which can enhance photosynthetic efficiency and support processes related to N and P removal (Whitton et al., 2015). Maximizing light exposure, both in terms of intensity and duration, is crucial for photosynthetic activity. Multiple design optimisations have enabled algae to utilize light more efficiently, increasing the photosynthetic efficiencies by 3-5% (Norsker et al., 2011).

Oxygen levels are indicative of photosynthetic activity and overall system health. Adequate oxygenation often maintained between 3 and 10 mg L^{-1} for most algae to enhance the treatment process of wastewater (Kazbar et al., 2019). DO above 20 mg L^{-1} can inhibit many algae species, prompting the development of designs and techniques of the system to mitigate high DO levels, where oxygen stripping has been found effective (Posadas et al., 2017).

The depth of algal cultivation ponds impacts light availability and algal access to nutrients. Shallow ponds, typically 20-30 cm deep, are preferred as they allow better light distribution and facilitate gas exchange, enhancing algal growth and nutrient uptake (McGinn et al., 2011; Prajapati et al., 2013). The minimum depth of the pond can be determined by the shortest HRT typically necessary for effective removal of pollutants (Posadas et al., 2017). Additionally, the depth of the pond significantly influences its temperature, suggesting that the design of both HRT and pond depth must take into account local climate conditions, regulatory standards, and the specific attributes of the effluent (Posadas et al., 2017).

Mixing, also one of the most important operational parameters, whereby proper mixing in the pond or reactor ensures uniform nutrient distribution, prevents algal sedimentation, and maintains a homogenous environment, which is crucial for consistent algal growth and nutrient absorption (Eriksen, 2008; Grobbelaar, 2004). In practice, mixing is often accomplished by a revolving arm or paddle wheel in open ponds and is the most energy-intensive step in the microalgal culture process (Grima et al., 2003).

2.16 Practical Examples of Algal Remediation Setups

2.16.1 Shallow ponds

Waste stabilization ponds (WSPs) were among the earliest large-scale wastewater treatment technologies (Geremia et al., 2021). They are shallow basins where polyculture are cultivated in wastewater to eliminate N, P and COD (Foladori et al., 2018). The ease of use and affordability of WSPs in terms of infrastructure and energy make it a top option for effectively removing organic matter, nutrients as well as pathogens (Butler et al., 2015). Nevertheless, one of the drawbacks of WSPs include the difficulty in regulating the level of wastewater purification, which relies entirely on climate and pollutant load rates. For example, in cases when the climate is not ideal, this constraint gives rise to odor issues and poor biological activity. The extended retention times and substantial land requirement of WSPs account for their extensive application in rural regions (González-Camejo et al., 2019).

2.16.2 Raceways

Open raceway ponds, also known as high-rate algal ponds (HRAPs), are shallow ponds with paddle wheels designed to enable microalgae to obtain light and nutrients by means of culture mixing, degassing, and recirculation at a pace of 15 to 30 cm s^{-1} (Prajapati et al., 2013). In 1950s, Professor Oswald invented HRAPs to treat domestic wastewater in California, and several modifications have been documented subsequently. *Chlorella* sp. and *Scenedesmus* sp. are commonly used as inoculum in treating primary effluents (Vaz et al., 2023). HRAPs are the widely used algal polyculture systems for treating wastewater, Most HRAPs feature a depth of 0.1-0.3 m, while other designs go as deep as 0.4 m. Distinguished by their minimal operation, low-cost design, lower energy consumption, which is 0.52 MJ m^{-3} against 3.6 MJ m^{-3} when compared to the conventional wastewater treatment (Ledda et al., 2015; Leong et al., 2021). Despite these advantages of HRAPs, the low light photosynthetic efficiency as a result of poor mixing (dark zones) and prolonged light pathway, the release of CO_2 into the atmosphere, high evaporation, substantial use of land, are some of the main drawbacks of HRAPs (Arbib et al., 2017; Kumar et al., 2017).

2.16.3 Photobioreactors

Closed systems, such as photobioreactors (PBRs), are considerably more expensive to set up and run than HRAPs. Nevertheless, they are able to operate in controlled settings while achieving higher photosynthetic efficiency, which enhances the nutrient removal and biomass production (González-Camejo et al., 2019). PBRs come in a variety of forms, often as horizontal or vertical, as they utilize different types of technology. The affordable production cost and compactness of vertical column PBRs offer enormous potential since minimal surface area per unit volume are required for optimal growth of the polyculture (Geremia et al., 2021). Furthermore, PBRs facilitate in the gas exchange, nutrition delivery, as well as provide sufficient mixing of the cell solution (Doria et al., 2011). Photosequencing batch reactors (PSBRs) are one of the most popular concepts for algal polyculture application as they have the benefits of batch feeding and less complex sequencing processes (Foladori et al., 2018).

2.16.4 Algal turf scrubber

Adeay and colleagues introduced algal turf scrubbing (ATS) technology with the aim to enhance natural wastewater treatment systems (Craggs, 2001; Oruganti et al., 2022). An ATS comprises of long, inclined beds that hold biofilm formation, which is made up of a diverse group of filamentous microalgae, bacteria as well as fungi. Nutrients are eliminated by absorption into biomass as the wastewater passes through the biofilm, The most common species found were *Cyclotella* sp., *Navicula* sp., *Nitzschia* sp., and *Oscillatoria* sp. (Craggs, 2001). ATS technology addresses numerous underlying problems related to growth and harvesting of microalgae. In other words, convenient harvesting, and reduction in total cost of biomass production as the scrapping the benthic algae formation on the turf scrubber is done occasionally (Park et al., 2018). Efficient TN removal was demonstrated by the hybrid ATS technology used in constructed wetlands (Celente et al., 2019).

2.16.5 Rotating algal biofilm

The rotating algal biofilm (RAB) is another type of biofilm-based technology for nutrient removal, where its attachment component (e.g., flat rotating disks, a cylindrical drum, or a rotating conveyor belt) is partially submerged in water and rotates continuously, while scraping the algal biomass when it is in the air studies (Gross et al., 2015). The most popular example of RAB in wastewater treatment is the rotating biological contactor (RBC), whereby a drum with algae cells attached is let to rotate using a drive shaft (Gullicks et al., 2011; Hassard et al., 2014). Modified RAB systems have been reported to enhance the removal of nutrients and biomass productivity in numerous studies (Gross et al., 2015).

2.17 Conclusion

Microalgae have become valuable microbes especially in the constantly evolving field of wastewater treatment for nutrients removal. This chapter has thoroughly explored algal polycultures, highlighting their importance in wastewater treatment, especially for dairy farm effluent. Starting with the basics of algae and microalgae, the review emphasized their role in wastewater treatment and detailed the advantages of polycultures over monocultures in nutrient removal. It also covered the limitations and research needs, setting the stage for future studies. Critical factors like temperature, pH, and light affecting algal growth were examined, alongside the nutrient removal mechanisms such as absorption and adsorption. The impact of excessive nutrients on water quality, particularly eutrophication, was discussed. Further, this review delved into the specifics of dairy effluent treatment using algal polycultures, including operational conditions and system setups like ponds and raceways, showcasing their application and efficiency. This review not only sheds light on the existing understanding but also outlines the potential for algal polycultures in enhancing the sustainability and effectiveness of wastewater treatment processes.

Chapter 3: Methodology

3.1 Overview of Experiment

Two sets of experiment were run to determine the nutrient removal—specifically NH_4^+, NO_3^-, NO_2^-, and PO_4^{3-}, along with the growth rate of the algae polyculture in dairy farm effluent in sterilized and non-sterilized settings. Each set of experiment comprised of a duplicate of polyculture samples and a control consisting of dairy farm effluent without added algal polyculture. The carbon and protein content were also analyzed. These experiments were carried out over a period of 28 days. All experimental analyses were conducted weekly to monitor the performance of the algal polyculture by withdrawing adequate volume of sample effluent.

3.2 Collection of Dairy Farm Effluent

Dairy farm effluent was sampled from the farm sited in Awakeri in Bay of Plenty, New Zealand (Figure 3.1), which generates approximately 21.2 m^3 of effluent per day (Hull-Cantillo, Lay, Glasgow, et al., 2023). Effluents from system 4 (milking shed) and system 5 (feed pad) passed through the weeping wall as illustrated in Figure 3.2, before being discharged into the pond. The effluent for this research study was collected at the end of weeping wall (also known as passive solid separator that is made up of two concrete bunkers with a perforated wall at one end which functions as solid retention while liquids are drained out).

Figure 3.1: Layout of the study farm sited in Awakeri, Bay of Plenty, New Zealand (Hull-Cantillo, Lay, Glasgow, et al., 2023).

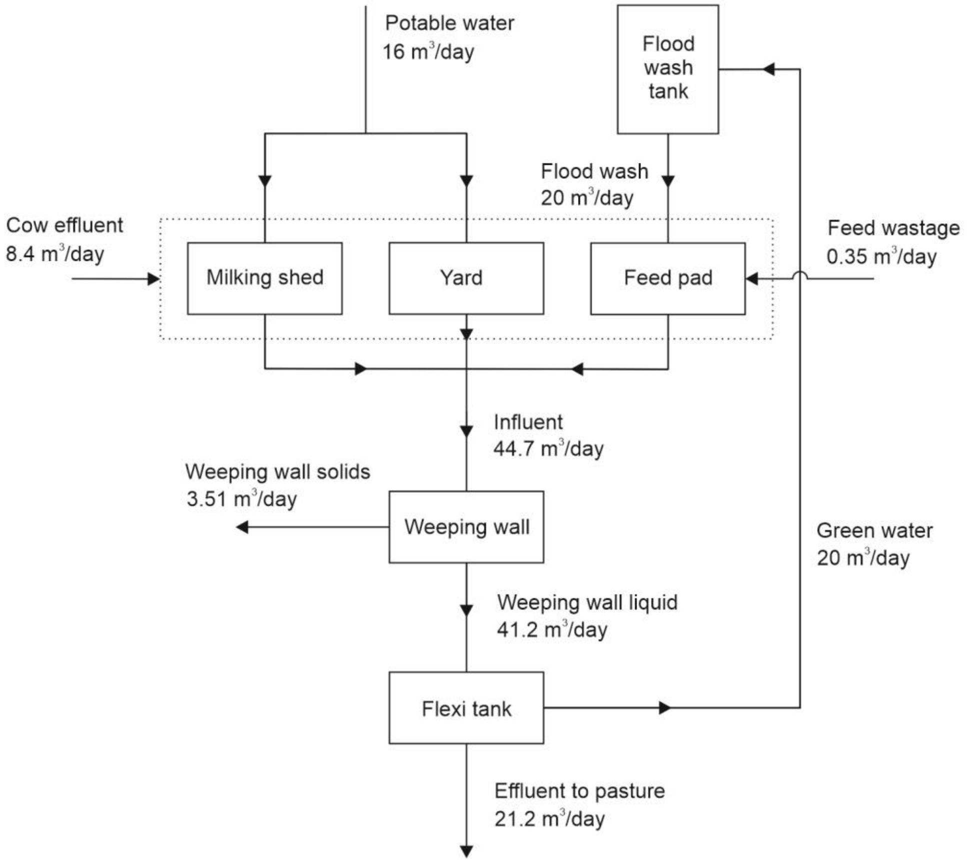


Figure 3.2: Flowchart of the study farm (Hull-Cantillo, Lay, Glasgow, et al., 2023).

The dairy farm effluent characteristics are tabulated in Table 3.1, which are based on previously reported data from a recognized laboratory (Hull-Cantillo, Lay, & Kovalsky, 2023).

Table 3.1: Characteristic of collected dairy farm effluent (Hull-Cantillo, Lay, & Kovalsky, 2023).

Parameter	**Content**
Acetic Acid	200 g m^{-3}
Acid detergent fiber (ADF)	9.5% DM[a]
BOD[b]	720 g O_2 m^{-3}
Butyric Acid	7 g m^{-3}
COD	3000 g O_2 m^{-3}
Density	1000 kg m^{-3}
Formic Acid	<5 g m^{-3}
Neutral detergent fiber (NDF)	14.7% DM
NO_3-N + NO_2-N	<0.10 g m^{-3}
Oil and Grease	310 g m^{-3}
Propionic Acid	137 g m^{-3}
Tannin	152 g m^{-3}
Total Kjeldahl Nitrogen (TKN)	290 g m^{-3}
TN	290 g m^{-3}
Total Ammoniacal-N	178 g m^{-3}
Total Carbon	1380 g m^{-3}
Total Solids (TS)	3300 g m^{-3}
Total VFA (as acetic acid)	320 g m^{-3}
Volatile Solids (VS)	1850 g m^{-3}

Note: [a] DM—dry matter; units are in percentage of DM; [b] carbonaceous biochemical oxygen demand (cBOD5)

3.3 Pretreatment and Preparation of Sample

Upon arrival to the laboratory, dairy farm effluent sample was stored at -20 °C for preservation until further analysis. Filtration, centrifugation, as well as autoclaving are the most used pretreatment methods in wastewater (Gupta et al., 2019). To separate particulate solids in the effluent, the effluent was first centrifuged at 4800 rpm for 20 min followed by vacuum filtration using Whatman filter papers. The dairy farm effluent filtrate was a coffee color. Half of the filtered effluent was then autoclaved for 20 min at 121 °C. The main purpose of autoclaving the effluent is to reduce the contamination of other microbes, while the non-autoclaved sample aims to preserve the indigenous microbes. Both autoclaved and non-autoclaved dairy farm effluent samples were used as culture media for algae polyculture and are categorized into four groups:

1. Polyculture (A): Autoclaved dairy farm effluent with algal polyculture E3.
2. Polyculture (NA): Non-autoclaved dairy farm effluent with algal polyculture E3.
3. Control (A): Autoclaved dairy farm effluent with no algal polyculture.
4. Control (NA): Non-autoclaved dairy farm effluent with no algal polyculture.

3.4 Algal Polyculture

Algae polyculture was previously isolated from the dairy farm effluent and grown under ambient conditions by a PhD student at The University of Waikato. Due to its rapid growth and high biomass observed from the preliminary screening, the algal polyculture (E3) was used in this study (Figure 3.3).

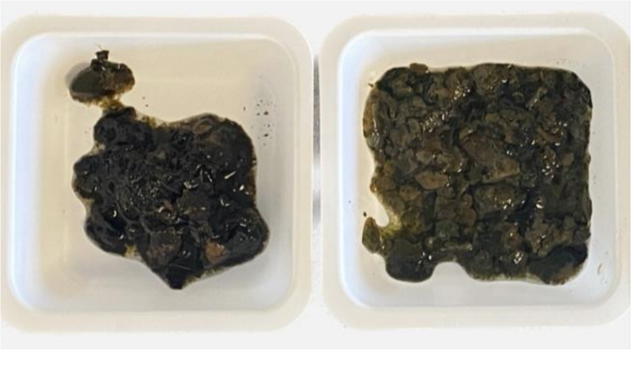


Figure 3.3: Algal polyculture E3 used in this study.

3.5 Experimental Setup

Three borosilicate bottles with a total volume of 500 ml were used in each set of experiment for the cultivation of algal polyculture (Figure 3.4). For each experimental unit, the experimental procedure was uniform: 12.5 g ww of algal polyculture (E3) were inoculated into an empty bottle and filled with 500 ml of dairy farm effluent as the culture media. Polyculture samples in each set of experiment were run in duplicate and were labeled as E3 (1) and E3 (2). An additional bottle of effluent with no algal polyculture added served as the control.

All bottles were continuously sparged with air through a clean air tube attached to a glass pipette to provide mixing with the other end of the tubing attached to the manual air flow control valves that was connected to a 3-W aquarium air pump. The bottles were illuminated under fluorescent lamps with an irradiance of 271.95 µmol m^{-2} s^{-1} at a single side and operated on a light dark cycle of 12 h:12 h.

During the course of the experiment, no nutrients were added. Distilled water was introduced into the bottles with a weekly draw-fill procedure. The experiment was carried out between January 2024 to February 2024 over a period of 28 days.

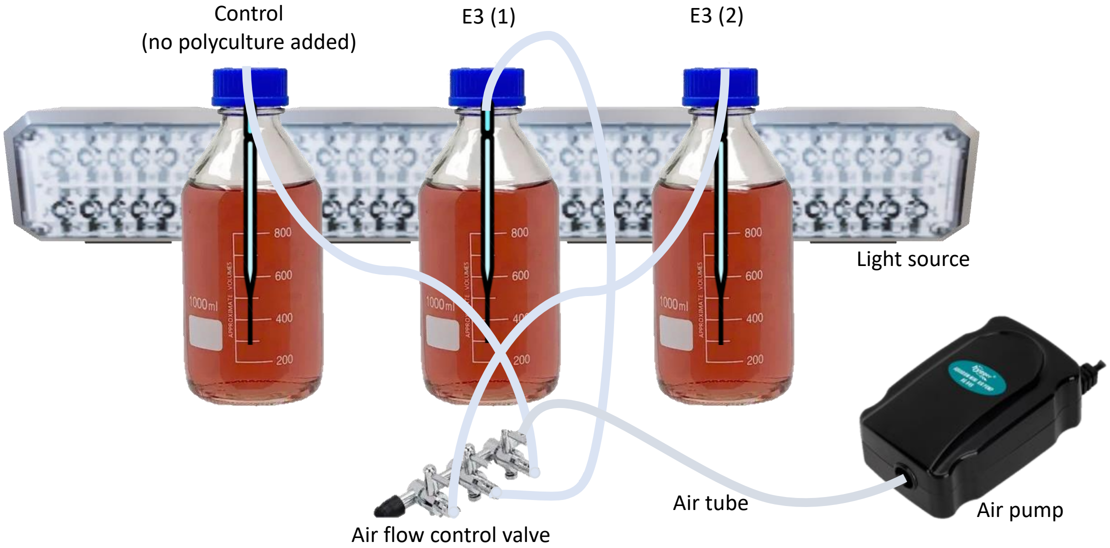


Figure 3.4: Experimental setup used in this study.

3.6 Analytical Methods

3.6.1 Operational parameters

Temperature, pH, turbidity, and DO were monitored and analyzed each week to characterize the growth conditions of the algal polyculture. The instruments used for analysis of water properties were tabulated in Table 3.2.

Table 3.2: List of instruments for analysis of water properties.

Analysis	Unit	Instrument
pH	-	Eutech Instruments pH 150
Turbidity	NTU	2100P Turbidity meter Hach
Dissolved oxygen	mg L^{-1}	Milwaukee MW 600

3.6.2 Algal polyculture growth

The growth of the algal polyculture was determined by dry-weight estimation to determine the biomass production, whereby a portion of the algal polyculture wet weight was dried in an oven at 105 °C. This was followed by cooling in a desiccator to achieve a constant weight (Becker, 1994). The final dry weight of the polyculture was noted, and the overall biomass was calculated using the formula (Eq. (6)):

$$Biomass\ Growth\ Rate\ (mg/L/day) = \frac{(Final\ Biomass - Intial\ \ Biomass)\ x\ Volume}{Time} \tag{6}$$

3.6.3 Nutrient analysis

Aliquot amount of sample for nutrient removal analysis were collected once a week. NH_4^+, NO_3^-, NO_2^- as well as PO_4^{3-} concentrations were determined using a Palintest photometer based on different test methods as shown in Table 3.3. Because of relatively high N and P content, which the photometer is sensitive toward, the samples were diluted prior to measuring.

Table 3.3: Palintest test method and dilution factor used for different nutrient analysis.

Nutrient	**Palintest test method**	**Photometer measurement**	**Dilution factor**
NH_4^+	Palintest Ammonia	Phot 62	1000
NO_3^-	Palintest Nitratest method	Phot 63	1000
NO_2^-	Palintest Nitricol method	Phot 64	1000
PO_4^{3-}	Palintest phosphate LR method	Phot 28	300

The nutrient removal efficiency was calculated on a percentage basis by the ratio between the difference of initial (Day 0) and final (Day 28) concentrations of the effluent by using the formula as given below (Eq. (7)):

$$Removal\ efficiency\ (\%) = \frac{\text{Initial concentration} - \text{Final concentration}}{Initial\ concentration}\, x\ 100 \tag{7}$$

Furthermore, to estimate the nutrient consumption rate, the following formula was used (Eq. (8)):

$$Nutrient\ Consumption\ rate\ (mg/g/day)) = \frac{\text{Initial concentration} - \text{Final concentration}}{Algae\ biomass\ x\ Time} \tag{8}$$

3.6.4 Carbon and protein content analysis

To determine the relationship between absorbance and protein as well as carbon content in the algal polyculture, UV spectrophotometer (Shimadzu Scientific Instruments) was used to measure the spectral absorbance of 280 nm (for protein) and 245 nm and 215 nm (for carbon). Due to limited time, it was not feasible to create standard solutions. Consequently, absorbance units (AU) were used for measuring carbon and protein concentrations in this study, rather than the conventional mg L^{-1}.

3.7 Algal Polyculture Visualization under Microscope

Some algal species in polyculture E3 were identified using a light microscope (Olympus BX53) with Olympus DP28 camera based on the cell structures and unique characteristics. The algal polyculture E3 was smeared on a clean glass slide, air dried and focused under the microscope at 40x and observed further at 100x magnification (oil emulsion) in bright light.

3.8 Process Simulation Model

A finite difference numerical simulation was developed to predict the impact of laboratory-scale growth experiments on a scaled-up system, using the differential equations below that represent algal growth and change in nutrient concentrations. The model assumes the reactor is a well-mixed tank and the inflow and outflow are the same.

The growth rate of algae can be simply described by the following equation (Eq. (9)):

$$\frac{dC_{algae}}{dt} = C_{algae}\ C_{NH4}\ k_{algae} - \frac{Q_{effluent}\ C_{algae}}{V_{reactor}} \tag{9}$$

where, C_{algae} is the concentration of algae (g m^{-3}), C_{NH4} is the concentration of ammonia, k_{algae} is the specific growth rate of algae (m^3 g^{-1}.d), $Q_{effluent}$ is the flow of effluent in and out of the reactor/pond (m^3 d^{-1}), $V_{reactor}$ is the volume of the reactor (m^3), and t is time (days).

The rate of change of a particular nutrient in the reactor can be determined using this equation (Eq. (10)):

$$\frac{dC_{nutrient}}{dt} = \left(\frac{Q_{effluent} \cdot C_{nutrientin} \cdot\ - Q_{effluent} \cdot C_{nutrient}}{V_{reactor}}\right) - k_{nutrient}\, C_{nutrient} \cdot C_{algae} \tag{10}$$

where, $C_{nutrient}$ is the concentration of a particular nutrient in the reactor (g m^{-3}), $C_{nutrientin}$ is the concentration of nutrient in the influent into the reactor (g m^{-3}), and $k_{nutrient}$ is the specific uptake or degradation rate for a particular nutrient (m^3 g^{-1}. d).

Parameters used in the model were based on the experimental data obtained, which were adjusted to fit the specific conditions of the model setup. Initial conditions, such as influent flow rate, starting algae and nutrient concentrations, were set and the system was simulated over time which would generate the model data output. To assess the accuracy of the models, the model data was compared against the experimental data using the Sum of Squares of the Error

(SSE) (described in the next section) and adjusting model parameters to reduce the SSE. Additionally, the modeling was extended to a larger farm sized scale, to predict outcomes relevant to practical algal cultivation and nutrient management scenarios.

3.8.1 Sum of squares

Furthermore, the SSE method, along with the Excel's Solver function was employed for optimisation, aiming to locate the best conditions for maximizing both nutrient removal and algal growth. This quantitative measure enables the evaluation of the model's performance by quantifying the extent of variance between the predicted and actual values. The lower the SSE, the closer the model's predictions align with the observed data, indicating a better model fit. The equation for the Sum of Squares of the Error is as follows (Eq. (11)):

$$SSE = \sum_{i=1}^{n}(\mathcal{Y}_i - \bar{\mathcal{Y}})^2 \tag{11}$$

where, $\mathcal{Y}_i$ represents the observed experimental values, $\bar{\mathcal{Y}}$is the predicted values based on the model.

Chapter 4: Results

4.1 Operational Parameters of Dairy Farm Effluent

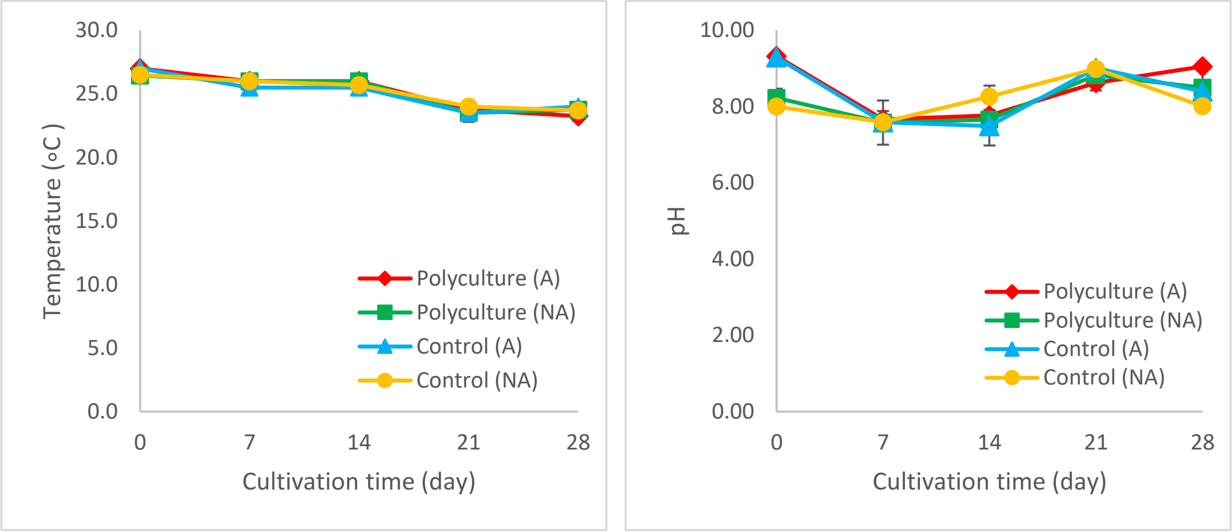
Table 4.1 summarizes the operational parameters across the entire 28-day cultivation period, averaging the values from both control and polyculture-treated effluent in the autoclaved and non-autoclaved settings. No significant differences were shown in terms of temperature, with an average value of 25.4 °C and 25.2 °C for both autoclaved and non-autoclaved dairy farm effluent, respectively. The pH was almost constant as the average value was 8.42 and 8.16 for autoclaved and non-autoclaved dairy farm effluent, accordingly. The average DO for autoclaved dairy farm effluent was 4.9 mg L^{-1} and non-autoclaved effluent had a slightly lower average value of 5.3 mg L^{-1}. Higher average turbidity was recorded in autoclaved dairy farm effluent (88.2 NTU) than the non-autoclaved effluent (51.9 NTU).

Table 4.1: Temperature, pH, DO, and turbidity off dairy farm effluent (Means ± S.D.).

Parameter	**Dairy farm effluent**	
	Autoclaved	**Non-autoclaved**
Temperature (°C)	25.4 ± 0.2	25.2 ± 0.1
pH	8.42 ± 0.05	8.16 ± 0.01
Dissolved oxygen (mg L^{-1})	4.9 ± 0.1	5.3 ± 0.2
Turbidity (NTU)	88.2 ± 1.6	51.9 ± 2.4

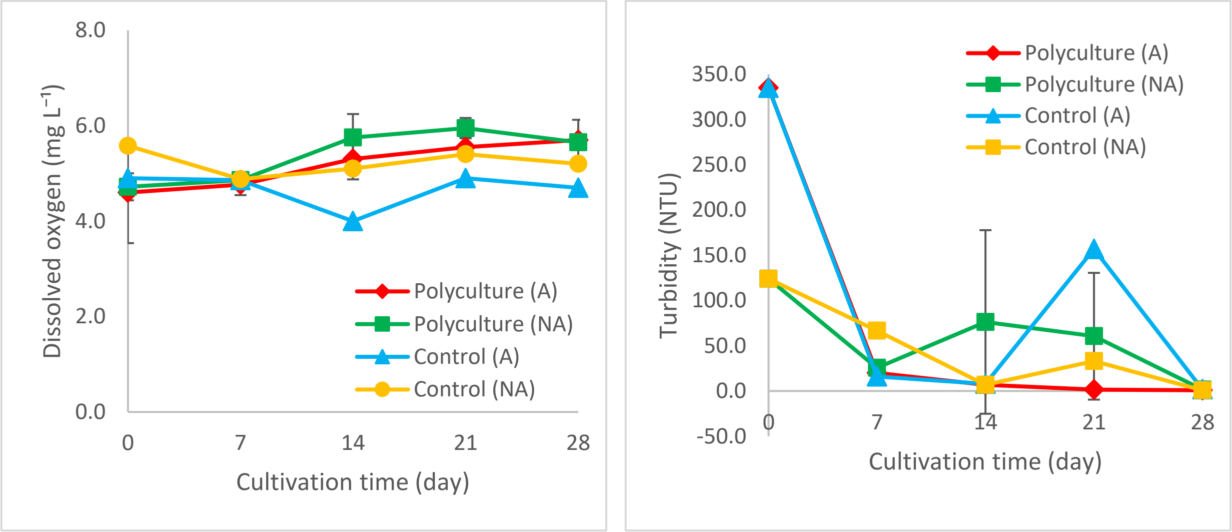
The trends in Figure 4.1 show how each parameter in the effluent changed over time. The temperature of the effluent in each bottle decreases over the course of the experiment with the minimum at 23.5 °C and maximum at 27.0 °C. The pH level appears stable with slight differences from 7.49-9.32. The initial high pH values were recorded: autoclaved effluent samples (>pH 9) and non-autoclaved effluent (>pH 8). DO levels of the effluent were between 4.0-6.1 mg L^{-1}. The sudden drop of DO from 4.7 to 4.0 mg L^{-1} in Control (A) was observed on Day 14. Finally, the turbidity of the effluent fluctuated significantly from 0.8 to 335 NTU, with trends declining over time, especially in autoclaved samples. The highest turbidity value was detected on Day 0 in

Polyculture (A). Moreover, the turbidity value in Control (A) showed a sharp increase (8.1 to 157 NTU) between Day 14 to 21.



(a) Temperature

(b) pH



(c) Dissolved oxygen

(d) Turbidity

Figure 4.1: Variation of (a) temperature, (b) pH, (c) DO and (d) turbidity of autoclaved and non-autoclaved dairy farm effluent by algal polyculture E3 and in controls over 28 days (Means ± S.D.).

4.2 Algal Growth

Biomass growth rates of the algal polyculture E3 between autoclaved and non-autoclaved dairy farm effluent, including controls across two distinct growth periods were compared as illustrated in Figure 4.2. During the initial growth phase (Day 0-14), Control (A) showed a slight growth (1.79 mg L^{-1} d^{-1}) in comparison to Polyculture (A), which exhibited significantly enhanced growth (7.46 mg L^{-1} d^{-1}). Conversely, in non-autoclave effluent, Control (NA) demonstrated a higher initial growth rate of 4.78 mg L^{-1} d^{-1} compared to Control (A). Polyculture (NA), however, showed an initial decrease in biomass growth rate. The subsequent growth phase (Day 14-28) revealed a continued a minimal growth for Control (A), which was almost consistent with its earlier performance. Polyculture (A) maintained a higher biomass growth rate of 8.07 mg L^{-1} d^{-1}. On the other hand, Control (NA) experienced a substantial decrease in growth rate while Polyculture (NA) resulted a sudden growth recovery of 10.13 mg L^{-1} d^{-1}.

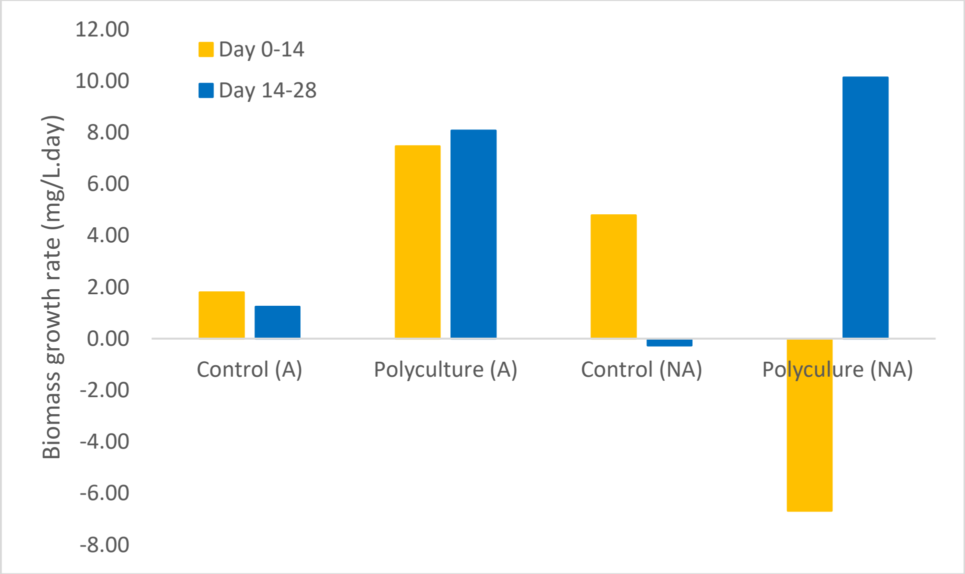


Figure 4.2: Biomass growth rate of algal polyculture E3 and controls in autoclaved and non-autoclaved dairy farm effluent (Means ± S.D.).

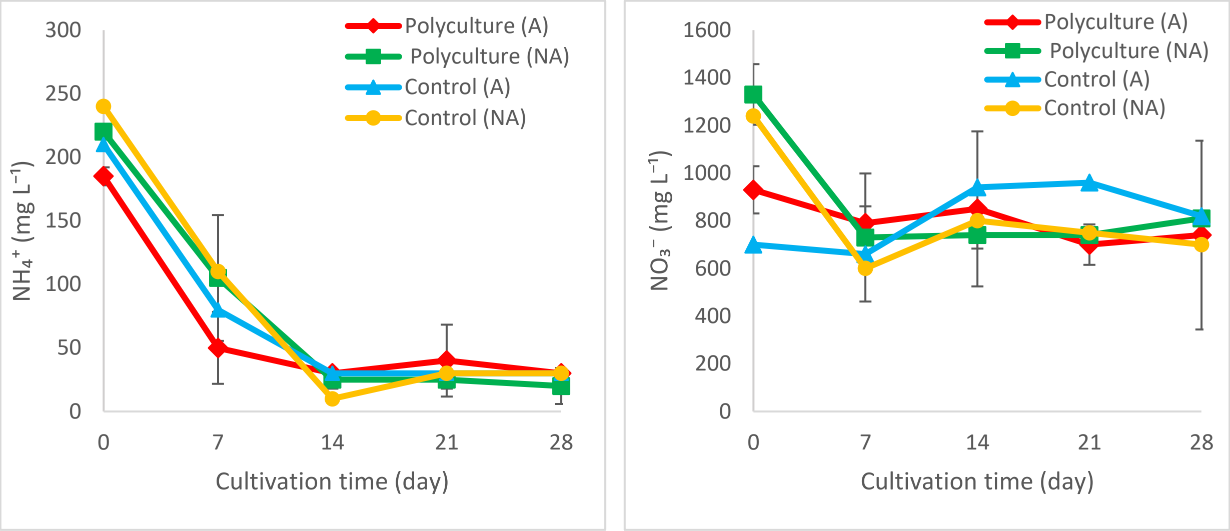
4.3 Nutrient Removal

Table 4.2 and Figure 4.3 show the nutrient removal efficiency by algal polyculture E3 and how each nutrient concentration (NH_4^+, NO_3^-, NO_2^-, and PO_4^{3-}) in both autoclaved and non-autoclaved dairy farm effluent changed over 28 days, respectively. In this regard, NH_4^+ decreases gradually over the cultivation period in both control and polyculture-treated effluent in the autoclaved and non-autoclaved settings, simultaneously presenting relatively high removal efficiencies of 83.8% and 90.9% in Polyculture (A) and Polyculture (NA), accordingly. NO_3^- levels generally show a decrease over time with a slight fluctuation. However, NO_3^- in Control (A) was seen to be increasing throughout 28 days of cultivation period from 700 to 820 mg L^{-1}. NO_2^- concentrations, on the other hand, exhibit a significant variability during the 28 days period. Some spikes in concentrations were recorded, particularly in Control (NA) with 110 mg L^{-1} on Day 14 and Polyculture (NA) with 70 mg L^{-1} on Day 21. PO_4^{3-} concentrations decline rapidly in both autoclaved and non-autoclaved conditions with high removal efficiencies of over 99.4% and 99.1% in Polyculture (A) and Polyculture (NA), accordingly.

Table 4.2: Nutrient removal efficiency by algal polyculture E3 in both autoclaved and non-autoclaved settings.

Nutrient	**Nutrient Removal %**	
	Polyculture (A)	**Polyculture (NA)**
NH_4^+	83.8%	90.9%
NO_3^-	20.4%	39.1%
NO_2^-	-	-
PO_4^{3-}	99.4%	99.1%

(a) NH_4^+ removal (b) NO_3^- removal



(c) NO_2^- removal (d) PO_4^{3-} removal

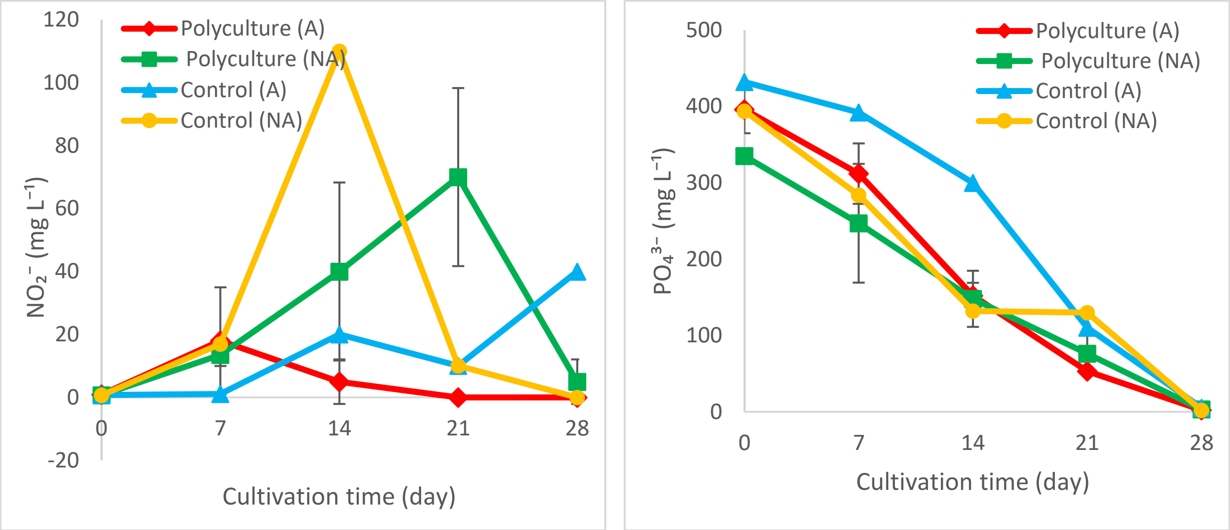


Figure 4.3: Removal of (a) NH_4^+, (b) NO_3^-, (c) NO_2^-, and (d) PO_4^{3-} from autoclaved and non-autoclaved dairy farm effluents throughout 28 days (Means ± S.D.).

4.4 Nutrient Consumption Rate

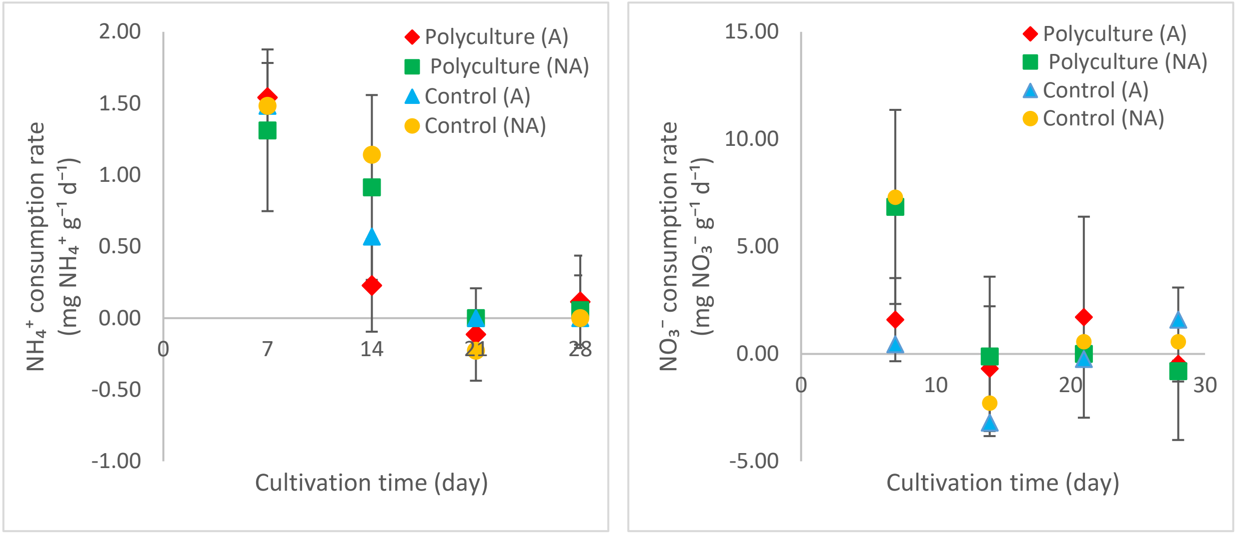
Figure 4.4 displays the nutrient consumption rates for Control (A), Polyculture (A), Control (NA), and Polyculture (NA) at different time points over the experimental period. All groups demonstrated varying degrees of reduction in NH_4^+ consumption rates. Polyculture (A) had a slightly higher initial consumption rate of 1.54 mg NH_4^+ g^{-1} d^{-1} than Polyculture (NA) with NH_4^+

consumption rate of 1.31 mg NH_4^+ g^{-1} d^{-1} on Day 7. On day 28, NH_4^+ consumption rate for Polyculture (A) and (NA) were 0.11 and 0.06 mg NH_4^+ g^{-1} d^{-1}, respectively. In contrast, the initial r consumption rates recorded on Day 7 showed that both Control (A) and Control (NA) exhibited identical NH_4^+ consumption rates of 1.48 mg NH_4^+ g^{-1} d^{-1}. By Day 28, both controls showed a substantial decline in NH_4^+ consumption rate at 0.

The NO_3^- consumption rate displayed variability across all samples over 28 days. Polyculture (A) and (NA) showed an average starting rate of 1.60 and 6.85 mg NO_3^- g^{-1} d^{-1} on day 7, respectively, while both experiencing minor fluctuations, and eventually decreasing to negative values of -0.46 and -0.08 mg NO_3^- g^{-1} d^{-1} by day 28, accordingly. Control (A) showed a minor increase in the initial consumption rate of 0.46 mg NO_3^- g^{-1} d^{-1} and subsequently dropped to negative rate (-3.20 mg NO_3^- g^{-1} d^{-1}) on Day 14, before increasing back to positive value of 1.60 mg NO_3^- g^{-1} d^{-1} by Day 28. Similarly, Control (NA) started with a high consumption rate of 7.30 mg NO_3^- g^{-1} d^{-1} but decreased to -2.28 mg NO_3^- g^{-1} d^{-1}, before stabilizing at 0.57 mg NO_3^- g^{-1} d^{-1} by Day 28.

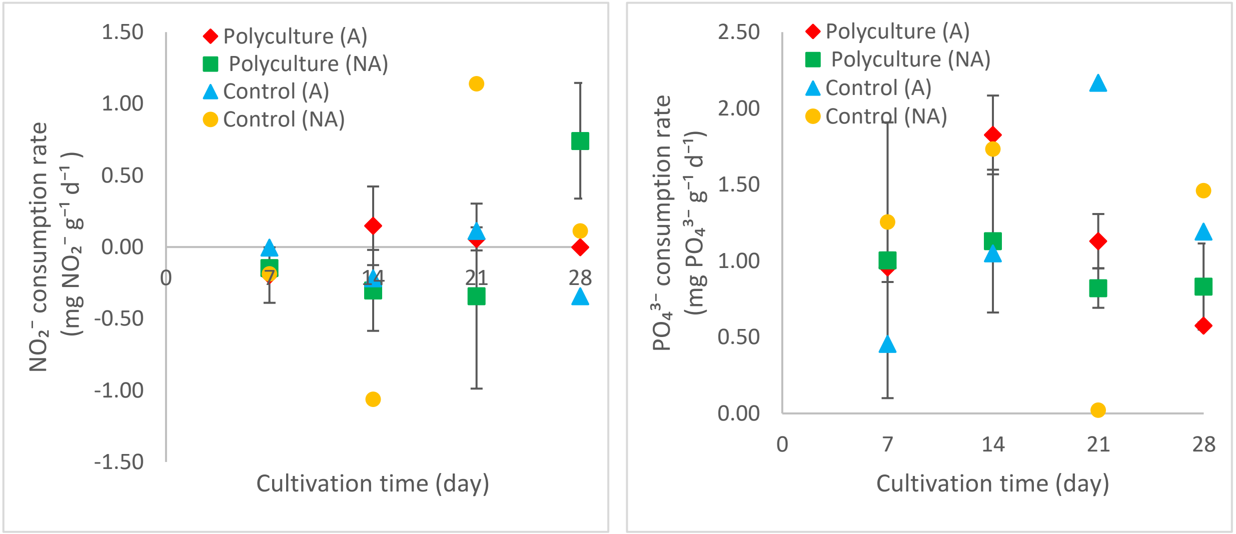
Relatively lower NO_2^- consumption rates were observed for all samples in comparison to NH_4^+ and NO_3^-**.** Polyculture (A) exhibited a slight improvement over time, ending with no change in NO_2^- consumption rate. Polyculture (NA) also displayed a significant improvement by the end of the experiment, with NO_2^- consumption rate of 0.74 mg NO_2^- g^{-1} d^{-1} at Day 28. On the other hand, Control (A) further decreased NO_2^- consumption rate from 0 to -0.34 mg NO_2^- g^{-1} d^{-1} between Day 7 to 28. Control (NA) demonstrated a considerably positive NO_2^- consumption rate of 1.14 mg NO_2^- g^{-1} d^{-1} on Day 21, followed by a drop at 0.11 mg NO_2^- g^{-1} d^{-1} on Day 28.

High initial r consumption rate of PO_4^{3-} was observed for Polyculture (A) on Day 7 (0.90 mg PO_4^{3-} g^{-1} d^{-1}), with the maximum rate at 1.83 mg PO_4^{3-} g^{-1} d^{-1} on Day 14. Polyculture (NA) showed an almost steady consumption rate and achieved 0.83 mg PO_4^{3-} g^{-1} d^{-1} on Day 28. Control (A) exhibited an increase rate from 0.46 to 1.19 mg PO_4^{3-} g^{-1} d^{-1} between Day 7 to 28, with a peak at 2.17 mg PO_4^{3-} g^{-1} d^{-1} (Day 21). Control (NA) had a high initial consumption rate of 1.26 mg PO_4^{3-} g^{-1} d^{-1}, followed by a sudden drop on Day 21 (0.02 mg PO_4^{3-} g^{-1} d^{-1}) before increased to 1.46 mg PO_4^{3-} g^{-1} d^{-1} on Day 28.



(a) NH_4^+ consumption rate

(b) NO_3^- consumption rate



(c) NO_2^- consumption rate

(d) PO_4^{3-} consumption rate

Figure 4.4: The nutrient consumption rate (a) NH_4^+, (b) NO_3^-, (c) NO_2^-, and (d) PO_4^{3-} (mg nutrient per gram of algae per day) in autoclaved and non-autoclaved dairy farm effluents over 28 days (Means ± S.D.).

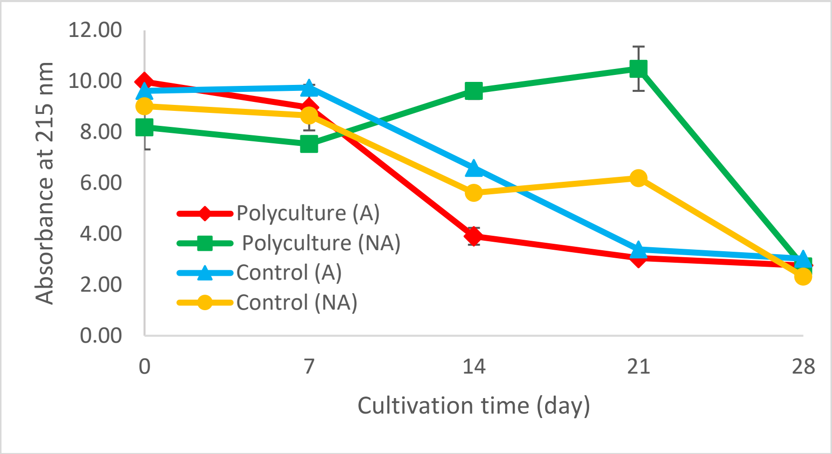
4.5 Carbon and Protein

Figure 4.5a shows the carbon content measured at 215 nm over a 28-day period of polyculture cultivation period, under autoclaved and non-autoclaved settings, including controls. A marked decrease in carbon concentration was observed in all samples over time. Control (A) and

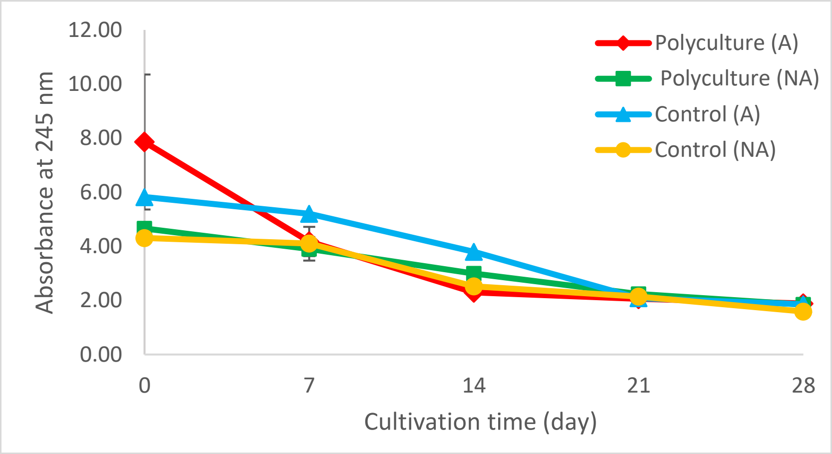
Polyculture (A) showed almost similar decline trend, from 9.62 to 3.03 AU and 9.98 to AU, respectively. Slightly lower initial carbon concentration was observed in Control (NA) at 9.02 AU and Polyculture (NA) at 8.19 AU. Interestingly, Polyculture (NA) exhibited a unique trend with an increase to 10.49 AU by Day 21, before declining to 2.73 AU.

Carbon concentration at 245 nm was also determined as illustrated in Figure 4.5b. Similar like carbon content measured at 215 nm, the overall trend showed a decreasing pattern across four samples. Initially, the carbon concentration was highest in the Polyculture (A) at 7.86 AU, significantly above the Control (A) at 5.82 AU. and both had a final concentration of 1.89 and 1.86 AU, accordingly. The carbon concentration from 4.66 to 1.85 AU was observed for Polyculture (NA) from Day 0 to 28, while Control (NA) reduced from 4.31 to 1.59 AU.

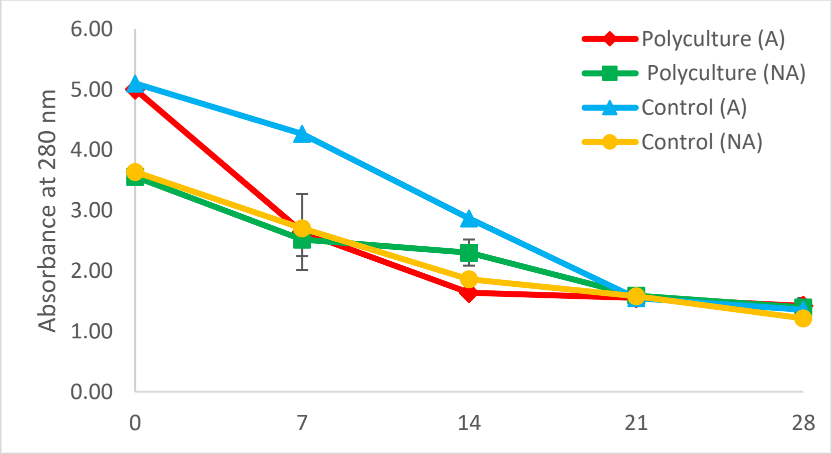
Besides carbon, protein levels were indicated by absorbance at 280 nm (Figure 4.5c). The initial protein was higher for autoclaved samples than non-autoclaved samples. Over time, both Polyculture (A) and (NA) showed a decrease in protein level, but with different patterns. By Day 28, Polyculture (A) decreased to 1.42 AU from an initial value of 5.00 AU, while the Control (A) showed a reduction to 1.36 AU from 5.10 AU. Conversely, in the non-autoclaved setting, the Polyculture (NA) samples ended at protein concentration of 1.39 AU and Control (NA)'s final concentration was 1.21 AU.



(a) Carbon (215 nm)



(b) Carbon (245 nm)



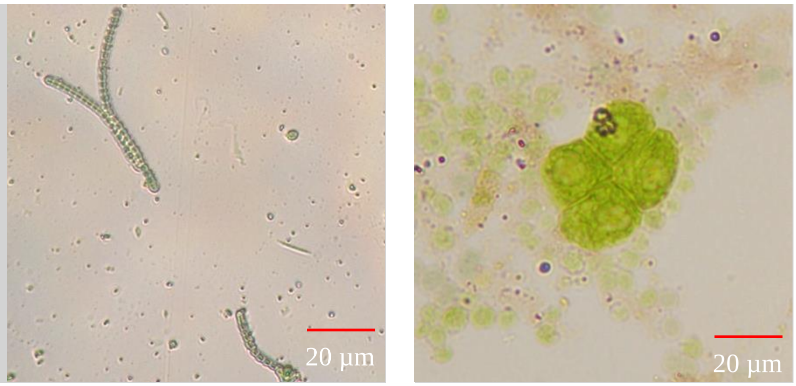
(c) Protein (280 nm)

Figure 4.5: Change in (a) Carbon at 215 nm (b) Carbon at 245 nm, and (c) Protein at 280 nm in dairy farm effluent throughout the cultivation period (Means ± S.D.).

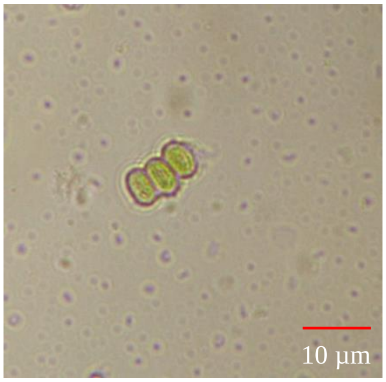
4.6 Identification of Microalgae in Polyculture

Figure 4.6 displays the microscopic images of some microalgae present in polyculture E3, which were observed and identified based on their unique morphological characteristics such as shape, structure, and coloration.

(a) *Chlorococcum* sp. (100x) (b) *Navicula* sp. (Diatom) (100x)



(c) *Cylindrospermum* sp. (40x) (d) *Cosmarium* sp. (100x)



(e) *Scenedesmus* sp. (100x)

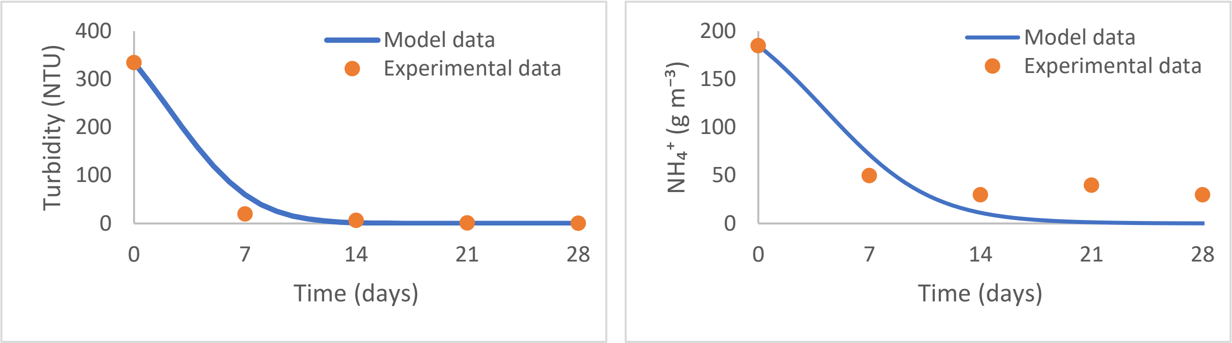
Figure 4.6: Identified species of microalgae found in polyculture E3 under microscope at different magnifications.

4.7 Process Simulation

4.7.1 Model data

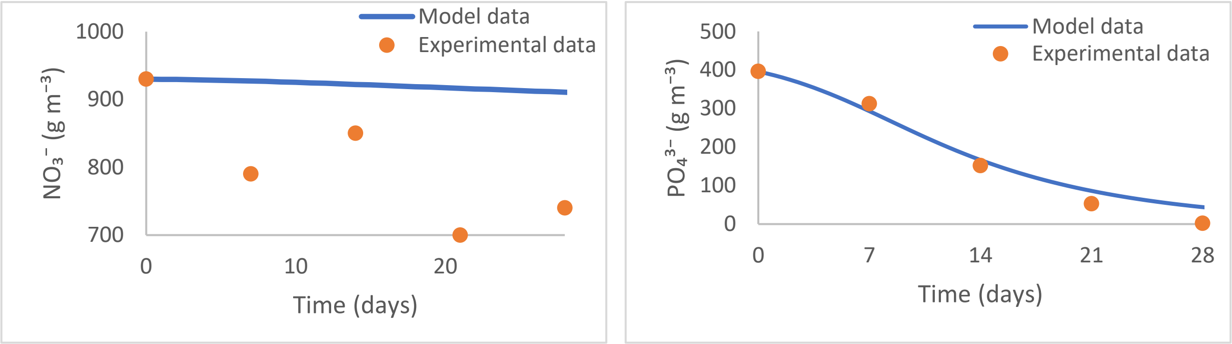
The model data predicts outcomes under controlled conditions over a 28-day period. Figure 4.7 shows the model data graphs with decreasing trends in turbidity, NH_4^+, NO_3^-, PO_4^{3-}, carbon and protein. Turbidity and NH_4^+ concentrations showed a sharp reduction, reaching a 100% removal efficiency before the end of the cultivation period. NO_3^-, on the other hand, showed a less pronounced decrease in concentration with only 2.1% removal efficiency. The algal growth was observed to be increasing steadily to 91.6 g L^{-1} on Day 28.

To further assess the model's accuracy, a Sum of Squares analysis was applied to both sets of data (experimental data and model data). The updated model in Figure 4.8 illustrates significant improved trends over the 28-day period, where a more stabilized decline observed in all parameters. Interestingly, the decline in NO_3^- becomes more consistent (26.4% removal efficiency) compared to pre-optimisation. Additionally, the algal growth curve demonstrates a gradual and realistic increase, reaching an equilibrium state at 50.8 mg L^{-1} by Day 28.



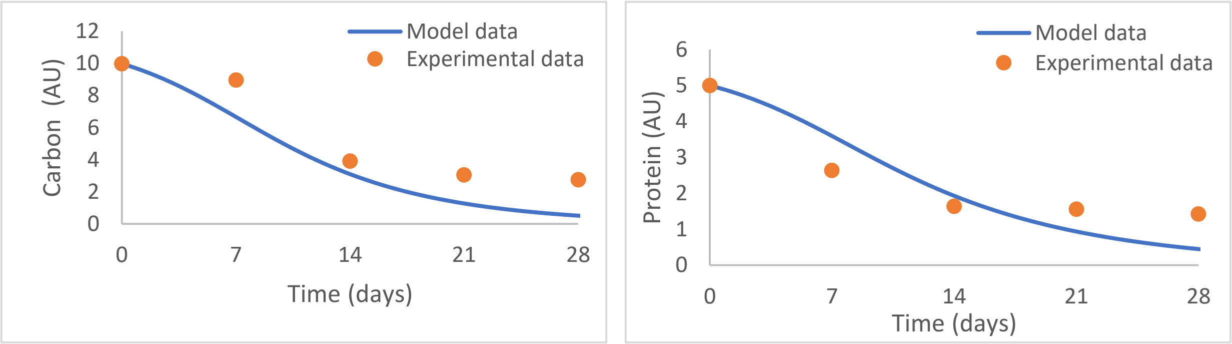
(a) Turbidity

(b) NH_4^+



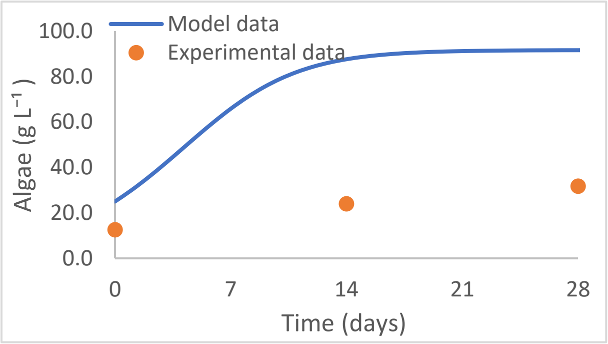
(c) NO_3^-

(d) PO_4^{3-}



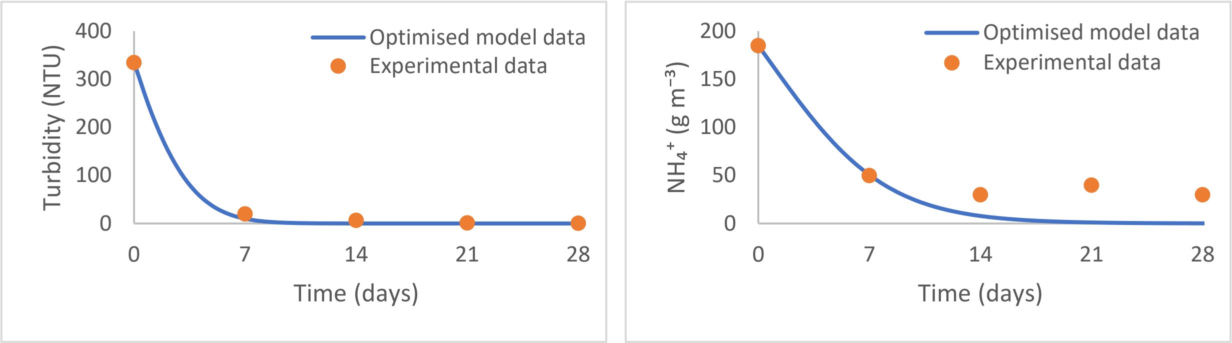
(e) Carbon (215 nm)

(f) Protein (280 nm)



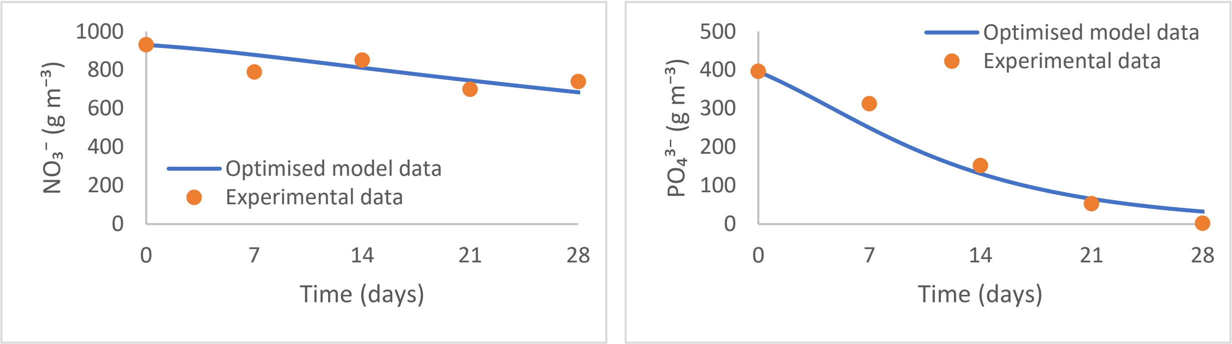
(g) Algal concentration

Figure 4.7: Changes in (a) turbidity, (b) NH_4^+, (c) NO_3^-, (d) PO_4^{3-}, (e) carbon, (f) protein, and (g) algal biomass over a 28-day cultivation period in model data and experimental data.



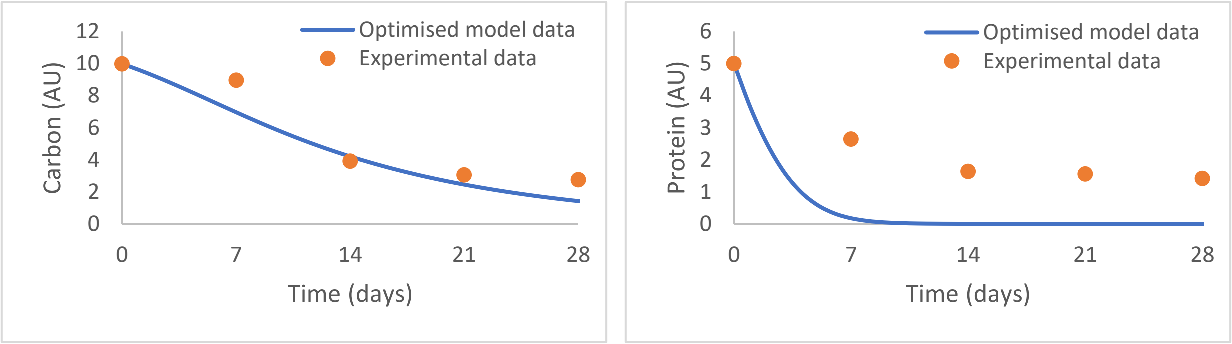
(a) Turbidity

(b) NH_4^+



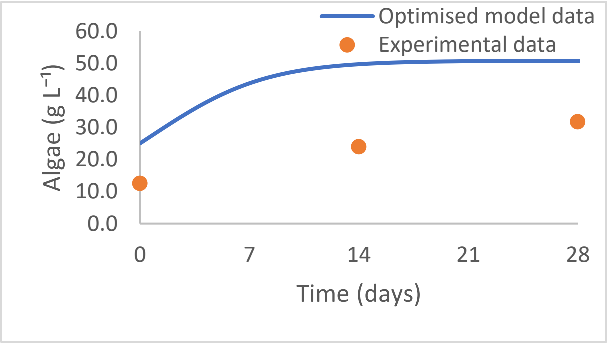
(c) NO_3^-

(d) PO_4^{3-}



(e) Carbon (215 nm)

(f) Protein (280 nm)



(g) Algal concentration

Figure 4.8: Changes in (a) turbidity, (b) NH_4^+, (c) NO_3^-, (d) PO_4^{3-}, (e) carbon, (f) protein, and (g) algal biomass over 28 days in optimised model data and experimental data.

4.7.2 Comparison between experimental data and model data

The experimental data and model data, before and after optimisation, exhibited variations and considerable improvements across different parameters over 28 days (Table 4.3). Carbon and protein removal efficiencies has shown an increase in both models in comparison to experimental data, with 100% of protein was removed in the post-optimisation model. While NH_4^+ removal efficiency remained exceptionally high across three conditions, NO_3^- removal efficiency showed an improvement in the optimised model data at 26.4%. PO_4^{3-} removal efficiency, in contrast, was higher in experimental data (99.4%) than in both model data, with 88.9% before optimisation and 91.8% after optimisation. Like NH_4^+, turbidity removal was consistently high across all scenarios.

The algae concentration as well as the growth rate displayed a contrasting picture, whereby the model data before optimisation showed highest values of algal biomass (91.5 g L^{-1}) and growth rate (1188.1 mg L^{-1} d^{-1}). However, the model data after optimisation predicted substantially lower values of algal biomass (51.0 g L^{-1}) and growth rate (464.3 mg L^{-1} d^{-1}) than in experimental data, with algae concentration of 63.5 g L^{-1} and 687.3 mg L^{-1} d^{-1} growth rate obtained by Day 28.

Table 4.3: Comparison of algal growth and removal efficiency for different parameters between experimental data and model data before and after optimisation for 28 days.

Parameter	**Unit**	**Experimental Data**	**Model Data (Before Optimisation)**	**Model Data (After Optimisation)**
Carbon Removal efficiency	%	72.3	94.9	85.8
Protein Removal efficiency	%	71.6	91.1	100
NH_4^+ Removal efficiency	%	83.8	99.9	99.9
NO_3^- Removal efficiency	%	20.4	2.1	26.4
PO_4^{3-} Removal efficiency	%	99.4	88.9	91.8
Turbidity Removal efficiency	%	99.8	100	100
Algal biomass	g L^{-1}	63.5	91.5	51.0
Algal growth rate	mg L^{-1} d^{-1}	687.3	1188.1	464.3

4.7.3 Farm pond system model

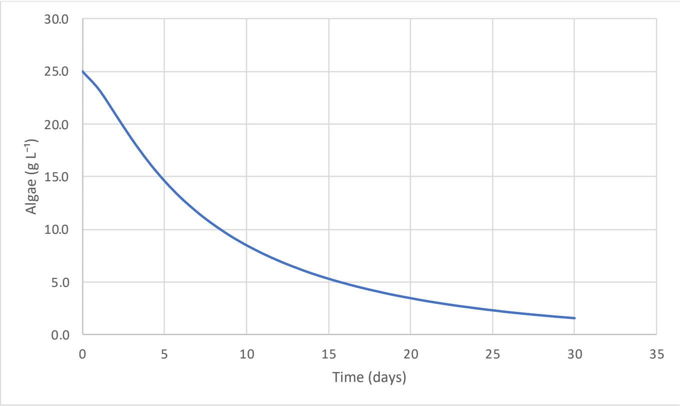
Table 4.4 display removal efficiencies of different parameters and algal growth, respectively, over time in a farm pond system under different HRTs. The removal efficiency for all parameters across HRTs of 2, 10, and 30 days increased as the HRT increased. For a 2-day HRT, the removal efficiencies were significantly low, with NH_4^+ achieving the highest removal at 10.2% and NO_3^- showing no removal. As the HRT extended to 10 days, removal efficiencies for carbon, protein, NH_4^+, and PO_4^{3-} significantly increased, demonstrating enhanced treatment capability. At an HRT of 30 days, the removal efficiencies further enhanced, particularly for NH_4^+ (92.8%) and turbidity (95.7%).

Figure 4.9 illustrates the distinct pattern of algae concentration over time at different HRT settings. The algae concentration showed a decrease with a 2-day HRT, whereas a significant increase in algal biomass to 114.1 g L^{-1} for the 10-day HRT. Further increase of algae growth was recorded at 147.2 g L^{-1} for HRT of 30 days, showing the highest steady-state algae concentration.

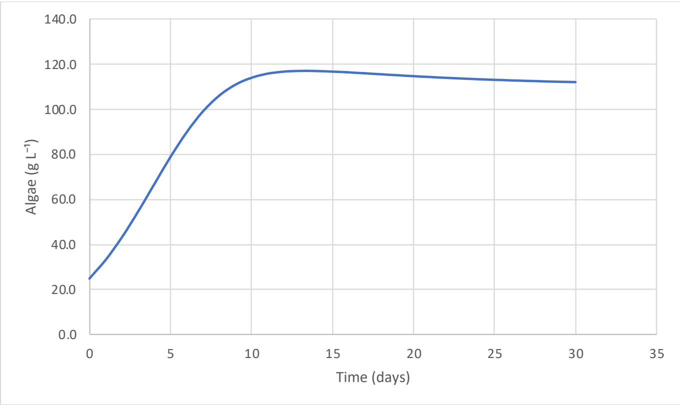
Table 4.4: Removal efficiency of parameters across different HRT in a farm pond system model.

Parameter	HRT=2 days	HRT=10 days	HRT=30 days
Carbon removal efficiency (%)	4.7	48	85.1
Protein removal efficiency (%)	3.9	41.9	82.3
NH_4^+ removal efficiency (%)	10.2	73.2	92.8
NO_3^- removal efficiency (%)	0.0	0.5	2.5
PO_4^{3-} removal efficiency (%)	3.5	39.3	80.8
Turbidity removal efficiency (%)	16.9	84.5	95.7

(a) HRT=2 days



(b) HRT=10 days



(c) HRT=30 days

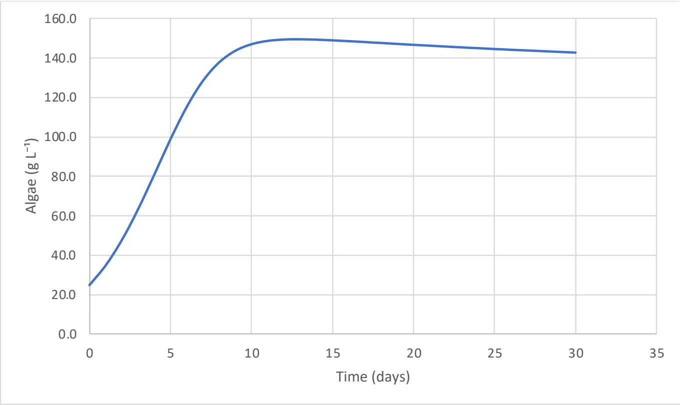


Figure 4.9: Algal growth in in a farm pond system model at three different HRTs: 2 days, 10 days, and 30 days.

Chapter 5: Discussion

5.1 Operational Parameters of Dairy Farm Effluent

The findings in Table 4.1 and Figure 4.1 demonstrate how algal polyculture interacts with and modifies the dairy farm effluent conditions in both autoclaved and non-autoclaved settings. The consistent temperature across both autoclaved and non-autoclaved conditions indicates that temperature control was effectively maintained while ensuring that any changes in nutrient removal or algal growth could be attributed to biological rather than temperature itself. The slight decrease in temperature over the course of the experiment suggests that external environmental conditions may have influenced the dairy farm effluent samples. Nevertheless, the temperature range used in this experiment remains within a suitable range for the algal polyculture E3 growth, which is consistent with the literature review by Singh and Singh (2015) on the optimum temperature range for the growth of different algal species between 20 - 30 °C.

The stability of pH values over time indicates a stabilization of the effluent's chemical environment, which could possibly be due to the buffering action of the algal polyculture from their photosynthetic and respiratory activities or natural equilibration processes for control samples. The initial high pH in Control (A) and Polyculture (A) suggest an adjustment phase where the effluent's physicochemical properties stabilize after autoclave process. Although pH 9 could inhibit certain biological activities in Polyculture (A) but it is typically favorable for algal growth in this experiment as different algal species have different favorable pH for growth. Given that the average pH values were 8.42 for autoclaved samples and 8.16 for non-autoclaved samples, this aligns with Huang et al. (2017)'s assertion that most algal species exhibit optimal growth within a pH range of 8.2 to 8.7, although they can tolerate a buffer range from 7 to 9.

The range of DO levels (4.0-6.1 mg L^{-1}) suggests that aerobic conditions were maintained throughout the experiment, which is crucial for both algal growth and the aerobic microbial processes involved in nutrient removal. Stable or higher DO levels in both Polyculture (A) and (NA) could be associated with the polyculture's photosynthetic activity, i.e., active algal growth and nutrient processing. Such phenomenon is in accordance with multiple literature studies, where a high abundance of microalgae significantly increases nutrient level and DO level (Lee et al., 2015; Renuka et al., 2013).

The overall fluctuation of the turbidity suggests that presence of certain suspended particulate matters which could not be captured or separated from filtration and centrifugation may contribute to such fluctuation. High initial turbidity in Polyculture (A) could result from the introduction of algal cells or other particulates in the polyculture, while the subsequent decrease on Day 7 suggests that algal polyculture E3 is able to reduce turbidity effectively in a sterile environment by utilizing the particulate matter as nutrient source (Renuka et al., 2013). Furthermore, the sharp rise in turbidity in Control (A) on Day 21 could also be due to unavoidable microbial contamination, such as oxygen introduction from the air leading to microbial growth, which could subsequently consume oxygen. Turbidity should ideally remain low to maintain the microbial communities, which depends on adequate light penetration for photosynthesis (Waikato Regional Council, n.d.-c).

The consistent temperature and pH levels across the experiment provide a stable environment that supports the potential efficacy of algal treatment. The changes in turbidity, particularly in polyculture treatments, along with DO fluctuations, reflect the ongoing biological processes, suggesting that algal polycultures can impact effluent characteristics significantly.

5.2 Algal Growth

The results shown in Figure 4.2 highlight the potential of polycultures in enhancing biomass production in autoclaved and non-autoclaved settings, along with the importance of the interaction between inoculated algal polyculture E3 and native microbial communities. A significant enhanced growth seen in Polyculture (A) over 28 days suggests that the inoculation of polyculture E3 may have a positive effect on biomass production in sterile conditions, which aligns with previous finding by Lage et al. (2021) whereby polyculture had higher biomass in treated wastewater. Furthermore, this supports the idea that algal polyculture E3, through their diverse metabolic capabilities, can exploit the sterile medium more effectively through nutrient demand complementarities between species as well as compensation of any catastrophic loss of one algal species by other species, as reported by Pires et al. (2013).

The negative growth during the first phase in Polyculture (NA) highlights the challenges that introduced polyculture E3 face in non-autoclaved environments, which are rich in native microbes. This adverse effect could be attributed to resource competition, or the production of inhibitory compounds by native microbes (Newby et al., 2016). However, the recovery and subsequent growth increase in Polyculture (NA) during the latter phase of the experiment is particularly noteworthy. This recovery may indicate a period of acclimatization where the polyculture starts to adapt to the new environment or establishes a symbiotic relationship with native organisms (Loganathan et al., 2021).

Moreover, the operational conditions of the effluent may provide a suitable environment that supported the growth observed in Polyculture (A) and Polyculture (NA), whereby the enhanced biomass growth could be attributed to the synergistic effects of optimal temperature recorded, a stable pH conducive to algal growth (pH 7 to 9) as well as increased DO from photosynthetic activity over the cultivation period in this study.

Since biomass growth was determined by dry weight estimation, this method does not differentiate between the actual algal biomass and suspended solids, which were not captured during centrifugation and filtration (Becker, 1994), hence this may contribute to the modest growth observed in Control (A) during Day 0-14. The increase in growth also indicates a contamination from the air as the air used for aeration in this study was not sterilized, therefore microbial contamination could be brought into the culture system inevitably (Qin et al., 2016). The presence of native organisms in Control (NA) may contribute to its higher growth rate when compared to Control (A). Factors like depletion of nutrients or accumulation of inhibitory substances may contribute to the decrease in growth rate of Control (NA) in the later growth stage.

Despite minimal biomass growth observed in control samples compared to Polyculture (A) and Polyculture (NA), the introduction of polyculture E3 into both sterilized and non-sterilized environments has demonstrated an increase in biomass growth, further revealing that environments with potentially higher microbial diversity and activity, as evidenced by the Polyculture (NA), lead to enhanced biomass production.

5.3 Nutrient Removal

Nutrient removal efficiency is an important indicator for evaluating the effectiveness of the algal polyculture wastewater cultivation system. From Table 4.2 and Figure 4.3, the high NH_4^+ removal efficiencies in Polyculture (A) and Polyculture (NA) may attributed mostly to the assimilation of NH_4^+ by algae for their growth and metabolic activities. Algae utilize NH_4^+ as a N source, converting it into organic nitrogen compounds (Yan et al., 2013). The presence of additional microbial communities in non-sterile conditions potentially aid in NH_4^+ removal for Polyculture (NA), either by direct uptake or by creating favorable conditions for algal growth (Cai et al., 2013). The removal efficiency in this experiment was supported by San Agustin et al. (2022)'s study where the algal polyculture was able to remove high NH_4^+ in non-autoclaved wastewater. Furthermore, the release of NH_4^+ in the form of gas due to aeration could play a role in the reduction of NH_4^+ over time (Silva-Benavides & Torzillo, 2012; Yan et al., 2013).

The general decrease in NO_3^- levels can be attributed to denitrification process and NO_3^- assimilation by algae in the polyculture, while the variations observed suggests that nitrification and denitrification might happen, along with the influence of environmental conditions. Lower NO_3^- removal efficiency than NH_4^+ removal by Polyculture (A) and Polyculture (NA) in this experiment, was in line with the study conducted by (Ma et al., 2023).

High NO_3^- concentration recorded in Control (A) suggests that the absence of denitrifying bacteria which are responsible in converting NO_3^- to N_2 due to autoclaving. However, there is a potential risk of microbial contamination in Control (A), where the presence of nitrifying bacteria converting NH_4^+ to NO_3^- may attribute to such result. Peter et al. (2000) mentioned that high light intensity might have inhibited bacterial nitrifiers as high irradiations inhibit nitrification process through photo-oxidation of the bacterial cytochrome. While the light intensity used in this experiment was above 150 µmol photons m^{-2} s^{-1} may inhibit nitrification to some extent, it may not completely stop the activity of all nitrifying bacteria (Qin et al., 2016).

NO_2^- levels are generally a result of nitrification, whereby NH_4^+ was first converted to NO_2^- before NO_3^-. Because NO_2^- is very unstable, this may cause the significant variability of NO_2^- levels including the observed spikes in Control (NA) and Polyculture (NA).

Like NH_4^+, PO_4^{3-} assimilation by algal polyculture, where PO_4^{3-} are incorporated into the cellular components for growth, is responsible for the high PO_4^{3-} removal efficiency observed in Polyculture (A) and Polyculture (NA). According to Zhou et al. (2012), the removal of PO_4^{3-} could be considered as assimilation by the microalgae when pH was around 8. Moreover, a study conducted by Yang et al. (2017) found the removal of TP (89–93%) in autoclaved wastewater was as good as that obtained with the non-autoclaved wastewater (96%), which is consistent with the high removal efficiency obtained in this experiment, both autoclaved and non-autoclaved effluent.

Because both control samples, particularly Control (A), demonstrated a decrease in nutrients, the enrichment of the microbial contamination along with the continuous aeration combined with the assimilation of nutrients by algae as well as the operational conditions, could lead to the substantial decline in nutrient concentrations observed for Polyculture (A) and (NA). Nevertheless, the introduction of polyculture E3 in both autoclaves and non-autoclaved settings has shown a remarkable potential in nutrient removal from dairy farm effluent.

5.4 Nutrient Consumption Rate

Results shown in Figure 4.4 indicate the potential of algal polyculture E3 in autoclaved versus non-autoclaved setups in treating various nutrients in dairy farm effluent. The initial high consumption rates observed in both Polyculture (A) and (NA) demonstrate active NH_4^+ consumption process at the beginning of the cultivation period, which suggests that algal polyculture E3 tended to utilize NH_4^+ for cell growth. Over time, Polyculture (A) and Polyculture (NA) maintain a steadier consumption rate, leading to a more sustainable consumption of NH_4^+.

Furthermore, the increase of NO_3^- consumption rate between Day 7 to 21 by Polyculture (A) features the role of algae in NO_3^- uptake, which aligns with findings from Ng and Chan (2021), whereby greater uptake of NO_3^- by polyculture was observed. The high initial consumption rate observed in Control (NA) suggests that some native microbes in the effluent may be effectively reducing NO_3^- through denitrification or assimilation for growth.

Although NO_2^- is generally less preferred by algae compared to NH_4^+ or NO_3^-, the slight increase in NO_2^- consumption rate observed for Polyculture (A) from Day 14 to 28 indicates NO_2^- uptake by algae in polyculture, which diminishes over time. Although very rare, some algal species are able to take up NO_2^-, especially when high NO_2^- is present in the wastewater (Abe et al., 2002). The fluctuation in NO_2^- consumption rates, especially in control samples, could be a sign of nitrification and denitrification processes occurring, which rapidly affect NO_2^- levels in the effluent.

The consistent PO_4^{3-} consumption rate observed in Polyculture (NA), which peaked at 0.83 mg PO_4^{3-} g^{-1} d^{-1} on Day 28, suggests an efficient PO_4^{3-} cycling in the system, for instance, the polyculture E3 could be actively removing higher levels of PO_4^{3-} and storing it as internal compounds for later use (Fallahi et al., 2020). Polyculture (A) had a gradual increase in PO_4^{3-} consumption rate from Day 7 to 21 revealed the algal polyculture able to utilize PO_4^{3-}, without experiencing competition of nutrients with other microbes.

The low nutrient consumption rates observed in both Control (A) and Control (NA) compared to Polyculture (A) and Polyculture (NA) reveal the potential of algal polyculture E3 for effective nutrient consumption in dairy farm effluent, by maintaining sustainable utilization of nutrients especially NH_4^+ and PO_4^{3-} throughout the cultivation period.

5.5 Carbon and Protein

While the absorbance at 215 nm is typically associated with a broader range of organic compounds, e.g., aromatic, and aliphatic carbon, the absorbance 245 nm is more specifically related with aromatic organic compounds like phenols. The decline in carbon content at both 215 nm and 245 nm in all samples indicate effective organic carbon removal from the dairy farm effluent. The Polyculture (NA) showing an increase in carbon content at 215 nm could be due to respiration by both algal polyculture E3 and native microbes, which tend to accumulate the organic compounds. The subsequent rapid decline of carbon suggests the microbes started to breakdown the organic compounds.

Similarly, the decrease in protein observed in Polyculture (A) and Polyculture (NA) reveal that algal polyculture E3 can assimilate the nitrogenous compounds for their growth. As N becomes limited, the algae species begin producing carbohydrates and/or lipids for energy reserves needed to overcome periods of nutrient limitations, whereby protein or peptides are forced to be converted into lipids/carbohydrates (Newby et al., 2016).

Cultivation of algal polyculture E3, both in autoclaved and non-autoclaved conditions, reflects effectual carbon and protein uptake, showing the potential for organic pollutant bioremediation in dairy farm effluent.

5.6 Identification of Microalgae in Polyculture

Five distinct microalgae species present in polyculture E3 were identified according to their morphological characteristics. The identification process in this study is crucial for understanding the ecological roles and potential impacts of these microalgae particularly in dairy farm effluents.

The microalgae depicted in Figure 4.6(a) are likely to belong to the genus *Chlorococcum*, as supported by comparisons with images from Roy et al. (2017) and Tan et al. (2023). *Chlorococcum* sp. is part of the Chlorophyceae class, known for its green algae species. They are typically unicellular, with spherical to oval shape and may aggregate into loose clusters, resembling the formation observed in the image.

The specimen observed feature a unique straight, central raphe within a bilaterally symmetrical, elongated form, suggests the presence of *Navicula* sp. or other types of diatom. The central raphe is known for its unique feature of many diatoms contributing to their silica-based cell wall. This morphology, highlighted by Kilroy and Sorrell (2013), is consistent with the diatom's characteristics shown in Figure 4.6(b).

The microalga in Figure 4.6(c) exhibit features indicative of the genus *Cosmarium*. The specimen appears to showcase the genus's bilateral symmetry with two mirrored semi-cells. The presence of green grass-like chloroplasts further corroborates the potential identification of this organism as a *Cosmarium* sp., as noted by (Roy et al., 2017).

The filamentous, chain-like structures observed in Figure 4.6(d) are indicative of filamentous cyanobacteria and algae, specifically pointing towards the genus *Cylindrospermum*. This genus is characterized by its linearly arranged cells, which are usually larger, round, or oval and transparent. The morphological features, including the straight or slightly curved filaments without significant branching or spiralling, align with descriptions provided in Pal et al. (2022)'s study.

The observed cluster of green and oval-shaped cells, which are neatly arranged in a line, support with the visual characteristics associated with the genus *Scenedesmus* (Roy et al., 2017; Tan et al., 2023). The side-by-side arrangement and the oval cells, typically 2 or more, are key visual indicators of *Scenedesmus* sp. based on known morphological traits.

While the initial identification of microalgae based on morphological characteristics such as shape and structure through light microscopy provides preliminary insights, it is often not sufficient for accurate classification. Therefore, additional analyses, including molecular techniques or advanced imaging methods, are required to ensure precise species identification.

5.7 Model Data

The observed trends in the initial model data suggest good nutrient cycling and algal cultivation under controlled conditions (Figure 4.7), where turbidity and nutrient levels decreased over time while algal polyculture exhibited an exponential increase. However, the overly increase in algal growth as well as insignificant decrease of NO_3^- indicate the discrepancies of the modeled system. This could be due to the model's hypothesis being overly simplified.

Following the sum of squares optimisation in Figure 4.8, the turbidity, along with nutrient concentrations, especially NH_4^+ and PO_4^{3-} highlights the solver's function in adjusting the model parameters to accurately imitate the actual event. The adjusted decline in NO_3^- concentration as well as the moderate algal growth curve may resolve the previous inconsistencies that are more align with empirical findings. The optimisation process, therefore, not only enhanced the model's accuracy but also provided deeper insights into the nutrient-algal interaction dynamics.

5.8 Comparison between experimental data and model data

The observed experimental data and model predictions highlight the importance of optimisation in environmental modeling for wastewater treatment systems. The dramatic increase in removal efficiencies for carbon and protein in the post-optimisation model reflects the model’s improvement, which align with the biological removal processes involving the breakdown and assimilation of organic matter by algal polyculture. The discrepancy in N and P removal efficiencies, especially for NO_3^-, could be due to the experiment taking place in conditions that were not entirely controlled, hence some external factors may influence the nutrient removal. Moreover, the model data did not incorporate some critical parameters such as light limitation, temperature, competition of nutrients, which might lead to the discrepancies noted. For example, the significantly higher algae concentrations and growth rates predicted by the model before optimisation could attributed to overestimation of growth conditions or nutrient availability in the modeled system.

Generally, the discrepancy reveals the complexity of balancing nutrient removal and algal productivity in controlled environments. While the post-optimisation adjustments resulted in a more realistic representation, further research on model predictions and experimental outcomes is required to ensure that models accurately mimic the real-world aquatic conditions.

5.9 Farm pond system model

The farm pond system model focuses the effects of different HRTs on algal polyculture growth and nutrient removal efficiencies. With influent flow rate of 22 m^3 d^{-1}, the size of the algal pond can be determined based on the HRT, resulting in pond sizes of 44 m^3, 220 m^3, and 660 m^3 for HRTs of 2, 10, and 30 days, respectively. According to Figure 4.9, shorter HRTs, particularly the 2-

day setting, were insufficient for substantial algal growth that leads to algae wash out, where the algal polyculture is flushed out of the system faster than they grow (Jalalizadeh, 2012). This subsequently led to lower nutrient removal efficiencies. Conversely, a 10-day HRT shows a pronounced algal polyculture growth, indicating this retention time allows for sufficient growth for the polyculture without the detrimental effects of washout and consequently reaching a stable population. The increase in algal growth demonstrated a positive correlation with removal efficiencies for carbon, protein, NH_4^+, PO_4^{3-} and turbidity as detailed in Table 4.4. However, NO_3^- showed only minimal removal efficiency across all HRTs. This suggests that the complex nature of NO_3^-, where its assimilation is achieved via NO_3^- conversion to NO_2^-, NH_4^+, and amino acids. Supply of N in the form of NH_4^+ involves less energy input because it is already in a reduced form; thus, more straightforward assimilation by algae than NO_3^- (Biswas et al., 2021). Moreover, although the 30-day HRT presents the highest steady-state algal polyculture concentration as well as enhanced removal efficiencies, this longer HRT may not be a cost-effective or efficient choice due to several problems such as nutrient depletion and algal self-shading which eventually reduce the light penetration and overall productivity of the pond system. To sum up, the 10-day HRT may represent a more balanced approach, optimizing both algal growth and nutrient removal efficiency in the farm system, while also acknowledging the need for additional strategies to reduce NO_3^- concentrations.

Chapter 6: Conclusion

This research investigated the effectiveness of algal polyculture in the bioremediation of dairy farm wastewater, focusing on the removal of nutrients along with the significance growth of algal polyculture. The notable aspect of the findings includes the comparative analysis between autoclaved and non-autoclaved conditions. The simulation models comprising of experimental model data and farm system model were also studied. In summary, the findings highlight the potential of using algal polyculture as an effective and sustainable method for wastewater treatment, offering a promising solution to nutrient pollution in dairy farm effluent.

The major findings of the thesis are:

1. The experiment's stable temperature and pH facilitated an ideal setting for evaluating algal polyculture treatment efficacy. The introduction of algal polyculture E3 to both autoclaved and non-autoclaved effluent samples resulted in more pronounced fluctuations compared to the control groups, which highlights the active biological processes, indicating that algal polycultures significantly influence the characteristics of the effluent.

2. The deployment of algal polyculture E3 across autoclaved and non-autoclaved settings resulted in increased biomass (8.07 and 10.13 mg L^{-1} d^{-1}, respectively). This reveals positive effect on biomass production in sterile conditions for Polyculture (A) while the recovery and subsequent growth increase in Polyculture (NA) shows adaptive strength and resilience of algal polyculture E3 in natural settings.

3. Polyculture (A) and Polyculture (NA) demonstrated high nutrient removal efficiencies, especially NH_4^+ and PO_4^{3-}, in dairy farm effluent, highlighting its effective bioremediation and sustainable approach in nutrient management. Polyculture (NA) was able to remove 90.9% of NH_4^+ and 99.1% of PO_4^{3-} in a period of 28 days.

4. High initial consumption rates across all nutrients in autoclaved and non-autoclaved effluent for Polyculture (A) and Polyculture (NA), reflect the effective nutrient utilization for algal polyculture growth. Both polyculture groups experienced NH_4^+ depletion at the end of cultivation period, which signifies effective consumption of excess NH_4^+. Polyculture (NA) showed an almost consistent PO_4^{3-} consumption rate suggests an efficient PO_4^{3-} cycling in the system.

5. The decline in carbon content as well as protein in dairy farm effluent, as evidenced in Polyculture (A) and Polyculture (NA), denote effective organic compounds removal from the dairy farm effluent.

6. Discrepancies between the experimental data and initial model predictions highlight the need for model refinement in nutrient removal efficiencies and algal growth rates. The post-optimisation in the model demonstrated its enhanced capability to replicate biological removal processes more accurately.

7. The investigation of different HRTs revealed that a 10-day HRT optimally balances algal biomass growth with nutrient removal, avoiding issues like washout seen in shorter HRTs and inefficiency in longer ones. Despite improvements, NO_3^- removal remains a challenge across all HRT settings.

6.1 Further Work

Throughout this study, several questions emerged that necessitate through further research. Although the polyculture demonstrated effective nutrient removal and growth in both autoclaved and non-autoclaved settings, the control groups exhibited similar patterns, though less pronounced than in the polyculture samples. This observation requires more thorough sterilization procedures and stricter control conditions. The current experiment relied solely on light microscopy for species identification, investigating the DNA profiles of the algal polyculture as well as determining the ideal species could allow a more effective approach for cultivation. Moreover, adjustment of existing parameters (e.g. optimisation) or additional of parameters like salinity and conductivity, to find the ideal conditions that support the algal growth and effective nutrient removal. Future work towards integrating other factors like temperature, pH, inhibition factors and decay rate in the kinetic expression could lead to a better prediction of algae growth and nutrient removal for simulation modelling.

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