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DECLARATION OF THESIS

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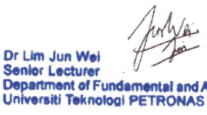

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DEDICATION

I would like to dedicate this thesis dissertation to my late father and family for staying strong and supporting my decision in pursuing further studies.

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ABSTRACT

Microalgae are regarded as potential feedstock for biofuel production while being able to sequester carbon dioxide into valuable bio-products. However, the challenges associated with microalgal cultivation are the high input costs coupled with infeasible integration into wastewater treatment. As such, a novel sequential flow baffled microalgal-bacterial (SFB-AlgalBac) photobioreactor was developed to exploit the synergistic microalgal-bacterial associations for enhancing microalgal biomass production while bioremediating nutrient-rich wastewater. The initial performance of photobioreactor was found to be optimum at the 5.0 L/d influent flow rate with records of the highest microalgal nitrogen assimilation rate (0.0271 /d) and biomass productivity (1350 mg/d). Further increase of flow rate had resulted in poor culture vitality evidenced by the 10% reduction in biomass productivity due to excessive cell washout and hydraulic stress from the continuous flow operation. A dual nutrient heterogeneity mode exploiting the nitrogen transformation and valorization mechanisms was subsequently introduced, resulting in total nitrogen removal efficiency up to 96.38% with maximum microalgal biomass production up to 792 mg/L under a balanced NH_4^+-N (60 mg/d) and NO_3^--N (58 mg/d) loadings. The microalgal lipid extracted via the Bligh and Dyer solvent extraction method using 1:2 (v/v) chloroform/methanol ratio was subsequently transesterified into biodiesel obtaining 228 to 281 mg/mg of biomass. The microalgal biodiesel constituted 97 - 100% in C16 to C18 fatty acid methyl ester (FAME) species thus, conforming to the requirements for quality biodiesel application. The FAME compositions which leaned towards higher unsaturated fatty acid (USFA) fractions lowered the biodiesel pour point, catering for the applications in cold climate regions. Energy feasibility studies revealed highly positive net energy ratio (NER) value (8.38) for producing microalgal biomass. However, the NER value dropped to a low value (0.23) for microalgal-to-biodiesel system, stemming from the high energy inputs incurred in the downstream processes for converting biomass into lipid and biodiesel. Nevertheless, the SFB-AlgalBac photobioreactor was anticipated to exploit the low-cost nitrogen sources from nutrient-rich wastewaters via bioconversion into valuable microalgal biomass while fulfilling the requirements of sustainable wastewater treatment technologies.

ABSTRAK

Mickroalgae merupakan biojisim yang berpotensi untuk pengeluaran biofuel di samping dapat menukarkan karbon dioksida ke bioproduk yang berharga. Walaubagaimanapun, cabaran dalam kultur mikroalgal adalah kos input yang tinggi ditambah dengan integrasi yang tidak dapat dilaksanakan ke dalam rawatan air buangan. Sebuah fotobioreaktor aliran berurutan berasaskan mikroalga-bakteria (SFB-AlgalBac) telah direka untuk mengeksploitasi sinergi mikroalga-bakteria dalam meningkatkan pengeluaran biojisim mikroalga dan merawat air buangan yang mengandungi nutrien. Prestasi awal fotobioreaktor didapati optimum pada kadar aliran influen 5.0 L/d dengan rekod kadar asimilasi nitrogen mikroalga (0.0271/d) dan produktiviti biojisim (1350 mg/d) tertinggi. Peningkatan kadar aliran seterusnya mengakibatkan kultur yang lemah dengan pengurangan sebanyak 10% produktiviti biojisim akibat aliran sel yang berlebihan dan tekanan hidraulik daripada operasi berterusan. Mod heterogeniti dwi-nutrien yang mengeksploitasi mekanisme transformasi dan valorisasi nitrogen kemudiannya diperkenalkan dalam meningkatkan kadar penyingkiran nitrogen sehingga 96.38% dan pengeluaran biojisim mikroalga yang maksima sehingga 792 mg/L dengan kadar NH_4^+-N (60 mg/d) dan NO_3^--N (58 mg/d) yang seimbang. Lipid yang diekstrak melalui kaedah pengekstrakan pelarut Bligh dan Dyer dengan nisbah kloroform/metanol 1:2 (v/v) seterusnya ditransesterifikasikan kepada biodiesel dalam 228 hingga 281 mg/mg biojisim. Biodiesel yang diekstrak daripada biojisim mikroalga membentuk 97 - 100% spesies C16-C18 asid lemak metil ester (FAME). Justeru, mematuhi keperluan untuk aplikasi biodiesel yang berkualiti. Komposisi FAME yang lebih kepada pecahan asid lemak tak tepu (USFA) boleh merendahkan takat tuang biodiesel dan sesuai untuk diaplikasikan di kawasan iklim sejuk. Kajian kebolehlaksanaan tenaga mendedahkan nilai nisbah tenaga bersih (NER) yang sangat positif (8.38) dalam menghasilkan biojisim mikroalga. Walaubagaimanapun, nilai NER ini menurun kepada 0.23 untuk sistem mikroalga-ke-biodiesel yang berpunca daripada input tenaga yang tinggi dalam proses hiliran untuk menukar biojisim kepada lipid dan biodiesel. Namun begitu, fotobioreaktor SFB-AlgalBac dijangka dapat mengeksploitasi sumber nitrogen kos rendah daripada sisa air buangan melalui biokonversi kepada biojisim mikroalga sambil memenuhi keperluan teknologi rawatan sisa kumbahan yang mampan.

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LIST OF ABBREVIATIONS

COD	Chemical Oxygen Demand
FAME	Fatty acid methyl ester
HRAP	High-rate rate algal pond
HTL	Hydrothermal liquefaction
NER	Net energy ratio
NH_4^+-N	Ammonium-nitrogen
NO_2^--N	Nitrite-nitrogen
NO_3^--N	Nitrate-nitrogen
PtCo	Platinum-Cobalt scale
SFA	Saturated fatty acid
USFA	Unsaturated fatty acid

CHAPTER 1

INTRODUCTION

1.1 Research Background

Sustainable living has been one of the main goals towards achieving global development as our needs and demands for such resources are increasing with the growing global population. Energy has been one of the most widely used resources, e.g., from generating heat and electricity in industries and homes to powering transportations, tends to be derived from non-renewable resources such as fossil fuels which are continuously depleting with the increasing energy needs and consumption. As such, renewable yet sustainable energy sources are being explored as alternatives to the non-renewable energy sources. Of having little sustainable alternatives, the world is turning to biofuels for answers to global energy woes. The well-known biofuel, i.e., biodiesel, is conventionally recognized to have combustible potential comparable with fossil fuels in addition to its renewability for incessant applications. Biofuels are derived from renewable fuel sources, e.g., various organic matters which can be categorized into two main categories, namely, primary and secondary biofuel sources. The primary sources use unprocessed organic materials directly as a fuel, whereas secondary sources are the resulting fuels (e.g., ethanol, biodiesel, etc.) produced from processing of various biomasses and used to power vehicles or for industrial applications [1]. Biodiesel in general is a synthetic diesel-like fuel composed of a mixture of fatty acid methyl ester (FAME) with quality commonly satiating the requirements decreed by either the European (EN 14214) Standard or American Society for Testing and Materials (ASTM D-6751) Standard. Biodiesel production has been gaining prominence as an alternative liquid fuel source and this is evident by the surge of biodiesel production demand throughout recent years where there is an increase of 13% in 2019 to 47.4 billion litres [2]. At present, oil products account for about 93% of energy consumption in the transport sector of which biofuels (bio-ethanol and biodiesel) contribute to 3% of the global transport fuel [2]. As biodiesel is a synthetic

diesel-like fuel, it can be directly used as a fuel or blended with petroleum diesel and used in diesel engines with little or no modification [3], [4]. In fact, the mechanisms and infrastructures needed for biodiesel applications have already existed and continuously developed, besides being simulated in existing diesel engines [5], [6]. The attractiveness of biodiesel in comparison with the conventional petroleum diesel fuel is that its applications are much safer and cleaner for the environment. While having virtually similar technical properties as diesel fuel, biodiesel is more advantageous due to its portability, ready availability, renewability, higher combustion efficiency and lower sulphur and aromatic contents [3]. Biodiesel also renders greater reductions in particulates and carbon monoxide upon the combustion as opposed to diesel fuel. Studies into evaluating the performances and emissions of pure biodiesel and blend mixtures with diesel oil in the non-modified and modified compression ignition engines have been implemented and reported in literatures [7]–[10].

Studies and developments into finding suitable renewable energy sources have brought us to the third generation of biofuels in which the sources are of microorganism feedstock origins particularly, the microalgal feedstock which had been hailed as the "supernova" of biofuels offering advantages over its predecessors for having higher oil yield to land usage efficiencies and of non-edible oil feedstock origin. Microalgae are photosynthetic microorganisms which just like any other higher plants, conducts photosynthesis for energy bioconversion into carbohydrates, proteins and lipids albeit at unicellular levels. Coupled with its ability to sequester atmospheric CO_2 into their cells, while storing lipid which in turn can be converted into biodiesels, the microalgal feedstock is no doubt, regarded as a clean energy source for possible commercialization [1],[2]. While microalgae have its own prospects, commercialization remains one of the main challenges due to its high input requirements, making both the upstream and downstream processes costly [12]. As the efforts into making microalgal feedstock more viable to be cultured both economically and environmentally sustainable, microalgal cultivation has been exploited into wastewater treatment as means to compensate for the nutrients feed and supply, owing to the presence of organic nutrients (nitrogen and phosphorus) availability in wastewaters.

Microalgal-bacterial cultivation system has been garnering attention as an alternative to the traditional wastewater bioremediation, i.e., relying either on the conventional activated sludge system or microalgae-based wastewater treatment system such as the high-rate algal ponds (HRAPs) and conventional photobioreactors (PBRs). The idea of introducing microalgal cultivation into wastewater treatment facilities is mainly due to the ability of microalgae to biologically abate the organic nutrient sources particularly nitrogen and phosphorus in wastewaters. Thereby, treating the wastewaters while also producing biomass for eventual biofuel production [13], [14]. Thus, saving the input costs incurred by the dedicated microalgal cultivation system. Besides, co-cultivation between microalgae and bacteria consortiums has become another viable option into further improving or optimizing the microalgal cultivation process. The approach behind this microalgal-bacterial cultivation system revolves around the symbiosis between the microalgal and bacterial populations, whereby there is the simultaneous exchange of organic and inorganic nutrients in mixed microbial consortium, resulting from the respective biological metabolisms performed by the microalgae (photosynthesis) and bacteria (respiration) [15]–[17]. As there is an effective exchange of such nutrients in the mixed microbial consortium, the biomass generation could be further increased, making the microalgal-bacterial system more viable option in generating biomass for biofuel production as compared with single strain culture.

Considering the microalgal-bacterial system approach, this study integrated microalgae into the conventional activated sludge process; thus, creating a mixed microalgae-activated sludge consortium capable of enhancing the nutrient removal efficiencies. The nutrients loaded in wastewater would be directly assimilated into the microalgal-bacterial biomass in this regard. The inorganic nutrients produced from the symbiosis system would also increase the respective microbial populations due to the enhancement of biological activities in the mixed microbial consortium. The change in population growth kinetics was dependent on the availability of the transformed and valorised forms of organic and inorganic nutrients, leading to the surge of biomass growth. As such, the characteristics of nutrient removal and/or assimilation by the microalgal while employing the symbiotic algal-bacterial system in the bioreactor would give further insights into the population growth kinetics. Hence, enabling

insights into predicting the optimum cultivation conditions for microalgal biomass growth in relation to the nutrient uptake and availability in wastewater.

To date, microalgal-based biofuel systems are not widely adopted due to many factors questioning on the economic feasibility and viability. While developed countries began to adopt microalgae technology to produce biofuels, more sustainable approaches in reducing the necessary input costs incurred are being researched into making the entire microalgal biorefinery systems more attractive to be adopted in terms of feasibility and viability along with carbon-zero initiative. Viewing in terms of sustainability and economic viability, the microalgal-bacterial system approach is expected to reduce the overall footprint, i.e., by incorporating microalgal cultivation into the existing activated sludge bioreactor, as well as nutrient input costs incurred by the conventional microalgal cultivation system. Also, the synergistic effects from the microalgal-bacterial symbiosis could be exploited in enhancing the microalgal biomass productivity via effective nutrient valorisation sourced from non-potable waters, i.e., wastewaters. Hence, making the entire microalgal technology more readily adoptable into existing wastewater treatment plants in line with sustainable oil-rich biomass production for feasible energy biorefinery systems.

1.2 Problem Statement

Microbial based feedstock particularly microalgae have been reported as a potential biofuel source benefitting from its multiple merits. However, the upstream processes required in maintaining a high, yet consistent biomass production remained a challenge in making it economically feasible. As such, microalgae cultivation is integrated into wastewater treatment facilities as a measure to reduce the input cost requirements. While there were studies into optimizing either the existing microalgae or conventional activated sludge (sequencing batch reactors) systems, there is still little to no development over a specific bioreactor catered to support microalgal-bacterial co-cultivation. Most studies focussed on optimizing the parameters which could exploit the microalgal-bacterial symbiosis relationship but the advances into developing an exclusive bioreactor for microalgae-bacterial co-cultivation is still close to none. At

best, there are studies in modifying existing bioreactors in ways to optimize the microalgal-bacterial system, e.g., photo-sequencing batch reactors, membrane sequencing batch reactors and biofilm reactors or carriers. However, concerns over the possible demerits of mixed microbial interactions were not addressed. For instance, both the microalgae and bacteria would pose detrimental effects to each other due to the production of inhibitory metabolites that may inhibit bacterial activity as well as the release of algicidal extracellular substances by the bacteria if the population ratio of microalgae and bacteria in the mixed consortium is not monitored and controlled carefully. To redress, this study proposed a new bioreactor design capable of supporting the microalgal-bacterial co-cultivation by exploiting the respective biological metabolisms in the microalgal-bacterial symbiosis in an integrated yet separated flow mode.

The mixed microbial interactions in the microalgal-bacterial co-cultivation have been proven to promote microbial biomass growth due to the exchange of organic and inorganic nutrients. However, the characteristics of the nutrient uptake and/or valorisation and interactions between the microalgae and bacterial activated sludge particularly the nitrifiers, required fundamental investigations to exploit the biological pathways in facilitating nutrient transfer and uptake efficiency. Microalgal growth kinetic models would be able to give an insight into the microalgal biomass production in relation to the nutrients of interest which is crucial in identifying and predicting the rate of nutrient removal, biomass growth and optimization of operating conditions. However, the heterogeneity of the nutrients in the wastewater may complicate the application of conventional kinetic models especially single substrate models due to the various nutrient availability in the medium coupled with other abiotic parameters. As such, the conventional kinetic models must be improved to better cater the targeted nutrients of interest under various conditions depending on the operating nature of an exclusive bioreactor.

The newly developed microalgal-bacterial bioreactor would be assessed for its economic feasibility in view of energy assessment for producing microalgal biomass, lipid, and biodiesel. To date, conventional microalgae cultivation systems, i.e., photobioreactors, open raceway ponds and high-rate algal ponds are the existing model

standards for microalgal biofuel biorefinery systems. The feasibility of these cultivation systems is often evaluated for energy or techno-economic assessments to further improv the entire microalgal biorefinery system in making biofuels production more competitively sustainable and feasible. In this case, the development of hybrid bioreactors, i.e., the microalgal-bacterial system would be assessed for its energy feasibility for potential commercialization in comparison with the conventional systems. As described previously, the approach of exploiting the microalgal-bacterial symbiosis in the form of a new hybrid bioreactor in the study is expected to edge out the conventional systems in terms of ease of technological adoptability and cost-competitiveness from wastewater bioremediation.

1.3 Research Objectives

The followings were the objectives achieved throughout the research study on the development of a new bioreactor for the inoculation of activated sludge and microalgae to simultaneously bioremediate wastewater and produce biodiesel.

1. To design a new bioreactor capable of integrating microalgal-bacterial activated sludge system for the simultaneous wastewater bioremediation and biomass generation leading to lipid for biodiesel production.
2. To remodel the population dynamics of microbial kinetic growths between the microalgae and bacterial activated sludge inoculating in newly designed bioreactor.
3. To evaluate the life cycle energy feasibility of the newly designed bioreactor for microalgal-bacterial activated sludge cultivation in comparison with the conventional microalgae systems in producing biodiesel.

1.4 Scope of Study

This study was centered on the applicability of newly designed bioreactor in supporting the cultivation of mixed microalgae and bacterial activated sludge whilst bioremediating wastewater and producing microbial biomass for eventual biodiesel production. The symbiosis between the microalgae and bacteria (activated sludge) communities was as well investigated to further understand how the biological interactions in the microalgal-bacterial consortium works. The synergistic associations between the microalgae and bacteria consortia involved prior nitrification in converting the NH_4^+-N species into oxidized nitrogen species (NO_2^--N and NO_3^--N) by the nitrifiers in activated sludge and assimilated directly by the microalgae consortia termed as the nitrogen nitrification-assimilation pathway. The bioreactor was designed in a such a way which direct the wastewater influent into designated columns separating the two consortia while exploiting the nitrogen nitrification-assimilation mechanism pathway in the microalgal-bacterial symbiosis allowing for more efficient nutrient removal and microalgal biomass production in a continuous manner. As the bioreactor was designed to accommodate a continuous flow mode, the total nitrogen nutrient concentrations introduced into the bioreactor was primarily investigated via adjusting the influent flow rate as well as diverting the flow partially in optimizing the ideal nitrogen nutrient growth conditions for maximum microalgal biomass production.

While factoring in the biological metabolism response merits of the microalgal-bacterial symbiosis, the existing microbial kinetic growth models were rederived for further simulation and optimization of the newly designed bioreactor. As such, the kinetics centering around the nutrient transfer from the bacterial activated sludge community into the microalgae in relation to their biomass growths were investigated in improving measurable input key parameters of existing kinetic models. The nitrogen nutrient was difficult to be removed from wastewater via conventional means and often relied on biological remediation process for removal. In this case, the biological pathways involving nutrients transfer, particularly the nitrogen species in relation to the population growth of the microalgal-bacterial consortium were investigated.

Other scope in this study included assessing the energy feasibility in terms of energy efficiency ratio as part of life cycle energy analysis of the newly designed bioreactor in comparison with the conventional microalgae cultivation systems. A multiple cradle-to-cradle system boundaries were identified consisting of microalgae-to-dry microalgal biomass and microalgae-to-lipid forming up the microalgae-to-biodiesel as cradle-to-grave system. The energy feasibilities of these bioenergy products were evaluated in terms of selected main energy output with the total energy inputs incurred in the respective system boundaries. Hence, allowing projections into the energy feasibility and identification of the energy demands required in each process incurred in the respective system boundaries.

1.5 Novelty of Study

Owing to the success of this research, a new bioreactor designed capable of integrating microalgae and bacterial activated sludge has been fabricated for wastewater bioremediation, while producing microbial biodiesel. The bioreactor was designed in such a manner which integrates the microalgal-bacterial consortium while keeping them separated via dedicated and designated flow columns in the bioreactor without any usage of separation nor purification layers. The proposed novel flow design would enable microalgal cultivation to be integrated into the secondary activated sludge process thereby, exempting the necessary denitrification process for converting the oxidized nitrogen species into atmospheric nitrogen but as low-cost nutrients for the microalgae in producing biomass while bioremediating nitrogen-rich wastewaters. Indirectly, paving the way for possible future commercialization of biofuels. In addition, a kinetic equation capable of describing the relationship between the nutrient uptake or removal and microbial biomass growth has been remodeled for further applications in bioremediating different types of nutrient-rich wastewaters, as well as for future upscaling intentions. The energy feasibility of employing the new bioreactor is as well vindicated through the life cycle energy analysis. Thereby, the newly designed bioreactor is suitable to be integrated into existing conventional wastewater treatment facilities.

CHAPTER 2

LITERATURE REVIEW

2.1 Wastewater treatment technologies

Wastewater treatment is mainly divided into three primary categories which are physical, chemical, and biological treatments (Fig. 2.1). As the primary objective of wastewater treatment is to remove contaminants to safe discharge levels, be it mineral nutrients, heavy metals or toxic compounds, each of these treatment methods are derived and integrated into the primary, secondary and tertiary treatment systems for effective and systematic removal of contaminants [18]. The physical treatment which utilizes mechanical forces, e.g., floatation, sedimentation, filtration etc., in removing the contaminant is the most basic method. Next, there is the biological treatment method which utilized microorganisms, e.g., bacteria, in breaking down organic matters and removing mineral nutrients from wastewater in normal cellular processes. On the other hand, the chemical treatment method as the name derives, involves the usage of chemicals mainly in expediating contaminant removal, e.g., precipitation, coagulation, oxidation, etc. Among the three treatment methods, the biological treatment method is the most effective method when considering environment and economic viewpoints as compared with the many of the physical and chemical processes. However, as the biological treatment method relies strongly on the performance of the microbial community, the inconsistent wastewater composition and presence of detrimental contaminants could very well affect the microbial population in the system.

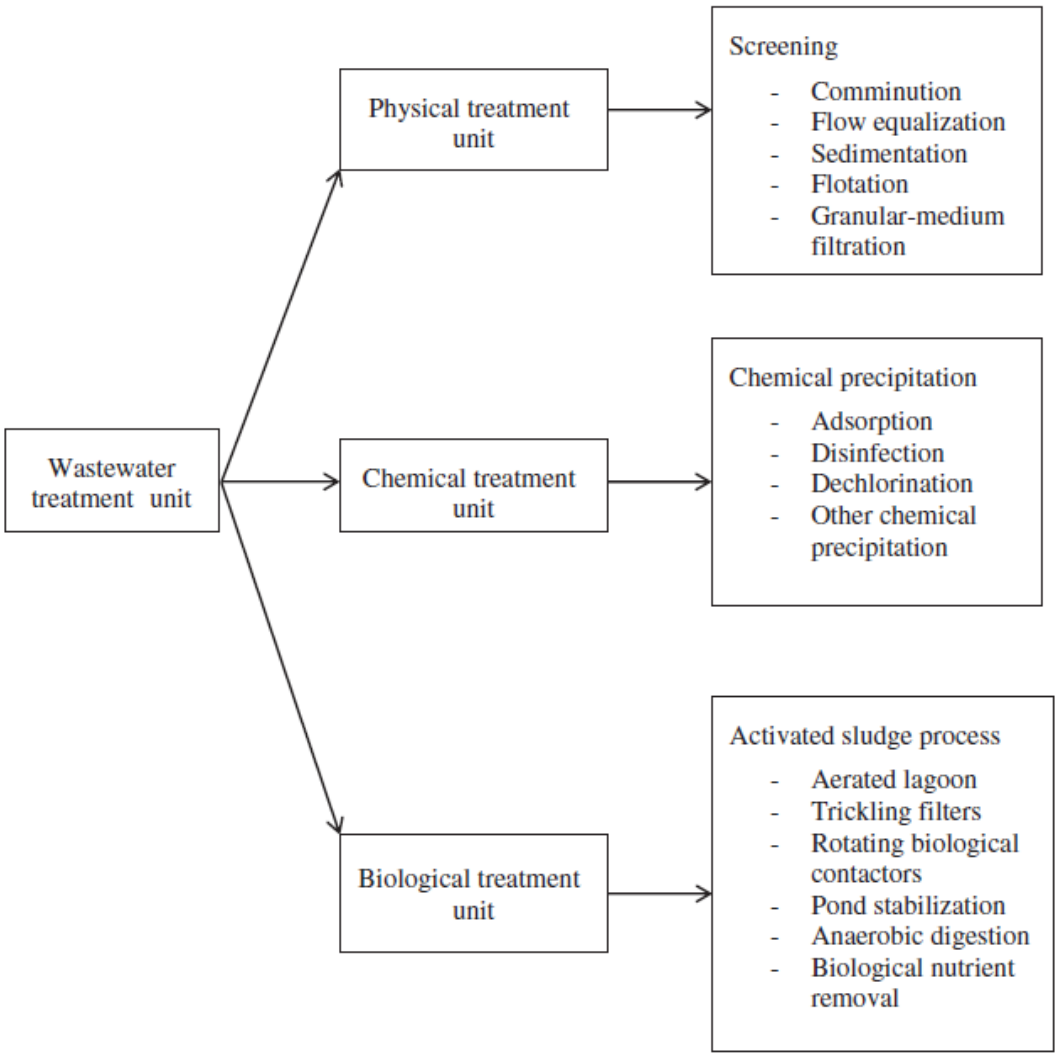


Figure 2.1:Wastewater treatment process [18]

2.1.1 Activated sludge process

A conventional activated sludge process deals with the treatment of various types of wastewaters from sewage to industrial discharges. As of now, there are many types or variations of activated sludge process designs such as package plants, oxidation ditch, deep shafts, and surface-aerated basins [19]. However, a functional activated sludge process in principle consists of three main components, namely an aeration tank

as bioreactor, a settling tank (clarifier) for the separation of activated sludge solids and treated or discharged wastewater and a return activated sludge equipment in transferring the previously settled activated sludge solids from the clarifier back to the aeration tank (Fig. 2.2) [19].

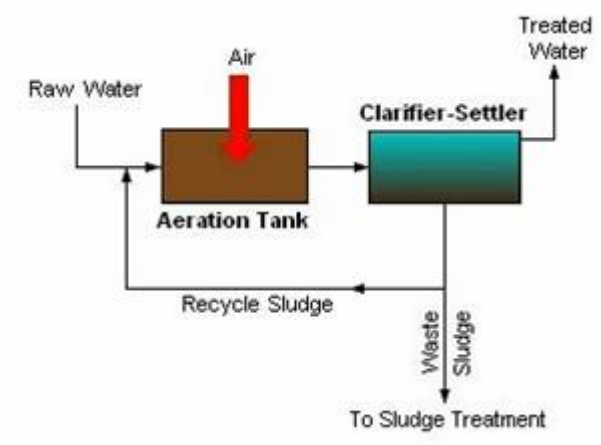


Figure 2.2: An activated sludge process [19].

The activated sludge process starts with the mixture of the raw sewage or influent wastewater and the biological microorganisms commonly known as activated sludge. The mixed liquor is then subjected to aeration with atmospheric air (oxygen). The biodegradable components in the influent will be reduced due to the biological processes occurring in the aeration tank. The mixed liquor will eventually be siphoned into the settling tank where the supernatant (now treated wastewater) is discharged into either a natural water source or undergo further treatment depending on the type of wastewater treatment facilities. The settled activated sludge solids are returned to the aeration tank to re-seed the new influent sewage or wastewater, ensuring a desired mixed liquor suspended solids (MLSS) concentration in the aeration tank. Eventually, the excess sludge will accumulate beyond the desired MLSS concentration in the aeration tank due to the biological growth. The excess solids known as waste activated sludge, have to be removed from the system in maintaining the balance of food to microorganisms (F:M) ratio in an appropriate range. These waste sludges will then be

stored away prior to land disposal but with proper management of such waste. It also could be potentially further utilized as useful by-product such as feedstock for biofuel production in curbing the rising energy production woes.

2.1.2 Microalgae-based wastewater treatment

Presently, there are many studies and developments into utilizing nutrient-laden wastewaters to serve as substitutes for water and nutrient resources in cultivating microalgae. In an attempt to reduce the energy input requirements particularly the nutrient sourcing for microalgae cultivation, algal wastewater treatment was initially proposed by Oswald et al. [20]; exploiting the potential of microalgae cultivation in wastewater medium. Since the wastewaters contain elevated levels of organic nutrients especially nitrogen and phosphorus elements, it serves as available nutrient sources for microalgae growth while also treating the wastewaters at the time. As a consequence, microalgae cultivation using wastewaters is judged to have economic and environmental prospects in producing biomass for biofuel industries while also biologically abating the organic nutrients in wastewater [13], [14], [21]. This strategy works in tandem with the treatment of wastewaters in which the removed nutrients will be assimilated into the microalgae biomass [14], [22]–[24]. This is especially advantageous for remediating nitrogen from wastewater as nitrogen could only be removed via biologically means unlike phosphorus which could be removed via physio-chemical processes, i.e., precipitation, adsorption and ion exchange thus, rendering ease and cost-effective phosphorus recovery and removal [25].

Albeit municipal and agricultural wastewaters do contain high amounts of nutrients required for microalgae growth, utilizing of these waste streams would incur high risks of contamination from potential pathogens, chemical compounds and heavy metals which could be detrimental to microalgae cells. Therefore, investigations into understanding the ecology as well as the pathology of microalgae cultivation ponds are

important to reduce the risks of contamination for further enhancement of commercialization, risk mitigation and improvement of bioremediation strategies.

2.1.3 Microalgal-bacterial consortium-based wastewater treatment

Co-cultivation between bacteria with microalgae has been recently explored as an alternative to bioremediation of wastewaters along with biomass generation for biofuel production. It has been reported that the co-cultivation between microalgae and bacteria had recorded improved nutrient uptake or removal coupled with improved biomass generation due to the symbiotic relationship between microalgae and bacteria. Symbiotic bacteria such as *Flavobacterium* sp., *Azosprillum* sp., *Azobacter* sp. and *Microbacterium* sp. were reportedly utilized in microalgal cultivations to enhance growth [26], [27]. The symbiotic relationship between microalgae and bacteria has been closely linked to the simultaneous exchange of organic and inorganic nutrients between the photosynthesis and respiration processes performed by the mixed microalgae and bacterial consortium respectively [15], [26], [28]. For instance, the co-culture of *Chorella vulgaris* with *Microbacterium* sp. had recorded an additional increase of 54% in dry cell weight and 24% in chlorophyll a content as compared with pure microalgae culture [26]. While most previous works had been reporting on the feasibility and final effects of practical application through algae-bacterial symbiosis, there is not much information available in identifying the mechanism of nutrient uptake or interactions between the co-cultured microorganisms in relation to the microbial population dynamics.

As an effort in improving the sustainability and economics of biofuel production from microalgae cultivation, utilization of wastewater as nutrient source for microalgal cultivation have been widely explored. However, microalgal cultivation system using wastewater mediums often have drawbacks as microalgae are very dependent on the nutrient compositions (nitrogen and phosphorus) available and might result in poor

growth due to insufficient nutrient conditions in maintaining the culture. Besides, maintaining a pure microalgae culture is rather difficult as wastewaters often have various microorganisms present that may very well provide contamination risks if not handled properly. As such, the microalgal-bacterial culture system is adopted and integrated with wastewater treatment instead of relying on sole microalgal cultivation system in wastewater treatment. A study conducted by Su et al. [29] revealed that the microalgal-bacterial culture recorded higher treatment efficiencies and removal rates in comparison with either culture of sole microalgae or bacteria. The microalgal-bacterial culture also recorded the highest COD, total nitrogen and phosphorus removal efficiencies (91.2%, 91.0% and 93.5%, respectively) within 10 days in their study. Narrowing into wastewater treatment facilities, microalgal-bacterial culture system which comprises of wastewater-tolerant microalgae strains and bacterial consortium in the activated sludge are explored for their synergistic effects in bioremediating wastewaters while amassing useful biomass for eventual biofuel production. It had been proven that the mixed algal-bacterial consortium had significant effects in improving carbon and nutrient removal in treating wastewater and biomass accumulation for lipid-based biofuel production [30]–[32]. As of late, the potential of the algal-bacterial co-cultivation system has been explored by varying and optimizing the culture conditions in an effort to further enhance the biomass productivity for lipid-based biofuel production. For instance, Lee et al. [15] found out that photoperiod control had significant effects on the mixed algal-bacterial consortium in which the ratio of nitrogen to phosphorus removal would decrease under prolonged dark conditions and vice-versa. On the other hand, Tsioptsias et al. [32] introduced biofilm carriers and electrocoagulation into the combined microalgae-activated sludge system which had resulted in high COD and total nitrogen removal rates (75% and 35%, respectively) due to the enhancement of microalgae growth.

2.2 Microalgae as third generation biofuel feedstock

2.2.1 Transition from first generation to third generation biofuel feedstock

Starting with the first generation of biofuels, the sources are derived from edible oil bearing crop plants such as palm oil, corn, soybean, sunflower, etc. [12]. However, there are serious concerns over food-versus-fuel debates when it comes to the first generation biofuels [1], [12], [33] . Issues over feedstock sourcing, impact on biodiversity, land availability for growing agricultural crops, and global food crisis are among the firm criticisms lambasted by environmentalists and non-government organizations [34], [35].With that, lignocellulosic feedstock from plant biomasses came into the development of second generation biofuels [1]. Indeed, the second generation biofuels cover a wider range of feedstock in the sense that they are mainly derived from non-edible feedstock such as lignocellulosic plant biomasses, agriculture residues (e.g., bagasses, straws, etc.), and waste products such as waste cooking oil [33]. These feedstocks are advantageous because it can counter the food-versus-fuel issues present in the first generation biofuels. Also, the examples of plants bearing oil-seeds that have been categorized under the second generation biofuels as well are *Jatropha curcas*, *croton megalocarpus*, *cerbera manghas*, etc. These plants were found to be an attractive alternative feedstock for biofuel production due to its similarities with the edible oils and capabilities to grow under non-arable lands, making way for effective land utilization [12], [33]. Nevertheless, growing these plants for second generation biofuels would entail regular irrigation and nutrient replenishment with good management practices in ensuring consistent oil yields [12], [36]. As a matter of sustainability, these feedstocks are not economically and practically viable for stable energy supply due to their low conversion rates and lack of sourcing materials [4], [10].

Microalgae are lauded to be a potential alternative feedstock for biofuel production as they are a sustainable energy source and do not compete with other edible feedstocks.

Cultivation of microalgae also creates a carbon sink for greenhouse gasses thus, making it greener in nature. Of all the lignocellulosic feedstocks, microalgae tend to be highly sought after in the biofuel production industries due to their ability in accumulating lipid up to 70% of their biomass weight while having a faster yet higher growth rate in comparison with terrestrial plants [12], [37]. Besides, the residual microalgae biomass also could be further converted into other value added products such as bio-methane, bio-oil, bio-ethanol, bio-hydrogen, etc. through series of biorefinery processes [37].

The annual productivity and oil content from microalgae are far superior than that of any oil-seed crop. For instance, a high-yielding oil crop i.e. oil palm would need 24% of total cropping area to meet 50% of the U.S. transport fuel demands whereas microalgae would only need 1 - 3% total cropping area in meeting the exact same demands for transport fuel; thus, cultivating microalgae in the same acreage devoted to oil crops would have easily sated the demands for petroleum diesel fuel usage, even with the modest microalgae productivity [38], [39]. Tilman et al. [40] termed the 'biofuels done right' to be feedstock with low greenhouse gas emissions while having little to no food competition. In this case, microalgae is proven to be favorable as it made way for an effective land utilization through degraded or abandoned land usage. This can minimize the direct and indirect land-clearing associated to biofuel expansion which would potentially lead to the creation of long-term carbon debt and biodiversity loss. Concisely, these benefits are impossible to be materialized with first and second generation biofuel feedstock [40], [41]. By taking sustainability and renewability into account, microalgae cultivation would be a more suitable and realistic approach towards future biodiesel production. However, the biofuel production from microalgae feedstock is still remained questionable since the intensive energy input is necessary resulted from nutrient supplementation, downstream processes involved, etc. As of now, commercialization of microalgae-based biofuel is still underway in further optimizing the available cultivation systems such as raceway ponds and closed photobioreactors in a cost-effective manner for biofuel production.

2.2.2 Microalgae cultivation systems

2.2.2.1 Open systems

Open microalgae cultivation systems are the typical "open" ponds that are freely exposed to the surrounding or external environmental conditions while being used for microalgae cultivation. Typical examples of open cultivation systems include raceway pond, shallow pond and circular pond whereby, the raceway type is the commonly preferred system employed to cultivate microalgae [4]. The raceway pond is generally comprising of single or multi- 'raceway' tracks or pathway channels where the mixture of microalgae, water and nutrients is circulated by the installed paddlewheel to promote the microalgae agitation and suspension in water as well as CO_2 utilization from the external atmosphere [4], [42] (Fig. 2.3). The depth of raceway ponds is designed to be shallow (up to a maximum of 70 cm in depth) to enable an efficient light penetration into the pond for maximum light capture by the microalgae to carry out photosynthesis process [43]. The nutrient medium will be continuously supplied at the front of the paddlewheel containing the microalgae culture (beginning of the flow) and eventually the microalgae biomass harvesting will be performed behind the paddlewheel (once the circulation of loop is completed) [18], [42]. The power requirements for the open raceway pond design may need to consider the following factors, e.g., culture agitation, feeding, harvesting, carbon dioxide supply, drainage, possible overflow and cleaning at a given velocity in a straight channel of hydraulic diameter [38].

In short, the power demand in operating the open raceway pond is dependent on the flow velocity whereby it must be kept to a bare minimum which is line to maintain a consistent yet satisfactory operation. It had been reported that the velocity of 0.05 – 0.1 m s^{-1} is required to prevent thermal stratification and cell sedimentation. However, a velocity of at least 0.2 m s^{-1} is necessary to keep the flow above the minimum acceptable speed in application [38], [42].

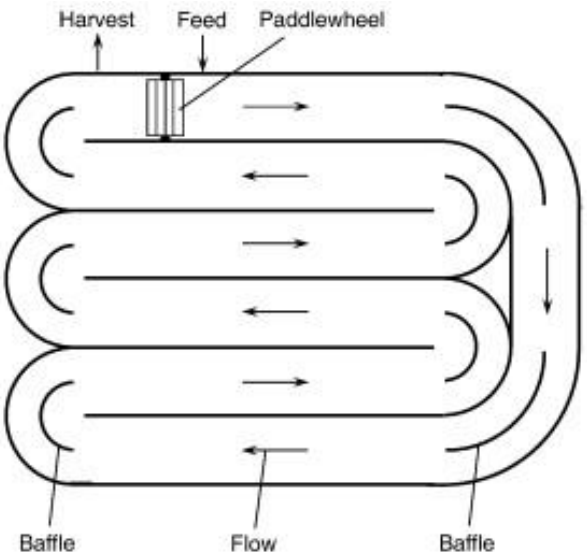


Figure 2.3: Raceway pond [18].

The main advantages of such open systems are that they are low cost and easy to operate, making them a more cost-effective as opposed to its counterpart; the closed microalgae cultivation systems which are more energy intensive. However, this system is very dependent on the external environmental factors, e.g., temperature and weather, as they are openly exposed to the surroundings. Thus, these abiotic factors will very well affect the microalgae biomass productivities [4]. Besides, there is high risk of possible contamination of unwanted fast-growing organisms, e.g., microalgae predators (rotifers) or heterotrophic organisms which may be detrimental to the overall microalgae cultivation, leading to the loss of microalgae biomass productivity [18], [43]. Excessive water loss by evaporation is also a prevalent problem in open systems, leading to inefficient dissolved CO_2 utilization by the microalgae and, resulting in low microalgae biomass productivity [18]. As maintaining a single or a monoculture proved to be rather difficult in such open systems due to the risk of exposure to the surroundings, cultivations are only limited to those microalgae strains or species that are resistant to extreme abiotic conditions such as high pH, salinity or nutrients. For instance, the *Spirulina* sp. can tolerate to high alkalinity (pH of 9 – 11.5), making it to outcompete the other possible organisms which may not thrive under such high alkaline

condition [42], [44]. Other common tolerant microalgae strains are *Dunaliella* sp. and *Chlorella* sp. These strains are usually selected for cultivation in open systems with high tolerance over salinity and nutrient, respectively [44]. Under the optimum culture condition, the biomass productivities in the range of 60 – 100 mg/L/day can be obtained from the open cultivation systems [4]. Several other microalgae strains and their respective biomass productions and productivities while being cultivated in open cultivation systems, e.g., open pond and open raceway pond are shown in Table 2.1. It is surmised that microalgal cultivation in closed systems often yield higher biomass productions in comparison with closed system under ideal, optimized culture conditions (Table 2.1). The microalgal species belonging to *Botrycoccus* sp., *Chlorella* sp., *Dunadiella* sp., *Haematococcus* sp., *Nanochloropsis* sp. and *Spirulina* sp. were often reported to produce high yet consistent biomass productions hence, making them ideal candidates for possible large-scale cultivation. However, the challenge into commercializing microalgal cultivation is still apparent due to the necessary inputs in maintain a high yet consistent biomass output from these reported microalgal cultivation systems. As such, integration of microalgal cultivation systems into wastewater treatment facilities to exploit the low-cost nutrients from wastewater as efforts in reducing the input costs necessitate in microalgal cultivation. Furthermore, the initial setup costs in microalgal cultivation would be nullified thereby, reducing the overall carbon footprint required for dedicated microalgal bioreactors in replacing the existing wastewater treatment facilities.

Table 2.1: Biomass productions and productivities derived from various microalgae strains having been cultivated in various systems.

Microalgae strains	**Cultivation system**	**Total volume (L)**	**Maximum biomass production, X_{max} (g/L)**	**Biomass productivity, P_{max} (g/L/d)**	**Reference**
Anabaena sp.	Open pond	-	0.23	0.24	[4]

	Open raceway pond	300	-	0.031-0.078	[4]
Botrycoccus braunii	Open raceway pond	2,000	-	0.114	[4]
	Continuous bioreactor	-	0.40	0.05	[45]
Botrycoccus sp.	Jar	9	-	0.07	[46]
Chlorella sp.	Flat plate	400	-	3.20-3.80	[47]
	Open pond	-	-	40.00	[4]
Chlorella sorokiniana	Inclined tubular	6	1.50	1.47	[47]
Chlorella vulgaris	Jar	9	-	0.07	[46]
Dunadiella tertiolecta	Culture flasks	0.65	-	0.39	[48]
Haematococcus pluvialis	Open raceway pond	100,000	-	0.122	[4]
	Open pond	-	-	0.20	[4]
	Parallel tubular	25,000	-	0.05	[47]
	Bubble column	55	1.40	0.06	[47]
	Airlift tubular	55	7.00	0.41	[47]
Nanochloropsis sp.	Raceway	2,000	0.50	-	[4]
	Flat plate	440	-	0.27	[4]
	Photobioreactor (PBR)	135	0.50	-	[4]
Phaeodactylum tricornutum	Open raceway pond	4,150	-	0.0028-0.13	[4]
	Airlift tubular	2,000	-	1.2-1.9	[4]
	Helical tubular	75		1.4	[4]

Spirulina sp.	Open raceway pond	135,000	-	0.006-0.07	[49]
Sprirulina platensis	Open raceway pond	282	-	0.183	[4]
		750	-	0.06-0.18	[4]
	Open pond	-	0.47	0.05	[4]
	Tubular type	5.5	-	0.42	[47]
	Flat type	1	-	0.16	[50]
	Conventional flask	1		0.08	[50]

2.2.2.2 Closed systems

Closed microalgae cultivation systems are the cultivation performed in an optimized controlled environment which favour the microalgae growth, e.g., closed photobioreactors (PBRs). In general, these closed photobioreactors involve microalgae cultures being cultivated in suspended form, usually in transparent tubes arranged either vertically, horizontally or inclined phases (whichever can provide maximum surface exposure) while having been supplied with the continuous artificial lighting and water circulation by pumps [1], [4]. Unlike the open cultivation systems, closed cultivation systems allow the cultivation of single dominant or monoculture microalgae strains in a closed environment, i.e., capable of reducing contamination risks [42]. Therefore, the cultivation systems of these types enable an effective control of cultivation conditions namely, pH, temperature, light intensity, CO_2 concentration, nutrients, etc., in creating an optimum condition for microalgae growth [4]. Initially, these systems are designed to overcome the limitations experienced by the open cultivation systems. Accordingly, the closed cultivation systems promote the prolonged cultivation period in which later, resulting in higher yet consistent biomass productivities as opposed to the open cultivation systems [4], [12]. The microalgae strains and their respective biomass productions and productivities having been cultivated in various cultivation systems

(open and closed cultivation systems) are also shown in Table 2.1. Although the closed cultivation systems can offer a consistent yet high biomass generation, the major drawbacks prevalent from this system are the high initial capital as well as the input costs [1]. While maintaining a closed microalgae cultivation system often demands high input and operational costs, this provides a gap for researchers to delve further into developing an economical, functional photobioreactor. Accordingly, the various photobioreactor designs also must meet the culture conditions requirement of maintaining an optimum microalgae growth, while producing high biomass productivities in making the whole upstream process ever to be more economically feasible.

As of now, the most common closed photobioreactor designs include the tubular, flat-plat and column photobioreactors [18], [44]. The tubular photobioreactor comprises of an array of transparent straight glass or plastic tubes termed as solar collectors. These solar collector tubes are usually 10 cm or less in diameter to enable a deep light penetration to the microalgae suspension broth [18], [42]. The microalgae culture broth is circulated to-and-fro between the solar collector tubes and a reservoir (degassing column and heat exchanger) with either a mechanical or air-lift pump which allows mixing and exchanging of CO_2 and O_2 gasses (Fig. 2.4). The tubular type is considered as the most efficient photobioreactor design as compared to the other photobioreactors due to its distinctive characteristic of available large surface area illumination geometry which enables efficient and maximum light capture. Therefore, the tubular PBR is deemed to be suitable for outdoor cultivations which can reduce input costs incurred in providing light illumination to the microalgae cultivation except for the air pumping costs as more energies are needed to extensively aerate the tubular PBR configuration to ensure high contact time between the gaseous and liquid phases. Another downside of the tubular PBR is that the configuration would lead to gradual increases in concentrations of dissolved oxygen. Due to the high photosynthetic activity resulted from the high irradiance rate ($\geq$ 10 $g/m^{-3}/min^{-1}$), the increase in oxygen

production may lead to the photo-inhibition or photo-bleaching [42]. Therefore, it is suggested that the level of dissolved oxygen be kept below 400% of air saturation in the bioreactor with the microalgae suspension broth being flowed back to the degassing column in removing the accumulated oxygen to the environment [18].

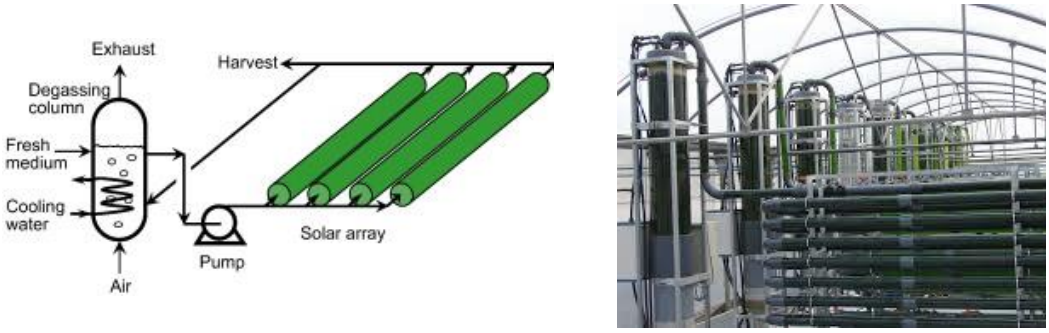


Figure 2.4: Tubular photobioreactor design configuration and facility [51].

Moving on, the flat-plate photobioreactor is consisting of flat-plate design made from transparent materials in providing a maximum light exposure to the microalgae cultivation (Fig. 2.5). To top it off, the thickness of these flat-plates is usually in the range of few millimeters only. Thus, allowing a high surface area illumination, resulting in high microalgal cell density. As opposed to the previous tubular photobioreactor design, there would be much lower dissolved oxygen accumulation in the medium while also having high photosynthetic capacity which deemed to be more suitable for mass microalgae cultivation [18], [44]. Other advantages of employing flat-plate photobioreactors are the low power consumption, high mass transfer capacity, absence of dark volumes and high photosynthetic capacity. However, the temperature control is rather poor or difficult in the flat-plate photobioreactors due to the high surface area to volume ratio, unlike the tubular photobioreactors which are usually equipped with cooling water or heat exchanger in the degassing column (reservoir) to facilitate the heat transfer.

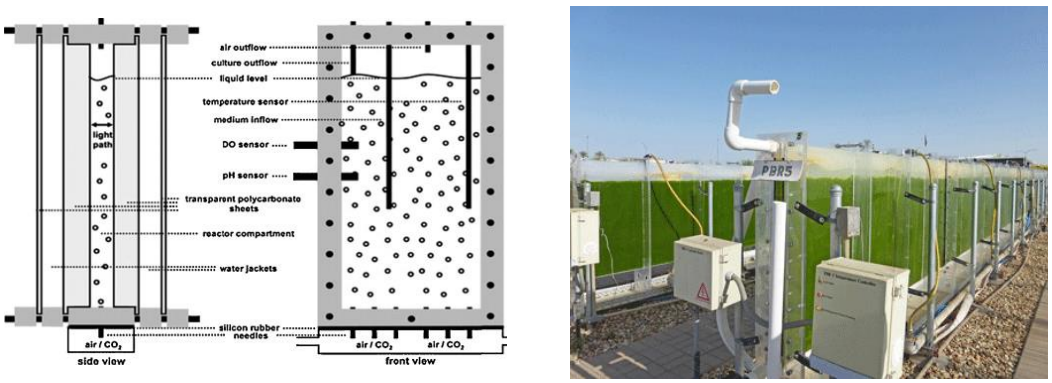


Figure 2.5: The flat-plate photobioreactor design configuration and facility [51].

Another closed photobioreactor design is the column photobioreactors. These photobioreactors revolve around to provide efficient mixing-saturation, better mass transfer rate (gas and liquid) and control over the cultivation conditions [18]. The design configuration of the column photobioreactors allows gas to flow vertically from the bottom to the top of the columns. Thus, rendering a better mixture circulation as the gas bubbles rise along the column and disperse as it reaches the top surface (Fig. 2.6). Such configuration will enhance the gas-liquid exchange. Besides, the mass transfer can be also easily manipulated by controlling the residence time of the gas bubbles residing in the photobioreactor. However, the column photobioreactor design has its flaws which are having smaller illumination surface area as compared to the other closed photobioreactor designs, e.g., the tubular and flat-plate photobioreactors, and more sophisticated requirements during the construction [1].

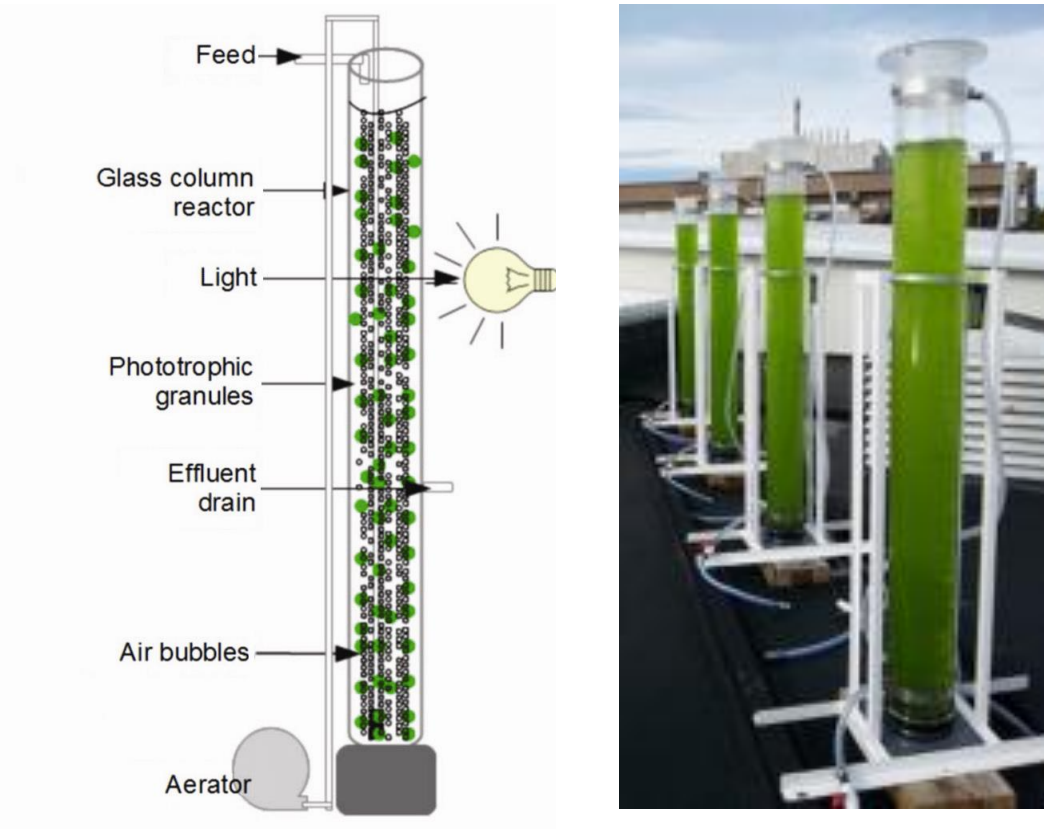


Figure 2.6: Column photobioreactor design configuration and facility [52], [53].

2.2.3 Physiological growth parameters

2.2.3.1 Light

Light supply combining both light intensity and photoperiod, is one of the most important growth parameters which directly influences the growth kinetics of microalgae. As microalgae are unicellular photosynthetic microorganisms, they rely heavily on the sources of light to perform their photosynthetic process to grow further. This has indirectly influenced the uptake of nutrients such as carbon source (CO_2 for autotrophic metabolism), nitrogen and phosphorus (macronutrients), etc. Depending on

the type of microalgal species, the light supply could be a major limiting factor especially for those autotrophic species. While light can be supplied via natural sunlight, artificial lightning or both, the growth rate of microalgae corresponds with the increase in light intensity until a certain value [44]. In this regard, too high incidence of light intensity may lead to possible oxidative stress and photoinhibition phenomenon which cause biochemical damage to the photosystems thereby, leading to the growth reduction or cell death [54]. The selection of proper incidence of light intensity is crucial, concerning monoculture which is more prevalent in the closed photobioreactor cultivation system and different microalgal species will have different optimum or threshold levels of light intensities. For instance, Kim et al. [26] found out that the microalgae, *Chlorella vulgaris,* experienced ceased in growth rate when exposed to 197 µmol photons m^{-2} s^{-1} which was likely due to photoinhibition. On the other hand, Ho et al. [55] found that the microalgae, *Scendesmus obliquus,* only experienced the similar phenomenon at higher light intensity which was 500 µmol photons m^{-2} s^{-1}. While exposing to high incidence of light intensity might backfire on the microalgae growth, this exposure could increase the cell lipid productivities as well. It was found that the lipid bodies of microalgae, *Scenedesmus dimorphus,* increased when the light intensity was increased from 50 to 1200 µmol photons m^{-2} s^{-1}. Nevertheless, the decrease in light intensity would reduce the numbers of lipid bodies and starch granules accompanied by the decrease in cell growth. It is assumed that the cell lipid accumulation was triggered by the production of photoassimilators whereby, the excess light energy is converted into chemicals. Thus, protecting the cells from possible photooxidative damage resulted from the high incidence of light intensity [54]. While considering the optimum light intensity for cell biomass growth as well as cell lipid accumulation, the microalgae cultivation growth phases have to be fully exploited in the sense that the light intensity can be adjusted based on the microalgal growth phases to maximize cell biomass growth and lipid accumulation.

Besides light intensity, the light/dark photoperiod cycle plays a significant role in regulating both the cell density and cell lipid accumulation. Long term exposure to high light intensity may cause cell photodamage or photoinhibition as described early. However, the microalgal cells can repair these "damages" during the dark cycle [44]. As the microalgal cells are constantly mixed in a photobioreactor, the cells near the irradiation source are mostly exposed to higher light intensity as compared with the cells that are shaded in the photobioreactor (lesser light exposure). In this case, the air-lift photobioreactor design is proven to be more advantageous as opposed to the other closed photobioreactor systems as there is the 'shading' phenomenon transpiring in the photobioreactor, allowing indirect application of light/dark photoperiod cycle. In general, it is often perceived that the photosynthetic rate increases with increasing of light/dark frequencies and that the microalgal cells will utilize the light energy more efficiently when there is a longer dark period. However, it is to be noted that the microalgae do not have a certain optimum light/dark photoperiod cycle and instead, depending on its acclimated cultivation state, specific frequency of the light/dark fluctuations and exposure duration [44]. For instance, Chandra et al. [56] had demonstrated that the microalgae, *Scenedesmus obtusus*, which was pre-acclimated to 24:0 hr (continuous illumination) recorded a higher cell growth rate accompanied with higher lipid productivity under the acclimated condition when compared to either 12:12 or 16:8 hr. Dittamart et. al [57] found that the cell lipid accumulation was the highest with 16:8 hr for the microalgae, *Scenedesmus* sp., cultivated under mixotrophic mode whereas, the highest cell density was obtained via a continuous irradiation. Therefore, when considering the overall light supply (light intensity and photoperiod cycles) in a photobioreactor design, the irradiance should be regulated based on the culture density. For instance, the supplied light intensity should correspond to the cell density of the culture in considering the limitations from possible photoinhibition due to high incidence of light intensity exposure as well as cell 'shading' phenomenon where there is limited light penetration (increase in dark volumes) in the photobioreactor.

Interestingly, there were studies investigating the impacts of light wavelengths on the microalgal growth and lipid accumulation as well. Hulbert et al. [58] found that the microalgae, *Chlorella vulgaris,* produced higher biomass under the yellow light than the other colours or wavelengths, e.g., white, red, blue, purple, etc. However, the light colours had no significant effect on the lipid production except for the green colour. It is generally known that the green light can neither contribute nor have any significant effect on microalgal growth due to the lack of necessary pigments to absorb the wavelength from the green light for growth [59]. Similar findings by de Mooij et al. [60] also reported that the maximum biomass growth of microalgae, *Dunaliella tertiolecta,* was transpiring under the yellow light. However, as different microalgal species would have different cell morphologies along with their biochemical characteristics, each species would absorb a specific range of wavelengths effectively due to its distinctive distribution of chlorophyll contents, namely, chlorophyll *a* (core pigment responsible for light absorption), chlorophyll *b*, carotenoids, xantophylls and anthocyanins. For instance, Rai et al. [61] had recorded the highest biomass and lipid production for the microalgae, *Chlorella* sp., when grown under the red light, whereas Rebolledo-Oyarce et al. [62] had demonstrated that the microalgae, *Dunaliealla tertiolecta*, cultured under the blue light had a comparable growth with the control culture illuminated with fluorescent light. Taking energy feasibility concern in bioreactor configurations or operations into account, the energy input for culture illuminations has to be considered while determining the best light colour with a specific range of wavelengths required for optimum yet comparable growth with the fluorescent light illumination, which is commonly used to illuminate closed photobioreactors. As chlorophyll a and b are the most prevalent chlorophyll contents in photosynthetic microalgal cells, the monochromatic blue light wavelength can serve as a good substitute for white light illumination. Findings by Kim et al. [63] had suggested that the microalgae cultured under the different ratios of red:blue lights had recorded a significantly higher biomass production than white light illumination due to effective absorption peaks of chlorophyll *a* and *b.* Similar findings by Rebolledo-Oyarce et al.

[62] also proposed that the blue and red lights had generated the highest biomass production due to the high distribution of chlorophyll *a* and *b* in *Dunaliealla tertiolecta* with the blue colour light being the most appropriate substitute for polychromatic fluorescent light.

2.2.3.2 pH

The pH culture is among the important growth parameters in maintaining a microalgal cultivation as well as inducing lipid productivity. While most microalgal species can grow in a wide range of pH values, an optimum growth medium is often required as different pH values have direct influence over the microalgal cell enzymatic function thus, affecting the overall cell metabolism [54]. For instance, the microalgae belonging to the genus *Scenedesmus* could grow in a range of pH values of 4 – 11 but recorded the highest biomass productivity at pH 7 and optimal lipid productivity at pH 6 – 10 [64], [65]. On the other hand, the microalgae, *Chlorella* sp., had recorded the highest biomass production at the initial cultivation pH of 7, but maximum lipid production at the initial cultivation pH of 8 instead [61]. As demonstrated by these two microalgal species, it is safe to assume that most microalgal species populating in the freshwater medium can tolerate a wide range of pH values while having an optimal growth condition at neutral or slightly alkaline pH.

As pH values have a direct influence over the microalgal cell activities, the nutrient uptake is typically depending on the type of carbon sources being supplied which is often associated to the change in pH culture. In autotrophic culture modes which use atmospheric CO_2 as the carbon source, the pH culture medium will increase with cultivation time due to the continuous assimilation of CO_2 [54]. Similar findings were also reported by Swarnalatha et al. [66], demonstrating the medium alkalization phenomenon is stemming from the utilization of carbonic acid (formed from atmospheric CO_2 sparging into culture medium) by the microalgae to carry out the

photosynthesis process. On the other hand, for heterotrophic or mixotrophic culture modes which have organic carbon sources in the culture medium, the pH culture may fluctuate close to the neutral pH range due to the release of H^+ ions from the organic carbon metabolism [67]. In this case, the pH control strategies such as introduction of pH buffers are often essential to maintain an optimal growth medium condition.

Concerning the mass microalgal cultivation, microalgal species which can tolerate the extreme pH conditions is much preferred especially those growing in the open cultivation systems, e.g., open raceway ponds. As these systems are often exposed to the outdoor surrounding, they are more prone to the contamination risks especially when these systems utilize wastewater effluent as their source of nutrients. A study completed by Tan et al. [68] had found that the high ammonia concentration could very well inhibit the microalgal growth under the high pH level. In addition, the microalgal growth could be reduced by 54 – 83% at pH of 8.3 – 8.8 due to the formation of free ammonia to retard the microalgal growth from ammonium ions. While the microalgal growth is not totally compromised, the extreme pH condition such as at the high pH is favourable to reduce the possible contamination risks and induce cell lipid accumulation. Therefore, the microalgal species which can tolerate the extreme pH values either acidic or alkaline conditions, are the much preferable species to be used in open cultivation systems. For instance, the *Spirulina* sp. which could tolerate a very high alkalinity (pH values of 9 – 11.5) would be more suitable for such cultivation system using wastewater effluent without compromising its biomass and lipid production [44]. However, maintaining a monoculture in open cultivation systems is still the much prevalent problem of late.

2.2.3.3 Temperature

Microalgae has tolerance over a wide range of cultivation or environment temperatures ranging from 15 to 30 °C [54] but manipulation or selection over a specific temperature

range may well optimize the biomass growth or lipid productions of most microalgal species. Depending on the environment or cultivation temperature, the microalgal cell basic properties, i.e., enzymatic activity, membrane fluidity and electron transport chain could be altered into adapting to the specific range of temperature for a more efficient metabolic processes, i.e., photosynthesis, respiration and nutrient uptake [69].

While most microalgal species achieve optimum biomass growth at the temperature range of 20 to 25 °C, regulating the temperature whether higher or lower may induce stressed conditions for the microalgae. Hence, increasing the microalgal cell lipid accumulation. Variations in temperature affect the intake of minerals and the chemical constitution of microalgal cells. Temperature stress has the potential to limit nutritional interactions in some circumstances. Lowering the temperatures will diminish photosynthesis process by inhibiting the carbon digestion, while increasing the temperatures will inhibit photosynthesis process by inactivating photosynthetic enzymes and disrupting the cells' energy equilibrium [70]. Cell size and respiration both will shrink when the temperature rises, declining the photosynthesis efficiency while resulting in a slow growth rate.

2.2.4 Carbon metabolic pathways

2.2.4.1 Autotrophic

The autotrophic mode fixes inorganic carbon sources, e.g., carbon dioxide (CO_2), under the presence of light through photosynthesis [11]. This mode is usually the most preferred for mass microalgal biomass production mainly due to the low upstream production costs, which only involve CO_2 sparging above the atmospheric concentration rather than supplying organic carbon sources (heterotrophic mode), in which the latter being costlier. Chandra et al. [56] had demonstrated that there was an increase in specific growth rate of the microalgal, *Scenedesmus obtusus*, with

corresponding increasing in CO_2 enrichment. But, the increase in lipid content eventually halted when the CO_2 concentrations were above 10%. It could be inferred that the microalgal cell growth would increase until an optimal CO_2 concentration which eventually halted any further cell growth, resulting from the competitive inhibition for binding to the carboxylating CO_2 enzyme (RuBISCO) under high O_2 and CO_2 concentrations [54]. However, it had to be noted that the addition of dissolved inorganic carbon via continuous CO_2 sparging would significantly increase the cost of upstream process in microalgal biorefinery. Besides the CO_2 sparging, there is also an option of adding soluble inorganic carbon sources, e.g., bicarbonate, to the cultivation medium. However, this procedure could affect the pH and osmolarity of the cultivation medium which may eventually impact the microalgal growth [71]. Abedini Najafabadi et al. [72] demonstrated that the microalgal species, *Chlorella vulgaris*, produced higher biomass when cultured under media supplemented with sodium bicarbonate, sodium acetate and molasses than CO_2, with the highest biomass production from sodium bicarbonate. Gardner et al. [73] on the other hand, showed the addition of small dosages of bicarbonates either solely or combined with CO_2 sparging could achieve similar biomass growth and lipid production yield which could decrease the required input costs incurred in continuous CO_2 sparging. Therefore, a proper and controlled ratio of bicarbonate addition combined with CO_2 sparging had to be further optimized along with its cellular responses in order to improve the carbon source utilization in a cost-effective manner. Other instances in reducing the cost of CO_2 sparging include utilization of industrial flue gasses rich in CO_2. However, the induction of flue gasses to the microalgal culture would also increase nitrogen and sulfur oxides (SO_x and NO_x) concentrations which could be detrimental to the microalgal culture. The presence of these NO_x and SO_x compounds induces acidity to the culture medium as well as direct inhibition to the microalgal growth. While SO_x from flue gasses can be removed via the chemical desulfurization system, the removal of NO_x is more of a concern due to the its low solubility in liquid phase [44]. As NO makes up most of the NO_x in flue gasses (about 90 %), control over the microalgal and oxygen concentration parameters would

be able to enhance the mass transfer of NO from gaseous to liquid phase. Thus, enabling the NO uptake by the microalgal cells via diffusion.

2.2.4.2 Heterotrophic

The heterotrophic mode on the other hand, utilizes organic carbon compounds as both its energy and carbon source [11]. Unlike in autotrophic mode, the heterotrophic mode does not require light; thus, enabling it to be cultivated in a closed bioreactor. Depending on the various organic carbon substrates used, the biomass and lipid productions also differ. For instance, Liang et al. [74] demonstrated that *Chlorella vulgaris* cultured in glucose as its carbon substrate favoured both the biomass and lipid productions in comparison with acetate, glycerol or autotrophic cultivation (inorganic CO_2 source). Similar findings by Ren et al. [30] also reported that glucose was the best for both microalgal growth and lipid accumulation in *Scenedesmus* sp. in comparison with fructose, maltose, sucrose, acetate, propionate and butyrate. While the heterotrophic mode does not require light for growth which could potentially reduce the energy input costs required for light supply, there is the expense of providing these organic carbon substrates for the cultures. Other disadvantages of heterotrophic mode include possible contamination by other microbes, e.g., yeast and fungi, growth inhibition by excess organic substrate and inability to produce light-induced metabolites [75]. In that sense, utilization of other carbon co-products could serve as alternatives in reducing the energy and cost expenses required for heterotrophic cultivation. For instance, glycerol which is the main co-product produced from biodiesel production could be utilized as the carbon substrate for heterotrophic microalgal cultivation [54]. Other alternatives include utilizing non-sugar substrates as potential low-cost carbon substrates for the heterotrophic cultivation [11]. Xu et al. [76] demonstrated the microalgal species, *Chlorella protothecoides*, growing in corn powder hydrolysate medium showing comparable biomass and lipid yields with glucose feeding.

2.2.4.3 Mixotrophic

The mixotrophic mode involves utilization of both the organic and inorganic carbon sources which is a combination of autotrophic and heterotrophic modes. Depending on the availability of the carbon sources, the microalgal cells could perform photosynthesis in the presence of light (autotrophic) as well as carbon capture through aerobic respiration (heterotrophic) [54]. Microalgal cultures grown mixotrophically tend to record much higher biomass and lipid yields in comparison with either autotrophic or heterotrophic modes, respectively, for its ability to simultaneously utilize both the photosynthetic and chemoheterotrophic pathways. Li et al. [77] demonstrated that the microalgal, *Chlorella sorokiniana*, recorded much higher biomass yields as well as cell lipid content when grown under mixotrophic mode as opposed to either autotrophic cultivation using CO_2 or heterotrophic cultivation using glucose. Similar findings also recorded higher biomass growths coupled with higher lipid accumulations in other microalgal species as well, namely, *Chlorella* sp., *Chlorella protothecoides, Chlorella saccharophilia* and *Scenedesmus* sp. [11]. As mixotrophic microalgal cultures could produce more biomass per unit time, the effect of either possible photodamage or photo-limitation induced by excess light penetration or lack of light and cell shading phenomenon, respectively, also could be eliminated indefinitely independent of the bioreactor design.

2.2.5 Nitrogen and phosphorus

Other than carbon, there are two other essential macronutrients required for microalgae growth, namely nitrogen and phosphorus sources. Nitrogen is mainly responsible in regulating protein synthesis and growth metabolites of microalgae which influence the overall growth rate whereas, phosphorous is responsible in regulating most of the cell activities and metabolism such as energy transfer and nucleic acid synthesis [37]. While these two nutrients do play significant roles in microalgae growth,

there were studies investigating on nutrient balancing and starvation with the intention to enhance lipid accumulation in microalgae cells. For instance, Xin et al. [78] reported that the microalgae, *Scenedesmus* sp., could accumulate higher lipid content when introduced with lower initial nitrogen and phosphorus concentrations. The study conducted by Mutlu et al. [79] also found that the lipid content in *Chlorella vulgaris* could go up to 35.6 ± 8% under complete nitrogen starvation as opposed to 12.29 ± 3% in the control system. However, the lipid accumulation and productivity rate in microalgae should not be related as both tend to be in contradiction from each other. Xin et al. [78] verified that the highest lipid content in *Scenedesmus* sp. was achieved under the nutrient limitation which was around 5% to 10% higher than in control system but its lipid productivity was not higher due to the offset of lower growth rate [0.32 ± 0.02 (x 10^6 cells/mL d)] under the nutrient limitation as compared with control system [1.34 ± 0.08 (x 10^6 cells/mL d)]. In light of considering both biomass and lipid productivities, a nitrogen to phosphorus (N/P) ratio of 5:1 to 8:1 range was proposed to be optimum for *Scenedesmus* sp. [78]. However, more in-depth studies of N/P ratios in relation to other species of microalgae are needed in enhancing their respective growths which positively impact the lipid productivities used for biofuel production.

2.3 Nitrogen transformation and valorization via nitrification-assimilation mechanism in microalgal-bacterial consortium

As nitrogen compounds in the wastewater usually exist in the form of ammonium, the high concentrations of ammonium in water bodies will generally lead to the eutrophication, causing water hypoxia and posing toxic hazards to the aquatic ecosystem and environment [80]. Also, the risk of toxicity stemming from the presence of free ammonia under alkaline conditions and unionised form of ammonium could inhibit certain microbial activities in bioremediating wastewaters, particularly the nitrification process. This is detrimental to the conventional biological treatment in

wastewater treatment plants as spearheaded by the activated sludge process [81]. While the ammonium concentration in wastewater leading to the accumulation of free ammonia is unavoidable, advanced biological treatment processes are deemed necessary to exploit the potential of ammonium source laden into wastewater. For instance, the combined biological treatment process from two microbial consortia, i.e., the microalgal-bacterial cultivation system, is beneficial in proffering low carbon requirements, effective nutrient removal via bioconversions and value-added bioenergy generation.

The approach behind the microalgal-bacterial cultivation system revolves around the symbiotic relationships between these two microbial populations. The simultaneous exchange of organic and inorganic nutrients in the mixed microbial consortia is stemming from the intrinsic biological metabolisms performed by microalgae and bacteria, namely, photosynthesis and respiration, respectively [69], [82]. In highlighting the nutrient removal processes in the microalgal-bacterial consortium particularly nitrogen, the mechanism behind the higher nitrogen removal rate was ameliorated by the prior nitrification process executed by nitrifiers in activated sludge where NH_4^+-N was converted into oxidized nitrogen species, namely, NO_2^--N and NO_3^--N. This prompted the assimilation rate of these oxidized forms of nitrogen species into the microalgal biomass for growth. As confirmed by a previous study [83], the nitrogen assimilation rates by microalgal species, *Chlorella vulgaris*, were found to be the highest when NO_3^--N (2.78 ± 0.07 mg/L/d) was the nitrogen source, followed by NO_2^--N (2.38 ± 0.00 mg/L/d) and lastly, NH_4^+-N (0.74 ± 0.05 mg/L/d). The nitrogen removal mechanism which initially started with the nitrification of NH_4^+-N into oxidized nitrogen forms would serve as a ‘shortcut’ in speeding up the nitrogen uptake into microalgal biomass; thereby, shortening the overall microalgal cultivation period especially in wastewater mediums.

2.4 Kinetics of biomass growth

The growth dynamics of a microorganism follows the sigmoid curve and is characterized by three phases namely, (1) the lag phase; (2) the exponential phase and (3) the stationary or linear growth phase [84]. In a typical batch culture, these phases often reflect the changes in the biomass and the environment to which the microorganism is exposed to. The lag phase is observed with little increase of cell density attributed to the period of physiological adjustment of the cell metabolism to grow due to changes in the culture conditions. Given time, the cells would have adapted to the new environment or culture conditions in which they would start to multiply and eventually entered the exponential growth phase. The cells would continue to multiply as an exponential function of time provided that there is sufficient saturation of nutrient substrates and energy. As the cells continue to multiply, the cell division would eventually slow down to a degree where the biomass accumulates at the constant rate (the stationary growth phase) whereby some substrates in the culture medium became the limiting factor such as nutrients, light, pH or any other physical and chemical factors that might limit the cell growth.

Various kinetic growth models are employed to evaluate the feasibility of microbial culture before being upscaled for biofuel production. These models simulate and/or predict the population growth of microorganisms under different cultivation conditions. The kinetic modelling would also help in understanding the behavioural growth patterns of microorganisms when subjected to various treatments which eventually lead to the selection of an optimum condition in enhancing the microbial biomass growth. One of the key parameters in evaluating or gauging the feasibility of the cultivation system is the specific growth rate. Most studies often employed specific growth rate in determining the suitability or efficiency of the cultivation or treatment method used [85]–[87]. The specific growth rate can be calculated from the slope of the linear

regression of time and nature log cell density in the 'exponential' growth phase in growth curve as shown in Eq. (2.1) below.

$$k = \frac{(ln\ X - ln\ Xo)}{(t - to)} \quad (2.1)$$

where k (1/d) is the specific growth rate in the 'exponential' growth phase, X_o is the biomass concentration (g/L) at the initial point of the 'exponential' growth phase (t_o) (d) and X is the biomass concentration (g/L) at time (t) (d) of the 'exponential' growth phase.

There are non-linear mathematical models which were employed in validating the biomass production in previous growth kinetic studies such as the Logistic (Eq. (2.2)), Gompertz (Eq. (2.3)), and Richard models (Eq. (2.4)) [88]. The advantage of these non-linear models lay in that it could determine the specific growth rate of microorganism population growth curve as a whole without excluding the 'lag' growth phase of the population growth curve as compared with the specific growth rate equation (Eq. (2.1)) commonly used in previous studies which only include the 'exponential' growth phase of the population growth curve. The differences between these models on the other hand are the number of parameters in which each of the respective models could take into account for.

Logistic model:

$$y = \frac{A+C}{1+exp^{-B(t-M)}} \quad (2.2)$$

where A is the asymptotic of ln X_t/X_o as t decreases indefinitely, C is the asymptotic of ln X_t/X_o as t increases indefinitely, B is the relative growth rate at time M (day^{-1}), t is the residence time (day), M is the time at which the maximum growth rate is reached (day), X_t is the biomass concentration at time (g L^{-1}) and X_o is the initial biomass concentration (g L^{-1}).

Gompertz model:

$$y = A + Cexp^{-\exp[-B(t-M)]} \quad (2.3)$$

where A is the asymptotic of ln X_t/X_o as t decreases indefinitely, C is the asymptotic of ln X_t/X_o as t increases indefinitely, B is the relative growth rate at time M (day^{-1}), t is the residence time (day), M is the time at which the maximum growth rate is reached (day), X_t is the biomass concentration at time (g L^{-1}) and X_o is the initial biomass concentration (g L^{-1}).

Richards model:

$$y = A(1 + vexp[k(T-t)])^{(-\frac{1}{v})} \quad (2.4)$$

where A is the asymptotic of ln X_t/X_o, t is residence time (day), v and k are shape parameters whereas T is time at the inflexion point (lag phase), X_t is the biomass concentration at time (g L^{-1}) and X_o is the initial biomass concentration (g L^{-1}).

While the described kinetic models had been widely used to describe the underlying population growth kinetics of the microorganisms in the culture, but often, these kinetic models failed to correlate the causal relationship between the behavioural growth and the underlying mechanism behind it defined as explanatory models. On the other hand, descriptive kinetic models have the advantage to provide insights into the population growth kinetics in relation to the underlying mechanism influencing it. For instance, cultivation growth parameters, i.e., organic/inorganic carbon source, nitrogen, phosphorus, light, pH etc., have direct influences over the culture growth kinetics which could not be uncovered by explanatory models. As such, kinetic models which have nutrient substrates and co-limitations on the population growth would be more suitable in describing and predicting the growth behaviour of microorganisms under a wide range of biotic and abiotic factors. The Monod-type model is an example of descriptive kinetic model which considers nutrient limitation conditions on the population growth

of microorganisms [70]. Owing to its simple formula, the Monod model has been extensively used to describe and predict the correlation between the microalgal growth and single substrate parameter (Table 2.2). While the Monod model is useful in describing the population growth kinetics for the single substrate studies, the model is limited to growth under low or moderate nutrient concentrations. Hence, cultivation conditions such as nutrient absence and/or higher thresholds would limit the application of Monod model. However, various studies of late had modified or expanded the Monod model to better facilitate the single substrate-dependent model under nutrient absence and/or higher thresholds (Table 2.2).

Table 2.2: Microalgal growth kinetic models based on Monod-type model and the modification of functions for the single substrate under various applications.

Application	**Formula**	**Parameter**	**Reference**
Nutrient limitation	$\mu = \mu_{max}\frac{S}{K_S + S}$	CO_2; N; P	[70]
Nutrient limitation and inhibition	$\mu = \mu_{max}\frac{S}{K_S + S + \frac{S^2}{K_i}}$	CO_2	[89]
Nutrient limitation and absence	$\mu = \frac{\mu_{m1}S + \mu_{m2}K_S}{K_S + S}$	P	[90]
Nutrient absence, limitation and inhibition	$\mu = \frac{\mu_{m1}S + \mu_{m2}K_S + \mu_{m3}K_S}{K_S + S + S^2/K_i}$	P	[91]
Light-limitation	$\mu = \mu_{max}\frac{l}{K_l + l}$	-	[92]
	$\mu = \mu_{max}(1 - e^{-l/K_l})$	-	[89]
	$\mu = \mu_{max}\frac{l}{(K_l^m + l^m)^{l/m}}$	-	[93]
	$\mu = \mu_{max} tanh\frac{l}{K_l}$	-	[94]

2.5 Techno-economic assessment of microalgae derived biofuel systems – Life cycle assessment

While the microalgae feedstock holding promising prospects in reducing the dependence over fossil fuels for energy production, technology scale-up and commercialization of microalgae feedstock still remain as the largest hurdle to be resolved in the biofuel industry. Of late, tremendous attempts to transform the laboratory scale technologies into commercialized operations had been made via assessing the life cycle assessments and refining various cultivation modes for microalgae. The economic feasibility of microalgae being used as biofuel feedstock in comparison with the other renewable and non-renewable sources is subsequently evaluated and this whole consideration approach is better known as techno-economic assessment (TEA) [95], [96]. The in-depth understanding on the TEA requirements by oleaginous microorganisms is foreseen to provide a specific corrective measure on profits-maximizing and investment risks-minimizing, cushioning the critical impacts faced by scale-up production. However, there were many contradictions and uncertainties concerning the microalgal-based biodiesels being reported in literatures, resulting in a broad range of values due to the complexity and inconsistent operation scenarios for various microalgae cultivation systems [97]. As such, TEA via energy-input analysis will be able to pinpoint and screen the feasibility of the microalgal cultivation system as it is primarily based on the energy output from the system, i.e., biofuel. Among the widely adopted methods in determining the feasibility and viability of the process and technology is the life cycle assessment.

Life cycle assessment (LCA) is a vital tool used to assess the environmental impacts of a product, process, or activity from cradle-to-grave. Cradle-to-grave is a term used to describe the life cycle process. A product needs raw materials (cradle) to be manufactured, and when the product is no longer of use, it is disposed (grave) [98]. There are inputs and outputs for every part of the cycle. Energy, water, and raw material

are inputs while waste and emissions are output examples. The source of raw materials in the life cycle is the environment like water, cotton, silver, clay, etc. The materials are manufactured at a production plant to become a product. The items created must be packaged and prepared to be distributed. Once the product is distributed, the people can begin using the product until it wears out. The item will eventually no longer be used, and it will be disposed or possibly recycled. For example, a pair of shoes need a ton of raw material (cradle) such as cotton, rubber, leather, etc., to be collected from the environment, and later manufactured. Then, the shoes must be packaged and distributed to different stores. People buy shoes and use them until they wear out and get disposed (grave). LCA will help to determine if there is a better alternative than the system currently in used. Further, if there is a better way to reduce waste by using fewer materials or turning to recyclable materials, the LCA will show that. The use of alternative materials could also expand the lifespan of the product.

The LCA has international standards set by the International Organization for Standardization (ISO). For LCA specifically, there is ISO 14040-14044, where each standard addresses a specific topic. ISO 14040-14044 are principles and frameworks, goal and scope definition and life cycle inventory analysis, life cycle impact assessment, life cycle interpretation, requirements, and guidelines (Fig. 2.7) [99]. The standards were hard to follow, as there were inconsistencies, so there were revisions to ISO 14040 and 14044. The readability was improved to be clear on the goals of LCA. ISO 14040 added these principles to remove inconsistencies in the previous one, life cycle perspective, environmental focus, relative approach and functional unit, iterative approach, transparency, comprehensiveness, and priority of scientific approaches. These will help the decision-making process while conducting the LCA. Indeed, the LCA is an important tool, so it must be standardized for optimal usability.

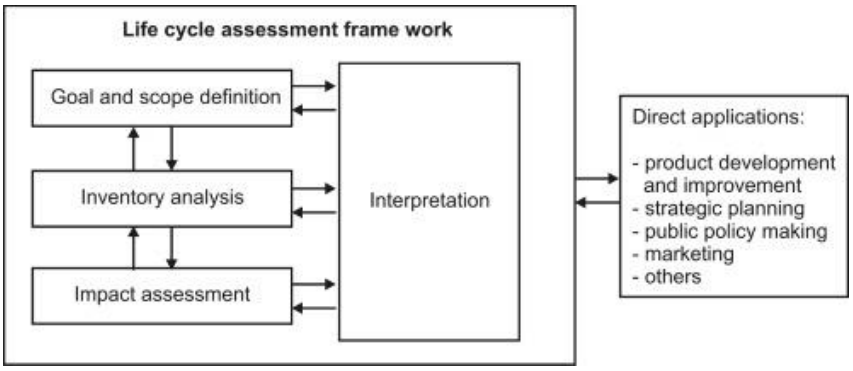


Figure 2.7: Life cycle assessment framework in accordance to EN ISO 14040 [99].

CHAPTER 3

METHODOLOGY

3.1 Source of domestic wastewater and simulated wastewater preparation

Sample of domestic wastewater was collected from the primary clarifier at the sewage treatment plant located in Silibin, Perak, Malaysia and instantly analysed for its characteristics following the Standard Methods for the Examination of Water and Wastewater [100]. The presence of metal trace elements in the domestic wastewater sample was also analysed using the Microwave Plasma Atomic-Emission Spectrometer (MP-AES, Agilent 4210). The composition of domestic wastewater is shown in Table 3.1. The synthetic medium simulating the composition of domestic wastewater was then prepared for the use in this study to ensure the consistency of wastewater characteristics being employed. The synthetic medium was also modified following the components from Lim et al. [101] (in mg/L): sucrose (109), $FeCl_3.6H_2O$ (10), $CaCl_2$ (42), $(NH_4)_2SO_4$ (118), KH_2PO_4 (18), K_2HPO_4 (90), $MgSO_4$ (49) and $NaHCO_3$ (354), yielding Chemical Oxygen Demand (COD) and NH_4^+-N concentrations of approximately 135 and 25 mg/L, respectively.

Table 3.1: Characteristics of domestic wastewater and synthetic medium.

Parameter	**Unit**	**Domestic wastewater**	**Synthetic medium**
pH	-	7.8	8.1
Colour	PtCo	515 ± 1.2	230
COD	mg/L	135 ± 14	135
BOD	mg/L	120 ± 6	120
Ammonium-nitrogen, NH_4^+-N	mg/L	25 ± 3	25
Nitrite-nitrogen, NO_2^--N	mg/L	0	0
Nitrate-nitrogen, NO_3^--N	mg/L	2 ± 0.6	0
Phosphate-phosphorus, PO_4^{3-}-P	mg/L	62 ± 6	61.63
Iron	mg/L	6 ± 0.3	5.51
Calcium	mg/L	15 ± 0.8	15.17
Potassium	mg/L	25 ± 1.3	25.37
Magnesium	mg/L	10 ± 1	9.89
Sodium	mg/L	97 ± 10	96.92

3.2 Activated sludge and *Chlorella vulgaris* stock cultures

The seed of activated sludge collected from a local sewage treatment plant was inoculated in a sequencing batch reactor (SBR) with a working volume of 18 L (Fig. 3.1). The activated sludge was then acclimated to the prepared feed medium of domestic wastewater. The SBR was operated with a cycle time of 24 hours via the following sequencing periods: instantaneous FILL, 0 h; aerobic REACT, 10 h; SETTLE, 1.5 h; DRAW, 1 h and IDLE, 11.5 h. During each cycle, 14 L of feed medium was introduced into the SBR and the same volume of treated effluent was siphoned off during the DRAW period. The excess sludge biomass was removed from the SBR to maintain the sludge age at 40 days.

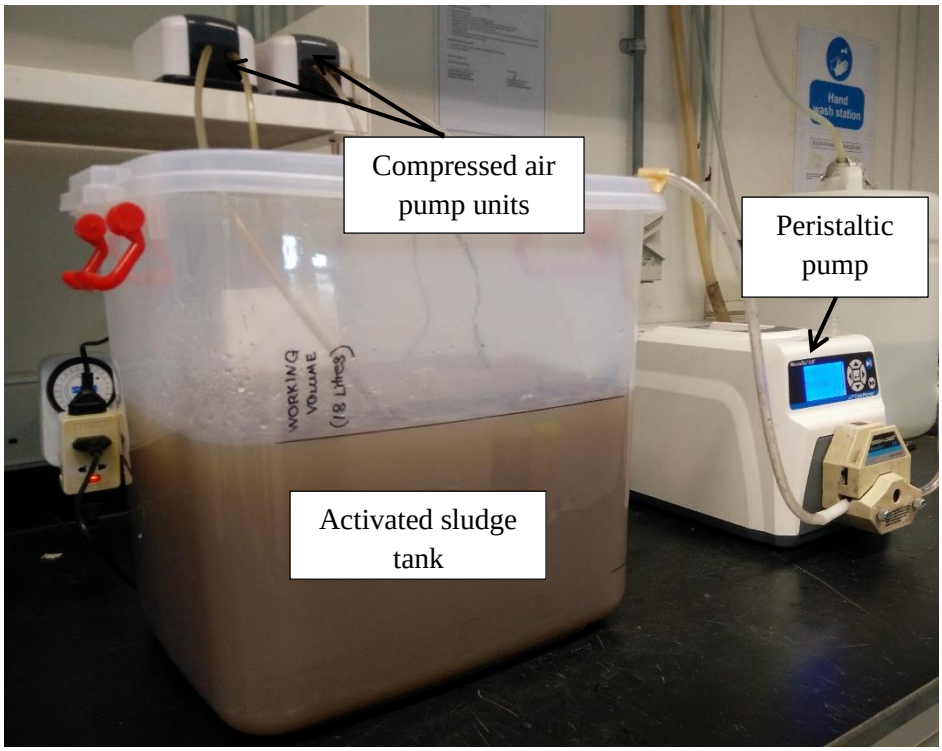


Figure 3.1: Activated sludge culture in the SBR system.

The freshwater microalgae species, i.e., *Chlorella vulgaris,* was acquired from the culture collections belonged to the Centre for Biofuel and Biochemical Research (CBBR), Universiti Teknologi PETRONAS and cultured in a 5-L bottle containing 4.5 L of the prepared feed medium of domestic wastewater composition, aerated with compressed air at a flow rate of 4.0 L/min and illuminated continuously with white light emitting diode (LED) light at light intensity of 1200 lux with initial pH being adjusted to 7.1 ± 0.1 (Fig. 3.2).

Figure 3.2: *Chlorella vulgaris* culture in the 5-L functional bioreactor.

3.3 Development of new microalgal-bacterial photobioreactor – Sequential Flow Baffled Algal-bacterial (SFB-AlgalBac) Photobioreactor

An integrated photobioreactor that could separate the bacteria in the form of activated sludge mixed liquor and microalgal culture via baffling has been named as “sequential flow baffled microalgal-bacterial photobioreactor” (SFB-AlgalBac

photobioreactor). The reactor design was based on the synergistic relationship involving the symbiotic exchange of organic and inorganic nutrients between microalgal and bacterial cultures namely, the nitrification-assimilation mechanism pathway as previously discussed in Section 2.3.

The newly designed SFB-AlgalBac photobioreactor utilized in the current study is presented in Fig. 3.3. This photobioreactor comprised of microalgal and bacterial cultures in a single baffled basin. However, instead of mixing both the microalgal and bacterial cultures directly, the photobioreactor had two baffled cultivation columns. The inner column was filled with bacterial activated sludge culture and had a working volume of 5.5 L. The outer column was occupied by microalgal culture with a working volume of 100 L. Between these two columns, a clarifier with a volume of 8.5 L was built to separate the treated effluent from the activated sludge. In this manner, during continuous flow, the raw wastewater would be initially introduced into the activated sludge column. Then, it would be separated in the clarifier and the activated sludge's effluent now would become an influent that would be subsequently treated by the microalgal culture prior to the final discharge through a weir. This design minimized the risk of bacterial contamination that could jeopardize the microalgal populations, which is commonly experienced by the high rate algal ponds. Based on the previously described 'shortcut' nitrogen removal mechanism, the wastewater fed into the activated sludge column would undergo nitrification, converting NH_4^+-N into NO_3^--N (trace NO_2^--N due to the complete nitrification process). Then, the effluent from the activated sludge containing the NO_3^--N would be collected in the clarifier and would overflow as an influent into the microalgal column for assimilation into microalgal biomass. The treated effluent by microalgae with reduced NO_3^--N concentration would be finally flowing into the weir for discharge.

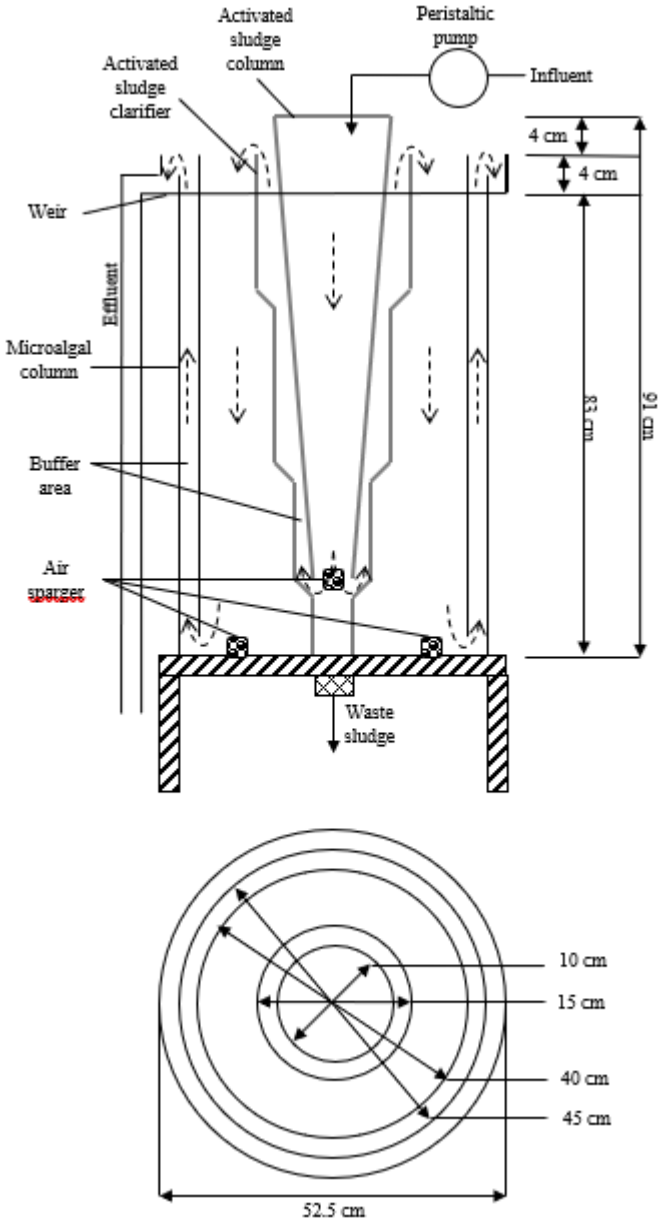


Figure 3.3: Schematic diagram of the Sequential Flow Baffled Microalgal-bacterial (SFB-AlgalBac) Photobioreactor.

3.4 Experimental setup

3.4.1 Synergistic associations between co-cultivation of activated sludge (nitrifiers) and microalgae

The activated sludge and microalgal cultures acclimated to the synthetic wastewater medium were used for the co-cultivation investigation. The bioreactors inoculated with axenic cultures of activated sludge (200 mg) and microalgae (200 mg) only were indicated as AS and MA, respectively while another bioreactor inoculated with the mixed co-cultivation culture of activated sludge (100 mg) and microalgae (100 mg) was indicated as AS/MA. A pre-determined volume ratio of the synthetic wastewater to total working volume (v/v) and inoculation culture to total working volume (v/v) were introduced into the Erlenmeyer flask with a total working volume of 1 L. The bioreactors were illuminated with white LED light at the light intensity of 1200 lux and aerated with compressed air, continuously. The cultivation temperature and pH of the bioreactors were maintained at 27 ± 1 °C and 7.1 ± 0.1, respectively until the stationary growth phase was attained indicating experimental termination. Samplings were taken on a daily basis for analysing the concentrations of COD, nitrogen species (NH_4^+-N, NO_2^--N and NO_3^--N) for effluent analysis and the microbial biomass concentrations for growth analysis. Once the stationary growth phase was attained, the microbial biomasses from the experimental bioreactors were harvested via gravitational sedimentation and washed with distilled water twice before being oven-dried at 105 °C for further analyses. All experimental runs were conducted in duplicate.

3.4.2 Flow rate optimization of SFB-AlgalBac photobioreactor for continuous microalgal biomass production and wastewater bioremediation

The operation of SFB-AlgalBac photobioreactor was started with a batch cultivation mode. Accordingly, the activated sludge and microalgae acclimated to domestic

wastewater were introduced into its respective cultivation columns in the photobioreactor and immediately filled with fresh domestic wastewater to the working volume levels of each. The activated sludge and microalgal cultures were continuously aerated with compressed air with the dissolved oxygen being maintained at 8.0 ± 0.5 mg/L. The microalgal culture was also continuously illuminated with a white LED light at a light intensity of 1200 lux; measured from the external surface of the photobioreactor. The batch operation was maintained until the activated sludge and microalgal cultures had both attained steady states. Then, the operation of photobioreactor was shifted to the continuous cultivation mode with the influent flow rates being increased stepwise in the order of 2.5, 5.0 and 10.0 L/d. Again, the activated sludge age was maintained at 40 days for all operations with the concentrations of mixed liquor suspended solids fluctuating within a range of 1.6 - 1.7 g/L at the mixed liquor pH of 7.5 ± 0.1. Samplings were performed at the regular intervals to analyse the COD, nitrogen species (NH_4^+-N, NO_2^--N and NO_3^--N) and microalgal biomass concentrations. The microscopic images of the microbial communities and flocculation efficiencies were also recorded at the steady state of each operation.

3.4.3 Dual nitrogen heterogeneity mode under continuous flow operation of SFB-AlgalBac photobioreactor

The optimized influent flow rate determined from Section 3.4.2 was selected to ensure the continuous operation of the SFB-AlgalBac photobioreactor could maximize the nutrient removal and microalgal biomass generation. Hence, the 5 L/d of flow rate was set to be the constant operational flow rate in the study, receiving activated sludge effluent containing NO_3^--N, i.e., being the sole nitrogen source for the controlled operation. The total nitrogen loading was regulated at approximately 115 mg/d for the controlled operation and other operations with various diverted wastewater loadings into SFB-AlgalBac photobioreactor as described hereafter. The nutrient-rich wastewater containing NH_4^+-N source would be loaded at 1, 2.5 and 3 L/d in pairing

with 4, 2.5 and 2 L/d of the activated sludge effluent containing NO_3^--N source, respectively, to attain the 5 L/d of flow rate for the corresponding DW-1, DW-2.5 and DW-3 operations. The nitrogen species loadings for each operation inclusive of controlled operation are shown in Table 3.3 with the total nitrogen loading range of 115 – 117 mg/d. The time courses for all operations continued until reaching its steady states, measured as the constant removal of NH_4^+-N and NO_3^--N from SFB-AlgalBac photobioreactor. Samplings for the analysis of concentrations of COD, nitrogen species (NH_4^+-N and NO_3^--N) and microalgal biomass concentrations were executed at regular intervals throughout the study.

Table 3.2: Various diverted wastewater loadings with respect to the nitrogen species into SFB-AlgalBac photobioreactor.

Operation	**Wastewater loading (L/d)**	**Activated sludge effluent loading (L/d)**	**Nitrogen species**	
			NH_4^+-N	**NO_3^--N**
Control	0	5	-	115
DW-1	1	4	24	92
DW-2.5	2.5	2.5	60	57.5
DW-3	3.0	2	72	46

3.4.4 Energy feasibility of SFB-AlgalBac photobioreactor in generating microalgal biomass for biodiesel production

3.4.4.1 Goal and scope

The life cycle analysis (LCA) for biodiesel production from microalgal feedstock was applied to gauge the energy feasibility of the cultivation system for potential commercial microalgae-based biodiesel production. Accordingly, the life cycle energies of various bioenergy products from different stages of system boundaries were investigated. This study analysed the microalgae-to-biodiesel production which comprising of cradle-to-cradle (microalgae-to-biomass and/or lipid) and cradle-to-gate (microalgae-to-biodiesel) boundaries as follows:

1. Microalgal cultivation followed by harvesting and drying to produce dry microalgal biomass.
2. Lipid extraction from dry microalgal biomass via solvent extraction.
3. Biodiesel production via transesterification of extracted lipid to produce mixed fatty acid methyl esters.

The various bioenergy products of interest formed throughout the microalgae-to-biodiesel production were dry microalgal biomass, lipid and biodiesel. Therefore, the functional unit for the study was selected to be 1 MJ of energy calorific value equivalent to the respective bioenergy products.

3.4.4.2 Life cycle inventory (LCI) and assumptions

The stages having been identified in the microalgae-to-biodiesel production in this study were cultivation, harvesting, drying, lipid extraction and biodiesel production in which were modelled in life cycle software, i.e., SimaPro® 9.3 with the Ecoinvent® 3.5 database, to obtain 1 kg of biodiesel. The life cycle system boundaries were illustrated in Figure 3.4. All the process parameters and input descriptions were defined hereafter.

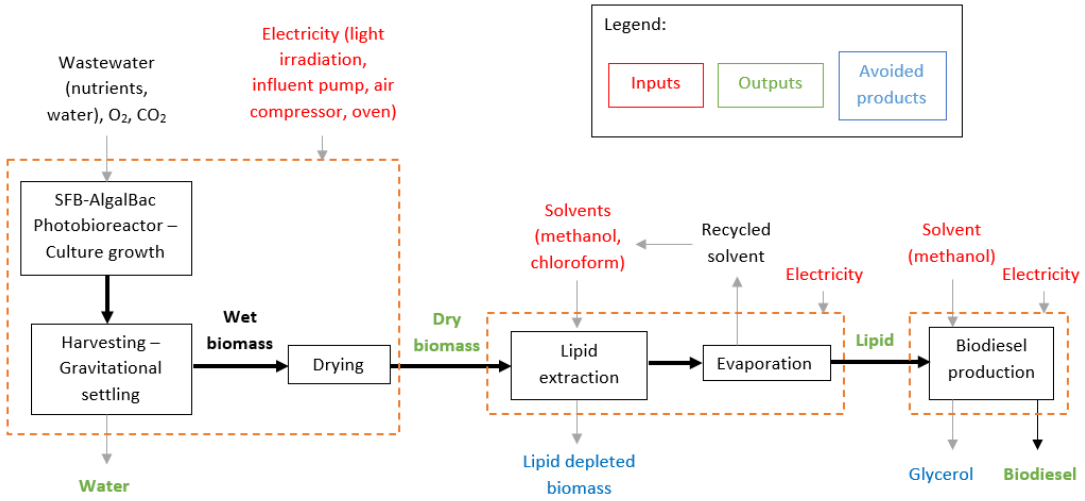


Figure 3.4: System boundaries for biodiesel production derived from microalgal biomass generated by the continuous flow mode of SFB-AlgalBac photobioreactor.

Microalgal biomass concentration was harvested at 0.8 g/L; Lipid content of microalgal biomass was attained at 30 %; Biodiesel transesterification rate was achieved at 90 %. Hence, to produce 1 kg of biodiesel, 1.1 kg of lipid from 3.7 kg of microalgal biomass was required.

Best case scenario: *Chlorella vulgaris* had an average doubling time of 16 h [102]. Starting a culture with exponential cells of 10 % to the final desired microalgal biomass density required ~52 h of cultivation. To produce 3.7 kg of microalgal biomass, an initial biomass density of 0.37 g/L of initial is required from 1000 L.

Real scenario: A 100-L pilot-scaled SFB-AlgalBac photobioreactor with microalgal biomass concentration at 0.8 g/L was operated at 5 % continuous flow rate (5 L/d), producing 4 g of biomass/d. Commercial culture system units of raceway ponds used for the production of microalgal biomass or wastewater treatment were ranging from 3 x 10^4 to 1 x 10^8 L, and about 1 x 10^4 L for tubular photobioreactors [103].

Assuming the bioreactor is upscaled to 100 times, 400 g of biomass/d could be produced. Thus, for producing 3.7 kg of biomass, 9.25 days ≈ 222 h are required.

3.4.4.3 Energy input calculations

Growth cultivation: The water and nutrients required for the microalgal cultivation was sourced from wastewater which contained the essential macronutrients for microalgal growth, namely, C, N and P elements, and was supplied via water pump with an electricity consumption of ~195 kW h of electricity/ton of algae biomass [97]. The O_2 and CO_2 supplies were provided at optimal air flow rate of 0.2 L/L min from an air compressor with 230 L/min capacity. The consumed electricity was 2.2 kW and the estimated energy consumption for 1 day ~0.29 MJ (not operated at maximum capacity) [97], [104]. The energy consumption for light irradiation was 5.4 W with luminance of 1200 lux at 15 % of light intensity [104].

Harvesting and dewatering: The harvesting of microalgal biomass was completed via gravitational settling in the settling chamber catered for the continuous operation of SFB-AlgalBac photobioreactor. The supernatant which was the treated effluent was eventually discharged from the photobioreactor. The drying energy via electrical oven to produce dry microalgal biomass was determined to be 0.0028 MJ/g dried biomass [104].

Lipid extraction: The optimum microalgal lipid content was attained at 30 % per dry biomass weight and considered in the current analysis. The lipid extraction was performed via solvent extraction using the mixed methanol and chloroform at the ratio of 2:1 (v/v). The amount of solvent to dry microalgal biomass required was in the ratio of 20:1. The homogenized extract was filtered to obtain the dissolved lipid in the solvent, while the leftover lipid-depleted biomass residue was defined as avoided

product. The solvents were removed and recycled via rotary evaporator to obtain the microalgal lipid again, and 90% of the solvent is expected to be recycled. The energy inputs for extraction and solvent evaporation were identified to be 152 MJ/kg biodiesel [105].

Biodiesel production: The final bioenergy product, i.e., biodiesel, was produced via transesterification reaction which required energy inputs from heating and electricity which were 4.05 MJ/kg biodiesel and 0.0336 kW h/kg biodiesel, respectively [106]. Methanol was required as the input for the transesterification reaction from lipid into biodiesel and glycerol (avoided product). In this regard, the estimated amount of methanol to produce 1 kg of biodiesel was 114 g [106].

3.5 Analytical procedures

3.5.1 Development of microalgal growth model

The kinetics of microalgal growth was derived from the Monod-type growth model for nutrient limitation which is usually applied to determine the specific growth rate of microorganisms as a function of major substrates [89]. As such, the growth rate of specific microorganism such as microalgae in the Monod growth model could be limited by the availability of the substrate as soon as its concentration was reduced. Therefore, the nutrient limitation could impact the maximum rate of the microalgal growth which can be expressed by Eq. (3.1).

$$u = u_{max} \frac{[S]}{K_s + [S]} \tag{3.1}$$

where u is the specific growth rate (/d), u_{max} is the maximum specific growth rate (/d), [S] is the substrate (mg/L) and K_S is the half-saturation constant of substrate (mg/L).

Taking the continuous process in SFB-AlgalBac photobioreactor into consideration, the kinetic growth dynamics of the microalgal biomass were assumed to be in equilibrium with the nutrient limiting factor at the given flow rate and nitrogen concentration such that the net mass rate of change of substrate = mass input rate – mass output rate – mass assimilation rate, which can be expressed relatively by Eq. (3.2).

$$\frac{dN_1}{dt} = FN_0 - FN_1 - \frac{u}{Y}XV \tag{3.2}$$

where dN_1/dt is the change in nitrogen mass with respect to time (mg/d), F is the nitrogen feed flow rate (L/d), N_0 is the influent nitrogen concentration (mg/L), N_1 is the effluent nitrogen concentration (mg/L), u is the specific growth rate of the microalgae (/d), Y is the microalgal biomass per unit of nitrogen assimilated (mg/mg), X is the microalgal biomass concentration (mg/L) and V is the volume of the microalgal cultivation column in the photobioreactor (L).

By substituting the specific growth rate of microalgae, u, from Eq. (3.1) into Eq. (3.2) will yield Eq. (3.3) as follows, where $[S] = [N_1]$:

$$\frac{dN_1}{dt} = FN_0 - FN_1 - \frac{u_{max}}{Y}XV\frac{[N_1]}{K_N+[N_1]} \tag{3.3}$$

At steady state, $dN_1/dt = 0$. Therefore, Eq. (3.3) can be rearranged to become Eq. (3.4).

$$\frac{F(N_0-N_1)}{V} = \frac{\frac{u_{max}}{Y}X[N_1]}{K_N+[N_1]} \tag{3.4}$$

The half saturation constant for nitrogen, K_N, was reported to be 31.5 mg/L (Eze et al., 2018). The kinetic parameters obtained were simulated in Eq. (3.4) with the aid of MATLAB R2020a software tool to obtain the values of u_{max}. By obtaining a value for u_{max} from Eq. (3.4), the specific growth rate of microalgae, u, can be calculated by using Eq. (3.1) for the respective photobioreactor operations.

3.5.2 Biomass determination

The microbial biomass concentration was determined gravimetrically by collecting 10 mL of sample in a pre-weighed glass vial and centrifuged at 3,800 rpm for 30 min. The supernatant was kept separately for the subsequent COD and nitrogen species tests whereas the residual biomass was dried in the oven at 105 °C until constant weight was attained.

3.5.3 Chemical oxygen demand (COD) determination

The collected supernatant from the biomass determination (Section 3.5.2) was analysed for concentrations of COD (Closed Reflux, Titrimetric Method), following the Standard Methods for the Examination of Water and Wastewater [100]. Approximately 1.5 mL of potassium dichromate digestion solution was placed in a digestion tube followed by 2.5 mL of supernatant (sample). Approximately 3.5 mL of sulfuric acid (H_2SO_4) reagent was then added into the digestion tube so that an acid layer is formed under the sample-digestion layer. The digestion tube was capped and inverted several times to allow complete mixing before being placed in aluminium block digestor (COD reactor) preheated to 150 °C and refluxed for 2 h. After 2 h, the digestion tubes were allowed to cool to room temperature before the contents were transferred to an Erlenmeyer flask for titration. The residue in the digestion tube was transferred thoroughly into the similar flask by washing with 2-3 portions of distilled water. About 1-2 drops of ferroin indicator was added followed by titration with standardized ferrous ammonium sulfate (FAS) titrant until the colour changed from blue-green to reddish-brown in the Erlenmeyer flask. The concentration of COD (in mg/L) was calculated as below (Eq. (3.5)):

$$COD\ (in\ mg/L) = \frac{(V_a - V_b) \times M \times 8000}{V_c} \qquad (3.5)$$

where V_a is the volume of standard FAS titrant titrated for blank (in mL), V_b is the volume of standard FAS titrant titrated for sample (in mL), V_c is the sample volume (in mL) which was 2.5 mL and M is the normality of standard FAS titrant.

3.5.4 Nitrogen species determination

The collected supernatant from the biomass determination (Section 3.6.2) was analysed for concentrations of NH_4^+-N (Titrimetric Method: 4500-NH_3), NO_2^--N (Colorimetric Method: 4500-NO_2^-) and NO_3^--N (Titrimetric Method: 4500-NO_3^-) species following the Standard Methods for the Examination of Water and Wastewater [100]. The standard error was determined to be 0.2-0.3 mg/L of nitrogen concentration per drop of sulfuric acid titrant (0.009N) from the burette hence the errors were not significant (0.8-1.2% error). Also, a recovery rate test was performed by preparing a known concentration of NH_4^+-N concentration and nitrogen determination analysis was done. The standard error from the titration method was less than 1% and the same was applicable for NO_2^--N and NO_3^--N species concentration at the second distillation protocol.

3.5.4.1 Ammonium-nitrogen (NH_4^+-N) determination

Approximately 5 mL of the supernatant collected was transferred to the distillation flask and added with 2.5 mL of borate buffer solution and 3 drops of 6 N of sodium hydroxide (NaOH) solution. The distillation flask was then placed into the distillation unit (KT200 Distillation Unit, FOSS) with the tip of the delivery tube situated below the surface of 10 mL of indicating boric acid solution in a 100 mL Erlenmeyer flask. Under a hot alkaline condition, the NH_4^+-N species in the sample was distilled and trapped in the boric acid solution. The distillation rate was set at 30 mL/min which enable 90 mL of distillate was collected in a period of 3 min, reaching a total volume of 100 mL in the Erlenmeyer flask. The concentration of NH_4^+-N (in mg/L) in the sample was

determined by titrating with standardized sulfuric acid (H_2SO_4) titrant until the colour of indicator was turned from green to pale lavender in the Erlenmeyer flask and calculated as below (Eq. (3.6)):

$$NH_4^+ - N\ (in\ mg/L) = \frac{(V_c - V_d) \times N \times 14 \times 1000}{V_e} \quad (3.6)$$

where V_c is the volume of standard H_2SO_4 titrant titrated for sample (in mL), V_d is the volume of standard H_2SO_4 titrant titrated for blank (in mL), V_e is the sample volume (in mL) which was 5 mL and N is the normality of standard H_2SO_4 titrant.

3.5.4.2 Nitrite-nitrogen (NO_2^--N) determination

The concentration of NO_2^--N in the supernatant was determined via the colorimetric method where a calibration curve had to be plotted first. The 0.5 mg/L standard nitrite solution was initially transferred into 25 mL volumetric flasks in the following volume: 0, 1, 2, 3, 4, 5, 6, 7 and 8 mL. Each flask was added with 1 mL of colour reagent before being diluted with distilled water to 25 mL. The flasks were mixed well and left for 10 min but not later than 2 h for spectrophotometric measurements. The developed colour obeyed Beer's Law up to 0.16 mg/L of NO_2^--N concentration with a light path of 1 cm at the optical density of 543 nm (OD_{543}). The calibration curve of absorbance versus concentration of NO_2^--N in the sample was determined by transferring a known volume of supernatant into 25 mL volumetric flask which was later added with 1 mL of colour reagent and diluted with distilled water to 25 mL. The spectrophotometric measurements of NO_2^--N concentration was determined based on the method obtained in the calibration curve as below (Eq. 3.7):

$$NO_2^-\text{-}N\ (in\ mg/L) = 0.3405 \times OD_{543},\ R^2 = 0.999 \quad (3.7)$$

3.5.4.3 Nitrate-nitrogen (NO_3^--N) determination

About 0.4 - 0.6 g of Devarda's alloy was added into the residual sample in the distillation flask after the NH_4^+-N species was removed through the distillation in order to reduce the remaining NO_2^--N and NO_3^--N to NH_4^+-N species (Section 3.5.4.1). The distillation flask was then placed into the distillation unit again and distilled for the second time into a 10 mL of indicating boric acid solution in a 100 mL Erlenmeyer flask. When 90 mL of distillate was collected, the total NO_2^--N and NO_3^--N concentration in the sample was determined in a similar way as for NH_4^+-N concentration; in which by titrating with standardized H_2SO_4 titrant until the colour of indicator was turned from green to pale lavender in the Erlenmeyer flask. The calculation of the total NO_2^--N and NO_3^--N concentration (in mg/L) in the sample is shown below (Eq. (3.8)):

$$Total\ NO_2^- - N\ and\ NO_3^- - N\ (in\ mg/L) = \frac{(V_f - V_g) \times N \times 14 \times 1000}{V_e} \quad (3.8)$$

where V_f is the volume of standard H_2SO_4 titrant titrated for sample (in mL), V_g is the volume of standard H_2SO_4 titrant titrated for blank (in mL), V_e is the sample volume (in mL) which was 5 mL and N is the normality of standard H_2SO_4 titrant.

The concentration of NO_3^--N in the sample was calculated by substracting the total NO_2^--N and NO_3^--N concentration (determined via the titrimetric method of second distillation) (Eq. 3.8) with the NO_2^--N concentration (determined via the colorimetric method in Section 3.5.4.2) (Eq. (3.7)).

3.5.5 Colour analysis

The colour of the collected domestic wastewater sample (Section 3.1) was measured based on the stored program 125, 465 nm in the spectrophotometer (HACH DR 6000) according to HACH Method 8025 (Platinum-Cobalt Standard Method) [55]. The stored

program is calibrated in colour units based on the American Public Health Association (APHA) recommended standard of 1 colour unit being equal to 1 mg/L of platinum as chloroplatinate ion. Test results for Program 125 was measured at the optical density of 465 nm (OD_{465}). Before taking measurements of the sample (supernatant), a blank was prepared using 10 mL of deionized water and set to Zero in the instrument. The contents of the blank sample cell were discarded and rinsed 2-3 times with the sample. The sample was read in the units of Pt-Co from the instrument.

3.5.6 Trace element determination

The collected domestic wastewater sample (Section 3.1) was analysed for trace elements using the Microwave Plasma Atomic-Emission Spectrometer (MP-AES, Agilent 4210) for characterization of the influent wastewater. Approximately 10 mL of wastewater sample was filtered using a 0.45 µm, 47 mm membrane filter with a sampling manifold. About 2 mL of filtrate was collected and analysed for the following trace elements based on the multi-element standard solution (*Trace***CERT**®, Merck) in 10% nitric acid.

3.5.7 Optical microscopy

At the steady state of each operation parameters, about 10 mL of microalgal suspension culture was pipetted out from the microalgal column of the SFB-AlgalBac photobioreactor. A few sample droplets from the homogenized microalgal suspension sample were taken using a dropper and a single droplet was placed onto the center of the slide. A cover slip was carefully placed onto the sample droplet by the edges to avoid creating any air bubbles. Any excess of liquid was wiped off using a paper towel. The slide containing the microalgal droplet sample was placed onto an optical microscope (Zeiss Axio Scope.A1) to observe the bio-floc sizes of microbial communities at different operation parameters of the SFB-AlgalBac photobioreactor.

3.5.8 Flocculation efficiency determination

The flocculation efficiency of biomass was determined following the method reported by Oh et al. [107]. The sample was homogenized via gentle stirring and the sample was measured immediately using spectrophotometer (Shimadzu UV-2600) at the optical density of 650 nm (OD_{650}). The sample was left unagitated for a period of 20 minutes and the aliquot of sample was then gently extracted at a depth of 2.5 cm below the sample's surface to measure for its OD_{650} again. The flocculation efficiency (%) of biomass was finally calculated based on Eq. (3.9):

$$Flocculation\ efficiency\ (\%) = \left(1 - \frac{F}{I}\right) x\ 100\% \quad (3.9)$$

where F is the OD_{650} of unagitated sample and I is the OD_{650} of homogenized sample.

3.5.9 Protein content determination

The protein content of microalgal biomass was calculated via a nitrogen-to-protein conversion factor of 6.25 [108] as in Eq. (3.10).

$$Microalgal\ protein\ content\ (\%) = N\ (\%) \times 6.25 \quad (3.10)$$

where N is the nitrogen amounts assimilated by growing microalgal biomasses from each operation of SFB-AlgalBac photobioreactor. It is calculated via Eq. (3.11), where N_i is the concentration of nitrogen species from influent (mg/L), N_o is the concentration of nitrogen species from effluent (mg/L) and X is the microalgal biomass concentration (mg/L).

$$Nitrogen\ assimilated\ (\%) = \frac{N_i - N_o}{X} \times 100\% \quad (3.11)$$

3.5.10 Lipid extraction and yield determination

An approximate of 0.2 g of dried microalgal biomass harvested from the bioreactor was subjected to the Bligh and Dyer method of total lipid extraction and purification using a mixed methanol-chloroform with volume ratio of 2:1 [109]. The extracted lipid was collected and the biomass residue was subjected to the repeated extraction twice using the same solvent. The solvent was evaporated under dry inert gas and the leftover crude microbial lipid was collected. The total dry lipid was measured gravimetrically and the lipid content (%) of microbial biomass was calculated by dividing lipid yield (g/L) over biomass yield (g/L) as in Eq. (3.12):

$$Lipid\ content\ (\%) = \frac{Lipid\ yield\ obtained\ (g/L)}{Biomass\ yield\ obtained\ (g/L)} \times 100\% \qquad (3.12)$$

3.5.11 Transesterification and fatty acid methyl ester (FAME) analysis

Approximately 1 mL of tetrahydrofuran (THF) was added to the extracted dried microalgal lipid as a co-solvent to promote sample mixing followed by 1 mL of concentrated sulfuric acid and 4 mL of methanol were introduced to initiate the transesterification process [110]. The optimized condition of methanol to THF to lipid molar ratio set at 60:15:1 with catalyst concentration of 21 wt % was executed in an incubator shaker set at 200 rpm and 60 °C for 3 h prior to cooling to room temperature. The acid transesterification was considered in the study due to the high concentration of free fatty acid in microalgal lipid [110] which was determined to be 2.7 wt % from *Chlorella vulgaris.* Then, 4 mL of hexane mixed with the internal standard at 0.6 mg C17:0/mL of hexane, 4 mL of sodium chloride solution (10 wt. %) and 4 mL of distilled water were subsequently added into the mixture and vortexed to achieve a homogenous mixing. The mixture was transferred into a centrifuge tube and centrifuged at 5400 rpm for 5 min for phase separation. The evident upper layer containing the fatty acid methyl esters (FAMEs) and hexane was pipetted out for subsequent FAME profile analysis. The FAME profile of microalgal biodiesel was analysed by using gas chromatography

(Shimadzu GC-2010) equipped with the flame ionization detector (FID), capillary column (BPX-BD20) and helium as a carrier gas. The initial temperature of the column was set at 150 ºC and programmed to increase to 240 ºC at a ramping rate of 15 ºC/min, while the temperatures for the FID and injector were set at 250 ºC. An injection volume of 1 µl sample containing FAME with internal standard (methyl heptadecanoate) was introduced into the sample port with a split ratio of 10:1.

The FAME composition, C_{FAME}, from the extracted microalgal lipid was calculated using Eq. (3.13) [111].

$$C_{FAME}(\%) = \left(\frac{A_{comp}}{A_T - A_{IS}}\right) \times 100\% \quad (3.13)$$

where A_T is the total peak area of C6 to C24, A_{IS} is the peak area of the internal standard (methyl heptadeconatoate, C17:0) and A_{comp} is the peak area of independent component existed in the FAME profiles of the microalgal biodiesel.

3.5.12 Life cycle assessment (LCA) – Net energy analysis

The net energy analysis of microalgal based biodiesel production from the SFB-AlgalBac photobioreactor was assessed via The Cumulative Energy Demand V1.09 method, part of the Ecoinvent 3.8® database in Simapro® 9.3 in assessing the energy requirements from each of the processes from microalgal-to-biodiesel production to obtain 1 kg of biodiesel. The data for key energy inputs and outputs, viz., electricity, water and chemical solvents, i.e., methanol and chloroform, were also obtained from the Standard of Ecoinvent 3.5® database (Table 3.4).

The net energy ratio (NER) which assessed the feasibility of the system was assessed via Eq. (3.14).

$$NER = \frac{Primary\ energy\ output}{Non{-}renewable\ energy\ input} \quad (3.14)$$

where NER < 1 resulted in negative energy return, NER = 0 indicated net energy return and NER > 1 indicated positive energy return from the system [112], [113].

Table 3.3: Process systems and input data for LCI to produce 1 kg of biodiesel.

Processes	**Relevant data**	**Known data inputs from technosphere (Ecoinvent 3.8®)**
Microalgal growth cultivation	Water pumping to the cultivation system: ~195 kW h of electricity/ton of algae biomass; Air compressor for gaseous supply and culture mixing: Optimal air flow rate of 0.2 L/L min with air compressor of 230 L/min capacity consuming 2.2 kW, estimated energy consumption in 10 days ~2.9 MJ (not operated at maximum capacity). Light irradiation: 36 W with luminance of 1200 lux for 10 days at 15% light intensity; 5000 L of treated effluent discharge from the cultivation system.	Electricity -Electricity, high voltage {MY} | production mix; Water - Tap water {RoW} | tap water production, conventional treatment
Harvesting and dewatering	Electrical oven drying: 0.0028 MJ/g dried biomass.	Electricity -Electricity, high voltage {MY} | production mix
Lipid extraction	Extraction and evaporation energy: 152 MJ/kg biodiesel; Solvents: methanol and chloroform in the ratio of 2:1 (v/v), amount of solvent to dry biomass required in the ratio of 20:1.	Electricity -Electricity, high voltage {MY} | production mix; Methanol {GLO} | for market;

		Trichloromethane {GLO} | for market.
Biodiesel production	Heating: 4.05 MJ/kg biodiesel, Electricity: 0.336 kW h/kg biodiesel; Solvent methanol: 114 g/kg biodiesel.	Electricity -Electricity, high voltage {MY} | production mix; Methanol {GLO} | for market.

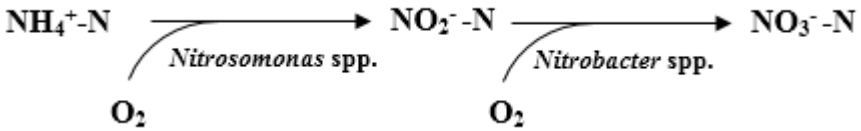
CHAPTER 4

RESULTS AND DISCUSSION

4.1 The synergistic associations of microalgal-bacterial culture in nitrogen removal and microbial biomass production

4.1.1 Nitrogen removal mechanisms and kinetics

The studies of nitrogen removal mechanisms by the co-cultures and axenic cultures of microalgae and activated sludge, respectively were based on time course profiles of NH_4^+-N, NO_2^--N and NO_3^--N species as shown in Fig. 4.1. The bioreactor inoculated with activated sludge culture demonstrated the typical trends of nitrogen species during the nitrification phase. The reduction of NH_4^+-N concentration would lead to the increase of total NO_2^--N and NO_3^--N concentrations. As the population size of *Nitrosomonas* spp. is always larger than *Nitrobacter* spp. [114], it caused accumulation of NO_2^--N concentration in the mixed liquor of wastewater medium in which the *Nitrosomonas* spp. performs nitritation of NH_4^+-N to NO_2^--N and *Nitrobacter* spp. performs nitratation of NO_2^--N to NO_3^--N during the progress of nitrification process (Eq. 4.1). As NO_2^--N is more toxic than other nitrogen species [114], the build-up of NO_2^--N would culminate in the retardation of nitrification process as observed in Control 1 bioreactor from day 5 onwards, i.e., all nitrogen species concentrations remained relatively plateau.



(4.1)

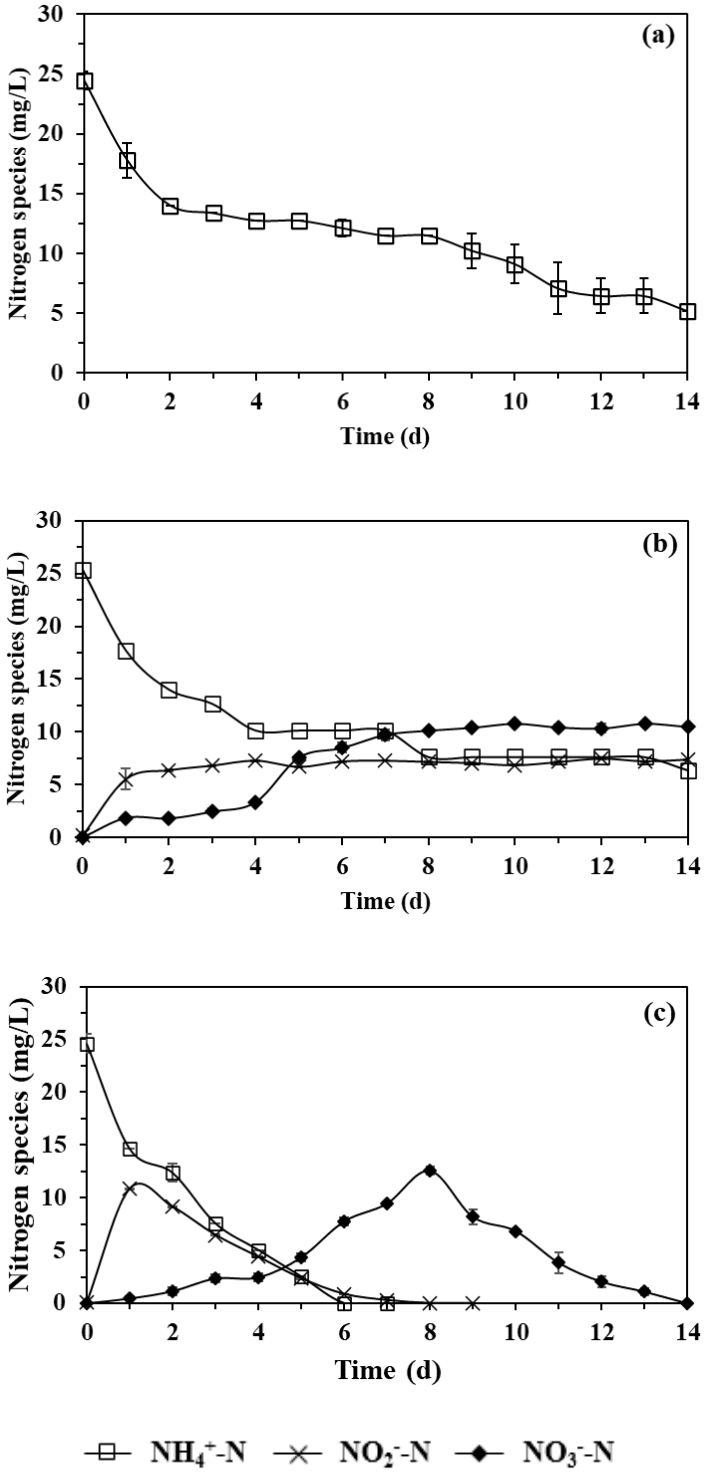


Figure 4.1: Profiles of nitrogen removal in bioreactors inoculated with microalgae (a), activated sludge (b) and co-culture (c).

In the case of bioreactor inoculated with only microalgae biomass, the initial drop of NH_4^+-N concentration was principally due to the mixotrophic nature of *Chlorella vulgaris* [75], [115]. The NH_4^+-N was rapidly assimilated while the sucrose and dissolved carbon dioxide from wastewater medium were simultaneously consumed and utilized as a carbon source for growing. From day 2 onwards, the descent of NH_4^+-N concentration was more gradual as the organic carbon source of sucrose was depleted from the wastewater medium. Accordingly, the assimilation of NH_4^+-N for growing was totally depending on the uptake of dissolved carbon dioxide source alone. On another note, throughout the cultivation period of axenic microalgae culture, the concentrations of NO_2^--N and NO_3^--N appeared below the detection limits.

In the co-culture bioreactor, the decrease of NH_4^+-N concentration was due to the simultaneous nitritation and assimilation (SNA) processes performed by activated sludge and microalgae, respectively. The build-ups of NO_2^--N and NO_3^--N concentrations from the nitrification process were also gradually assimilated by microalgae biomass. These decreases, NO_2^--N and NO_3^--N concentrations from the co-culture bioreactor, could be evidently viewed when their precursor species were depleted from wastewater medium. For instance, the depletion of NH_4^+-N would lead to the gradual decrease of NO_2^--N and the depletion of NO_2^--N would lead to the gradual decrease of NO_3^--N as manifested in any co-cultivation bioreactor of activated sludge and microalgae biomasses. Indeed, the assimilation of all nitrogen species by microalgae were advancing concurrently as reported by Collos and Berges [116] while producing new biomass.

Apart from that, the preferential source of nitrogen species for spurring assimilation process to grow microalgae biomass was then identified. The k_A values attained using NO_2^--N and NO_3^--N to grow microalgae biomass were 2.38 ± 0.00 and 2.78 ± 0.07 mg/L/d, respectively. These k_A values were much higher than the k_A value using NH_4^+-N source to grow microalgae biomass, i.e., merely 0.74 ± 0.05 mg/L/d as demonstrated by the microalgae bioreactor. Better yet, the high k_A values using NO_2^--N and NO_3^--N

to grow microalgae biomass also allayed the inhibitory effect of NO_2^--N on nitrifiers, permitting the nitrification process to occur incessantly in the co-culture bioreactor. The bioconversion of NH_4^+-N into NO_2^--N and NO_3^--N by nitrifiers from activated sludge would directly promote the assimilation process by microalgae biomass for growing. The symbiotic interactions between the activated sludge and microalgae biomasses in co-cultivation bioreactors had consequential impact on biomass production used to produce lipid feedstock for biofuel industries.

4.1.2 Biomass growth patterns and kinetics

The patterns of biomass growth over time in bioreactors inoculated with co-cultures and axenic cultures of microalgae and activated sludge, respectively were shown in Fig. 4.2. The initial upsurge of biomass concentrations in all bioreactors was mainly imparted by the presence of organic carbon source of sucrose, being rapidly assimilated to produce new biomass. Once this biodegradable COD source was used up, the growth of activated sludge biomass in the activated sludge bioreactor was virtually ceasing with relatively constant biomass concentration which was measured throughout the cultivation period. In the bioreactor inoculated with only microalgae, the growth of its biomass was rather constant and in commensurate with the gradual assimilation of NH_4^+-N source (Fig. 4.1). The co-cultivation bioreactors, on the other hand, were experiencing the exponential growth swiftly after the NO_2^--N and NO_3^--N sources were generated from the nitrification process executed by the nitrifiers in activated sludge. As discussed in Section 4.1.1, the oxidized nitrogen species of NO_2^--N and NO_3^--N were the preferential sources for assimilation over NH_4^+-N for microalgae biomass growth. While it is generally known that the phytoplankton tend to prefer NH_4^+-N over oxidized nitrogen due to its more reduced state which makes it easier for assimilation into the cells, this is however not necessarily true as revealed in this study. In this case, the microalgae biomass exposed to only NH_4^+-N source was growing slower than the microalgae in the co-culture bioreactor in which the oxidized nitrogen species were

available (Figs. 4.1 and 4.2). The uptake mechanism of NH_4^+-N into the microalgae cells is usually accompanied by a pH decrease caused by the translocation of protons from the cells in maintaining the cells neutrality during the cations uptake [117]. The decrease in medium pH will in turn inhibit the microalgae cells growth when NH_4^+-N is utilized as the nitrogen source. While this is only applicable if there is only NH_4^+-N presents in the cultivation medium, that is not the case when there are two and more nitrogen sources present at the same time. The initial assimilation of NH_4^+-N by microalgae biomass and later shifted to NO_2^--N and NO_3^--N as the preferential nitrogen sources had resulted in the enhancement of biomass growth in the co-culture bioreactor. The increase in cell densities of phytoplankton under the similar condition were also noticed by other researchers [118], [119].

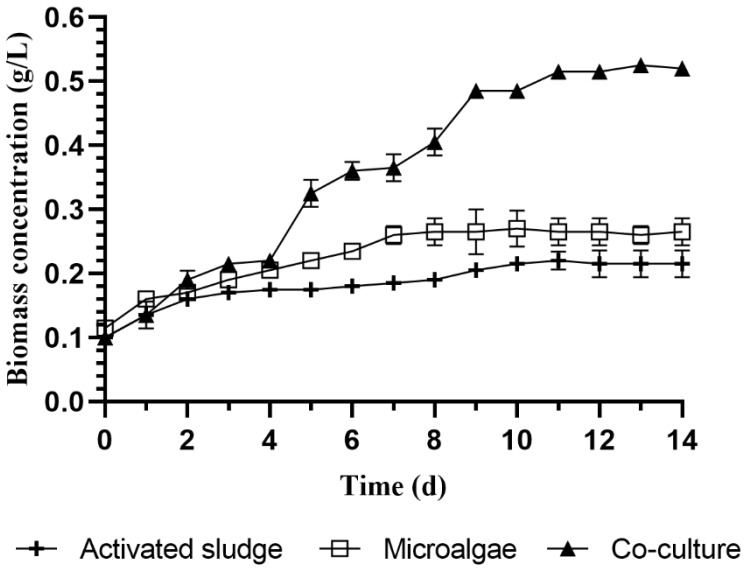


Figure 4.2: Profiles of biomass growth in bioreactors inoculated with microalgae, activated sludge and co-culture.

The measurements of biomass productivity unveiled the activated sludge bioreactor showing inconspicuous new biomass production. The bioreactor inoculated with microalgae biomass alone gained the least new biomass, bearing the biomass

productivity value of only 0.01 ± 0.001 g/L/d. This value was tripled when the activated sludge and microalgae biomasses were co-cultivated in the same bioreactor (0.03 g/L/d) until the stationary growth phase, preponderantly resulted from the presence of oxidized nitrogen (Fig. 4.2). Uggetti et al. [120] had also substantiated that the high microalgae biomass concentrated at the beginning of the experiment would generate more biomass. The importance of bacteria co-cultivated with microalgae biomass had as well been documented by Ma et al. [17] proving the presence of bacteria could improve the algal growth rate via the uptake of more nutrients. As such, concurrently serving as an impetus in treating nutrients containing wastewaters through the continuous cultivation process.

4.2 Flow rate optimization of SFB-AlgalBac photobioreactor for continuous microalgal biomass production and wastewater bioremediation

4.2.1 Nitrogen profiles during various flow rate operations

The overall nitrogen removal efficiencies fluctuated between 56% and 53% for all the flow rates studied in the SFB-AlgalBac photobioreactor. While the overall nitrogen removal efficiency was lower in comparison with a batch system, the later could only treat limited volume of wastewater. In light of continuous cultivation in SFB-AlgalBac photobioreactor, the initial nitrification to convert NH_4^+-N into NO_3^--N by the nitrifiers in the activated sludge was deemed to be more effective for rapid assimilation by the microalgal biomass later. This was determined in a previous study, where microalgal culture showed preferential to assimilate nitrogen species in the NO_3^--N form rather than the other nitrogen species forms. The dominant form of nitrogen species found in almost all wastewaters is usually NH_4^+-N and high NH_4^+-N concentrations under alkaline conditions could inhibit the microalgal growth due to ammonia toxicity [68].

Thus, the nitrification process initially executed in the activated sludge cultivation column of SFB-AlgalBac photobioreactor was crucial to protect and promote the microalgal growth.

The nitrogen profiles of the activated sludge and microalgal cultures in their respective cultivation columns under various influent flow rates are shown in Fig. 4.3. From the activated sludge nitrogen profile (Fig. 4.3A), it was observed that the nitrification rate increased exponentially, corresponding to the increase in influent flow rates from 0.00221 /d (batch) followed by 0.00458 /d (2.5 L/d), 0.0101 /d (5.0 L/d) and 0.0202 /d (10.0 L/d). The increase in influent flow rates would mean more NH_4^+-N source being nitrified, following the exponential increase in NH_4^+-N being supplied into the photobioreactor. Therefore, the change in the influent flow rates did not adversely affect the nitrification activity of the activated sludge. In fact, it strengthened the nitrification activity with corresponding increase in influent flow rates. While the nitrification activity of the activated sludge was enhanced despite the increase in influent flow rates, the NO_3^--N having been produced from the nitrification process was only amounted to 68.18 % – 73.08 % of the influent NH_4^+-N supplied into the photobioreactor. Meanwhile, there was absence of NO_2^--N concentration being detected in the activated sludge effluent for all of the influent flow rates assessed in the study, signifying the nitratation process of NO_2^--N into NO_3^--N during the progress of nitrification was complete. The remaining NH_4^+-N concentrations were plausibly lost to the assimilation process in maintaining the sludge age at 40 days. An insignificant amount of NH_4^+-N maybe be as well lost to the simultaneous nitrification and denitrification processes with the dissolved oxygen concentration set at above 4 mg/L and the biodegradable COD was completely depleted in the activated sludge culture immediately after being introduced [83]. The NO_3^--N produced from nitrification process in activated sludge cultivation column (effluent) would be then flown into the microalgal culture cultivation column (influent).

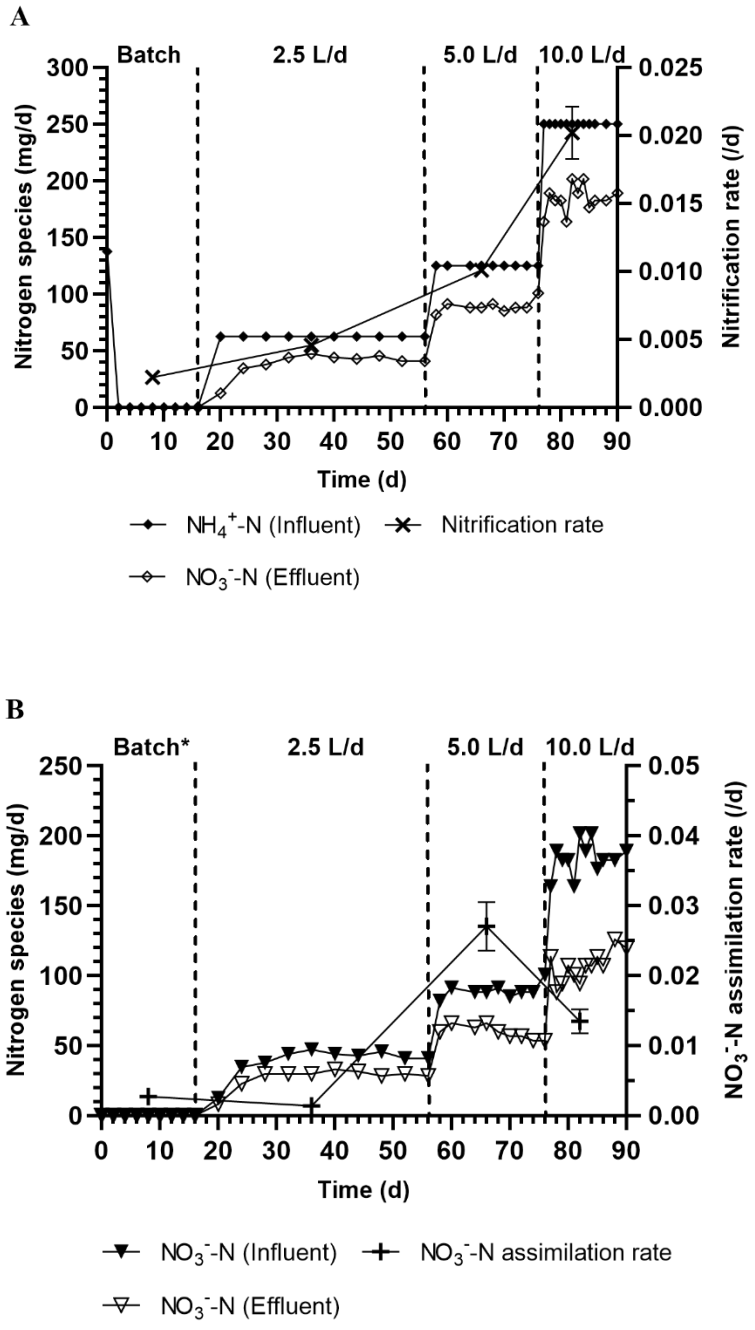


Figure 4.3: Nitrogen profiles of activated sludge (A) and microalgal (B) cultivation columns under batch and various influent flow rates. (*: indicates NH_4^+-N as nitrogen source)

From the microalgal nitrogen profile (Fig. 4.3B), it was determined that the 5.0 L/d flow rate had appeared to be the most optimum for the microalgal biomass to effectively assimilate the available NO_3^--N for growing as opposed to the other flow rates which were 2.5 and 10.0 L/d. Accordingly, the microalgal culture under the 5.0 L/d flow rate had recorded the highest assimilation rate which was 0.0271 /d as compared with the assimilation rate values of 0.00274 /d in batch, 0.00138 /d in 2.5 L/d and 0.0135 /d in 10.0 L/d. While there was an increase in terms of amount of NO_3^--N being removed via assimilation with increasing flow rates, the NO_3^--N being effectively assimilated into the microalgal biomass eventually dwindled when the influent flow rate was increased from 5.0 L/d into 10.0 L/d. For instance, the amount of NO_3^--N assimilated for microalgal biomass growth had increased about 170.96 % with increasing flow rates from 2.5 L/d into 5.0 L/d. But then decreasing to 86.67 % when the flow rate was further increased into 10.0 L/d. It could be concluded that any further increment in flow rates higher than 5.0 L/d would possibly jeopardize the microalgal biomass to effectively assimilate the available NO_3^--N source in the medium. This was evidenced by the NO_3^--N concentrations detected in the effluent in which showing a slight increase pattern towards the end of culture in 10.0 L/d flow rate (Fig. 4.3B), resulting in poor microalgal biomass productivity as discussed in the section after this.

4.2.2 Microalgal biomass production under various flow rate operations

Fig. 4.4 showed the time courses of the SFB-AlgalBac photobioreactor in terms of microalgal biomass concentration and productivity under batch and various influent flow rates. The photobioreactor unit was started with a batch cultivation mode to cultivate the microalgal cells up to the steady state. Also, serving as a comparative study with a continuous flow mode of increasing influent flow rates. A maximum microalgal biomass concentration of 270 mg/L was attained at the stationary growth phase in the batch cultivation mode with a biomass productivity of merely 8.125 mg/d. Thereafter, the continuous flow mode began with the lowest influent flow rate of 2.5 L/d. During

this period, the microalgal biomass concentration was fluctuating in a range of 250 to 270 mg/L for 24 days (day 16 – 40). The concentration was eventually experiencing a slight decrease to around 230 mg/L in day 44, before attaining a steady microalgal biomass concentration at 245 mg/L later with an average microalgal biomass productivity of 612.5 mg/d towards the end of 40 days of HRT. While the cultivation mode was changed from a batch mode into a continuous mode, the microalgal culture was expected to be affected; but, such change was only prevalent between day 40 and 44. With the starting influent flow rate of 2.5 L/d in which accounted for 2.5 % of the whole volume of microalgal cultivation column, the effect on the microalgal cultivation as indicated by the slight decrease in biomass concentration was only started when at least 60% of the medium volume had been replaced. Nevertheless, the difference in biomass concentrations between batch mode and continuous mode remained indifferent, vindicating that the microalgal culture could keep up with the biomass production at the influent flow rate of 2.5 L/d. Indeed, the microalgal biomass productivity for 2.5 L/d flow rate was 75 times higher than the batch cultivation mode, stemming from the presence of more NO_3^--N in the continuous cultivation mode.

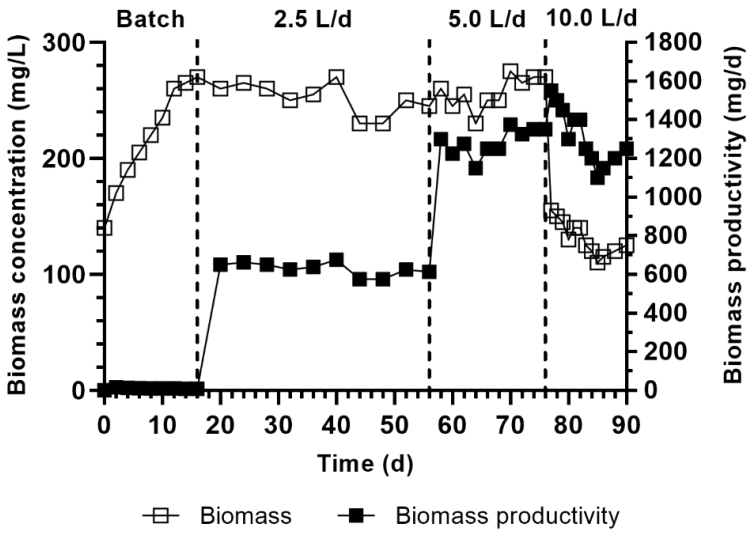


Figure 4.4: Time courses of microalgal biomass concentration and productivity under batch and various influent flow rates.

The microalgal biomass under the influent flow rate of 5.0 L/d showed an increase in concentration after an initial relatively plateau production pattern in the range of 230 – 260 mg/L between day 56 and 68. The concentration was then increased to about 275 mg/L before achieving a steady state at an average biomass concentration of 270 mg/L. The microalgal biomass productivity was obtained at 1350 mg/d during the steady state (day 70 – 76), which was more than twice the productivity achieved at previous influent flow rate. Although, the influent flow rate was doubled from 2.5 to 5.0 L/d, twice the hydraulic washing out strength, the microalgal culture was still able to retain and even improve its biomass production with a little increase in concentration (270 mg/L) as opposed to 2.5 L/d flow rate (245 mg/L) and equivalent to batch mode at steady growth phase.

Unfortunately, the biomass production of microalgal culture under the 10.0 L/d flow rate was observed experiencing a rapid decrease in concentration from 270 mg/L to only 110 mg/L (day 76 – 85), before showing a slight increase and eventually achieving a steady state at the microalgal biomass concentration of about 120 mg/L. The increase in influent flow rate from 5.0 to 10.0 L/d had engendered the entire system of microalgal culture being intensely disturbed via excessive washing out, resulting in poor culture vitality. The average microalgal biomass productivity was recorded at 1200 mg/d had also shown a substantial decrease as compared with the previous 5.0 L/d flow rate, i.e., more than 10 % of reduction. This signified that, any further increase in influent flow rates would further retard the microalgal culture as the biomass production could not keep up with an extreme increase in influent flow rate. Thereby, resulting in an unhealthy growth performance of microalgal culture.

4.2.3 Kinetic modelling of microalgal biomass growth under various flow rate operations

The kinetic growth parameters of microalgal biomass derived from increasing influent flow rates are epitomized in Table 4.1. The kinetic model was improved from a Monod

growth model to accommodate the continuous flow mode of SFB-AlgalBac photobioreactor with nitrogen serving as the nutrient limiting factor. The specific growth rate attained could be used as an indicator to determine the optimum flow rate of the photobioreactor under continuous flow mode. Accordingly, the specific growth rates were observed increasing with the increase in influent flow rates. Likewise, the maximum specific growth rates followed the similar trend. Despite recording higher microalgal biomass concentrations in both 2.5 and 5.0 L/d flow rates with the values of 245 and 270 mg/L, respectively, its specific biomass growth rates derived were still lower than that of the 10.0 L/d. While the microalgal cultivation under the 10.0 L/d flow rate may have achieved the highest specific growth rate due to the highest amount of influent being introduced into the culture medium, also reflecting more nitrogen availability for growing microalgal biomass, the nitrogen source, however, was not effectively assimilated for building more microalgal cells. The strong hydraulic strength experienced by 10.0 L/d flow rate would forestall the microalgal cells from growing to maturity using the assimilated nitrogen source, i.e., the immature cells had been washed out from the photobioreactor through weir. This was further evidenced by the microalgal biomass per unit of nitrogen assimilated values with both the 2.5 and 5.0 L/d flow rates had secured higher values, namely, 15.14 and 16.69 mg/mg, respectively, as opposed to merely 7.73 mg/mg in 10.0 L/d flow rate. Moreover, the differences in specific growth rate were about 3.7 times between 5.0 and 2.5 L/d flow rates and only about 1.4 times when the influent flow rate was increased from 5.0 and 10.0 L/d. In light of considering both the specific growth rate and microalgal biomass per unit of nitrogen assimilated as the parameters to justify the optimum influent flow rate for SFB-AlgalBac photobioreactor, the 5.0 L/d flow rate was concluded to be appropriate in generating decent specific growth rate while ensuring effective nitrogen assimilation for growing more microalgal biomass. Under the optimized 5.0 L/d flow rate employed in the study, the maximum biomass productivity and specific growth rate recorded were 0.275 g/L/d and 0.1151 /d. A study by Lam et al. [13] recorded higher specific growth rate (0.3 /d) but much lower biomass productivity (0.0409 g/L/d) from cultivating

Chlorella vulgaris using nutrients from domestic wastewater. While specific growth rate is one of the key parameters for measuring microalgal growth, it is only measured at the log growth phase and did not account for the overall microalgal biomass production. Also, it is worth noting that higher specific growth rate does not reflect higher biomass productivity and only limited to batch cultivation studies. Similarly, Lu et al. [121] cultivated *Chlorella* sp. using diary wastewater and observed lower specific growth rates but higher biomass productivities in outdoor cultivations compared with indoor cultivations. The indoor cultivation revealed maximum biomass productivities and specific growth rates of 0.338 g/L/d and 0.274 /d, respectively while outdoor cultivations observed 0.475 g/L/d and 0.098 /d, respectively. In light of considering a high yet continuous microalgal biomass production, the biomass productivity is a more relevant parameter when it comes to efficiency in producing a high yet consistent biomass production in a continuous manner.

Table 4.1: Kinetic growth parameters of microalgal biomass derived from continuous flow rate operations in SFB-AlgalBac photobioreactor.

Influent flow rate, F (L/d)	**Microalgal biomass, X (g/L)**	**Microalgal biomass per unit of nitrogen assimilated, Y (mg/mg)**	**Maximum specific growth rate, μ_{max} (/d)**	**Specific growth rate, μ (/d)**
2.5	245	15.14	0.0294	0.0078
5.0	270	16.69	0.1151	0.0292
10.0	125	7.73	0.1555	0.0428

In this study, the nitrogen source was found to be the main nutrient contributing to the kinetics of microalgal biomass growth in the SFB-AlgalBac photobioreactor. While other nutrients such as carbon source in which usually comprised most of the microalgal, *Chlorella vulgaris*, cell content (46 - 51 wt %) [122], the change in carbon

nutrients, either the organic or inorganic form, was not significant in contributing to the microalgal kinetic modelling. Since the influent medium was passing through the activated sludge cultivation column to allow prior nitrification before flowing into the microalgal cultivation column, the presence of organic carbon sources in influent medium would be immediately consumed by the activated sludge community to maintain its biomass growth via the aerobic activity. This was evidenced by the low COD concentration in the activated sludge clarifier. Instead, the nitrification activity executed by activated sludge culture would increase the dissolved CO_2 concentration in its clarifier to serve as an inorganic carbon source for the microalgal culture. Also, the dissolved CO_2 concentrations were found increasing with the increase in flow rates due to the increasing influent medium volumes being subjected to the nitrification pre-treatment in the activated sludge cultivation column. The dissolved CO_2 concentrations in the microalgal culture were measured at 123.82 mg/L for batch mode, and later increasing from 175.22 to 616.08 mg/L with the influent flow rate rising from 2.5 to 10.0 L/d. Nevertheless, the microalgal biomass production did not seem to be in tune with the increase of dissolved CO_2 concentrations especially amidst the influent flow rate transition from 5.0 to 10.0 L/d (Fig. 4.4). Thus, it could be inferred that the inorganic carbon source in the form of dissolved CO_2 could not be assimilated effectively into the microalgal biomass at higher flow rates, albeit higher dissolved CO_2 concentrations were available in the culture medium. The low impact of dissolved CO_2 concentrations on microalgal growth had as well been predicted via statistical modelling [123]. The concentrations of dissolved CO_2 above 200 mg/L were confirmed to have inconspicuous impact on maximum microalgal biomass concentration as presented in the perturbation plot modelled by the Design-Expert, Centre Composite Design tool.

4.2.4 Bio-floc community under various flow rate operations

The microscopic images of culture medium samples collected at the steady state operations of the SFB-AlgalBac photobioreactor are presented in Fig. 4.5. Under the batch cultivation mode, the size of microalgal cells was observed to fall in the range from 1 to 20 µm (Fig. 4.5) with a flocculation efficiency being measured at merely 1.23 ± 0.03 %. This had caused poor settleability, a trait that is common in all microalgal cultures, with a microalgal biomass density being close to that of the water medium. Fortunately, the microalgal settleability can be enhanced through the formation of microalgal-bacterial aggregates [124], [125]. As such, the microalgal-bacterial cultivation approach is often adopted to improve the settleability of microalgal cells through the formation of mixed microbial flocs, besides treating nutrients in wastewater which is usually exploited by the high-rate algal ponds. Nevertheless, this cultivation approach has led to a high contamination of bacteria in harvested microalgal biomass. Thus, the function of SFB-AlgalBac photobioreactor designed for current study was also to separate the bacterial culture in the form of activated sludge from the microalgal culture in minimizing the contamination by bacterial population.

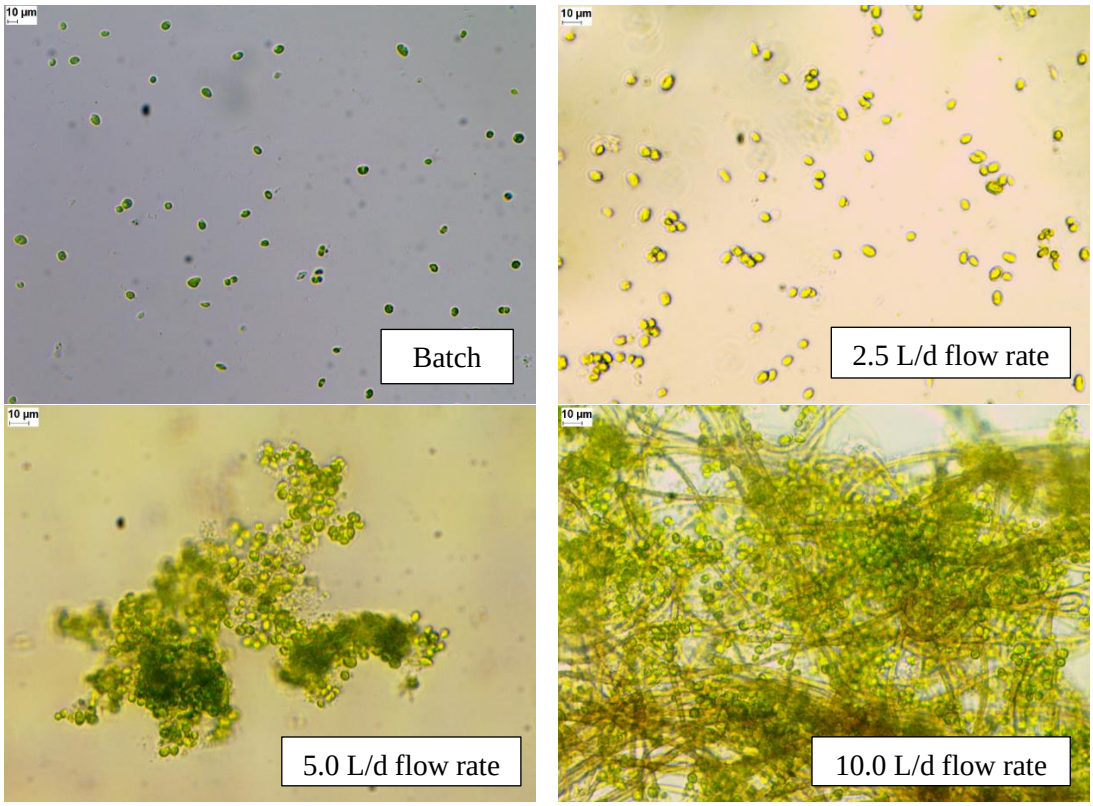


Figure 4.5: Microscopic images of culture medium samples collected from microalgal cultivation column during each steady state operation.

In transitioning from batch into continuous cultivation modes, the activated sludge treatment was initially introduced before the medium flowing into the microalgal cultivation column. As observed in Fig. 4.5, the microalgal cells formed increasingly bio-floc sizes when the influent flow rate was increased from 2.5 to 10.0 L/d. As a result of that, changing the bio-floc communities that eventually would impact the flocculation efficiency parameter. The bio-floc communities in 2.5 and 5.0 L/d flow rates were comprising of unicellular microalgal cells, clumping together to form flocs with the latter flow rate possessing bigger bio-floc sizes. Correspondingly, the flocculation efficiencies were measured at 10.52 ± 1.32 % and 25.72 ± 1.44 % with respect to 2.5 and 5.0 L/d flow rates. The formation of bio-flocs in the microalgal cultivation column was attributed to the two main factors, namely, (1) the presence of extracellular polymeric substances (EPS) in the medium exuded by the activated sludge

culture and (2) the induced stress stemming from increasing microalgal biomass washout at higher flow rates in the continuous cultivation mode. Since the microalgal biomass concentrations between 2.5 and 5.0 L/d flow rates were not much varied (Fig. 3), the formation of bio-flocs was mainly contributed by the EPS availability in the medium. At higher influent flow rates (5.0 against 2.5 L/d), there were more available medium volume treated by the activated sludge culture, resulting in higher EPS availability in which later flowing into the microalgal cultivation column. The hydrophobicity and positively charged surface of the EPS would promote self-aggregation among the microalgal cells; thus, inducing the bio-flocs formation. The flocculation efficiencies attained were also in conformity with the increasing sizes of bio-flocs. Thus, the increase in influent flow rate to 10.0 L/d had further promoted the flocculation efficiency to tune of 37.01 ± 3.25 %. Interestingly, the bio-flocs structure as seen for 10.0 L/d flow rate was surrounded by the filamentous microorganisms, either cyanobacteria or fungi, in which also accompanied by the highest flocculation efficiency value. While the microalgal cultivation column in the SFP-AlgalBac photobioreactor was designed to facilitate the microalgal cultivation without mixing with the bacterial culture, the possibility of suspended bacteria being inevitably introduced into the microalgal culture was still not to be ruled out preponderantly at the highest influent flow rate of 10.0 L/d. Moreover, coupling with the induced stress phenomenon as experienced by the microalgal culture due to the excessive biomass washout at 10.0 L/d flow rate (Fig. 4.4), the growth of filamentous microorganisms would bloom since the competition with microalgal cells for common nutrients had been abated. Besides, the filaments contained cell chains, comprising of inter-connected daughter cells by their cell walls in which could effectively prevent the washout of smaller cells [126]. This had also been reported by the other studies, demonstrating the filaments were often found in settleable microalgal bio-flocs either in the microalgal-bacterial aggregates or microalgal consortia [125], [127]. In this case, the filamentous microorganisms coupled with the high EPS availability in the medium had prompted into creating a microenvironment which facilitated the synergistic associations between

autotrophic and heterotrophic microorganisms as well as protection against prevalent predator that is common in wastewaters, e.g., rotifers [128], which can be observed in Fig. 4.6.

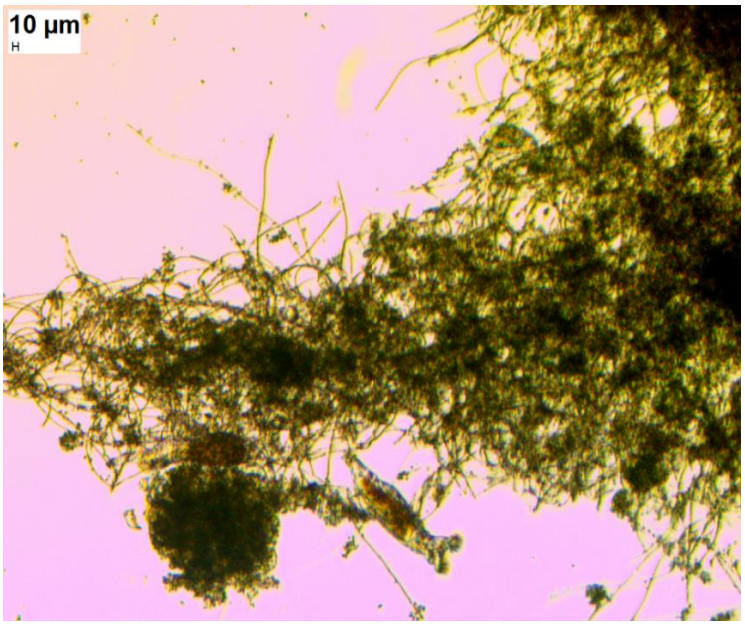


Figure 4.6: Microscopic image of rotifers presented in the culture medium sample collected from microalgal cultivation column during the 10.0 L/d flow rate operation.

4.3 Dual nitrogen heterogeneity mode via continuous flow diversion of wastewater feed and activated sludge influent

4.3.1 Mechanism and profiles of nitrogen removal under various diverted wastewater loadings

The nitrogen removal mechanism in the SFB-AlgalBac photobioreactor was designed to exploit the by-products generated in activated sludge effluent in blending with flesh nutrient-rich wastewater for growing microalgae via nitrogen assimilation. In this regard, the prior nitrification by the nitrifiers in activated sludge would convert the NH_4^+-N, which constituted as the dominant nitrogen species in nutrient-rich wastewaters, into oxidized form, primarily NO_3^--N for the rapid uptake by microalgal cells. Also, the blending of activated sludge effluent laden with NO_3^--N would dilute the NH_4^+-N concentration from nutrient-rich wastewater, reducing the impact of ammonia toxicity to the microalgal cultivation. While previous studies reported high nitrogen assimilation rates were observed in the freshwater microalgal species, i.e., *Chlorella* sp., when NO_3^--N was the sole nitrogen source [129], other investigations had postulated that the microalgal cells would prefer to assimilate NH_4^+-N directly due to lesser energy requirement in the uptake mechanism as opposed to the other nitrogen species [130]. However, the recent studies had pointed out on the complexity of nitrogen species assimilation into microalgal cells that was not supporting the universal preference because of the other interactive effects stemming from the various cultivation conditions as well as the microalgae species itself would behave differently [129], [131].

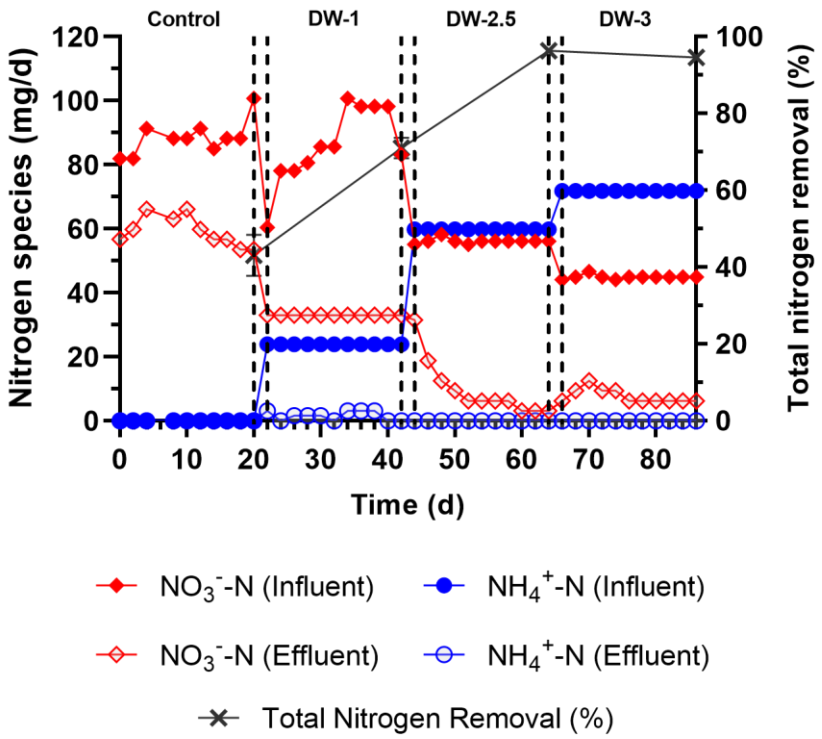


Figure 4.7: Microalgal assimilation profiles of NH_4^+-N and NO_3^--N in the SFB-AlgalBac photobioreactor under various diverted flows from blending nutrient-rich wastewater and activated sludge effluent together with the respective total nitrogen removal efficiencies.

The profiles of different nitrogen species assimilated into microalgae in the SFB-AlgalBac photobioreactor under various diverted flows from blending nutrient-rich wastewater and activated sludge effluent are presented in Fig. 4.7, together with overall total nitrogen removal efficiencies. When the photobioreactor was operated at controlled condition, the sole nitrogen source was originating from the activated sludge effluent and containing only NO_3^--N due to the complete nitrification process. Nevertheless, the total nitrogen removal efficiency was recorded at merely 40.91% amidst this operation at the steady stage of microalgal assimilation. With the increase of NH_4^+-N loadings from nutrient-rich wastewater directly into microalgal column of SFB-AlgalBac photobioreactor (DW-1 < DW-2.5 < DW-3), the total nitrogen removal

efficiencies had improved considerably from 66.85 % for DW-1 to 96.38 % for DW-2.5. However, it decreased to 94.60 % for DW-3 albeit the descent was rather insignificant as the total nitrogen removal had been recorded at almost complete (>94 %). Whilst the NO_3^--N was the sole nitrogen source used for growing microalgae in the controlled operation, the nitrogen assimilation rate recorded for this operation was at 0.0021 /d. The nitrogen assimilation rate by microalgae was subsequently increased to 0.0032 /d with the introduction of NH_4^+-N loading (24 mg/L) for DW-1. The complete removal of NH_4^+-N was observed during this operation of DW-1 with the remaining NO_3^--N concentration reached at around 33 mg/L in the effluent discharged by the photobioreactor. It was surmised that with the presence of NH_4^+-N mixing with NO_3^--N in the microalgal cultivation medium, the nitrogen uptake mechanism by the microalgae would shift into assimilating NH_4^+-N first before NO_3^--N due to the reduced metabolic energy requirement during the uptake process, which could be then utilized for other metabolic cellular processes [132]. When the NH_4^+-N loadings from nutrient-rich wastewater increased to 60 mg/L and 72 mg/L for DW-2.5 and DW-3 operations, respectively, the NH_4^+-N was still completely depleted at the steady stage with the remnant NO_3^--N in the effluent fluctuating within 4 - 6 mg/L for both operations. Better yet, the assimilation of nitrogen sources by microalgal cells was evidenced to be more effective with increasing NH_4^+-N loading into the NO_3^--N mixed medium, serving as a dual nitrogen species cultivation mode as opposed to the sole NO_3^--N species in the controlled operation. While both the DW-2.5 and DW-3 operations had recorded similar nitrogen removal performances with comparable nitrogen assimilation rates of 0.0015 /d and 0.0014 /d, respectively, further increasing of NH_4^+-N loadings into the mixed medium would defeat the purpose of the photobioreactor design, i.e., blending the nitrified effluent from activated sludge with nutrient-rich wastewater for growing microalgae and reducing nitrogen sources simultaneously. Therefore, the DW-2.5 operation with approximately equal amounts of NH_4^+-N (56 mg/L) and NO_3^--N (59 mg/L) concentrations loaded into the mixed medium was deemed to be optimum for

the microalgal cells to completely assimilate the nitrogen sources loaded into the photobioreactor.

4.3.2 Microalgal biomass productions and growth kinetics under various diverted wastewater loadings

The operation of SFB-AlgalBac photobioreactor was designed to generate the microalgal biomass in a continuous mode, exploiting the nitrification process performed by the activated sludge to convert NH_4^+-N into oxidized nitrogen species, i.e., NO_3^--N which was the preferable nitrogen species for the microalgal assimilation. This would reduce the retention time required for the microalgal biomass growth via a rapid nitrogenous nutrient nitrogen-assimilation mechanism. In enhancing the microalgal growth further, the nutrient-rich wastewater containing NH_4^+-N was blended with the activated sludge effluent laden with NO_3^--N to allow a more effective nitrogen utilization-cum-assimilation by the microalgal biomass.

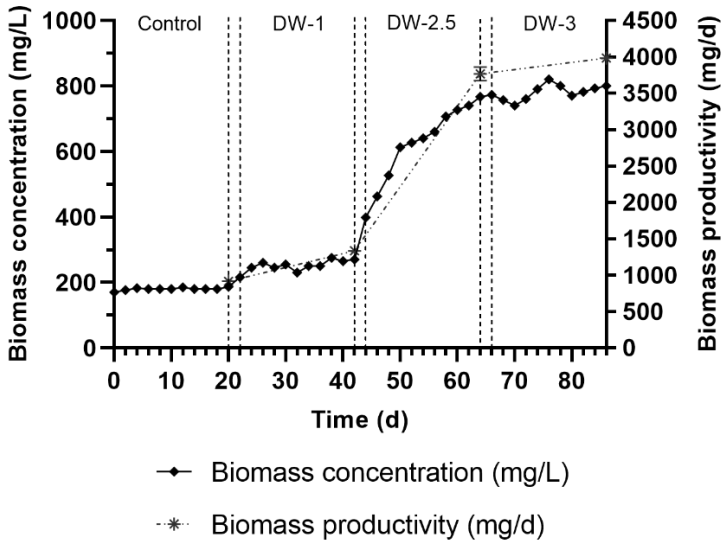


Figure 4.8: Microalgal biomass concentrations and productivities derived from the SFB-AlgalBac photobioreactor under various diverted flows from blending nutrient-rich wastewater and activated sludge effluent.

The microalgal biomass growth patterns and productivities under various diverted flows from blending nutrient-rich wastewater with activated sludge effluent are shown in Fig. 4.8. The microalgal biomass concentration under the controlled condition with influent solely from activated sludge had recorded at 182 mg/L upon reaching the steady state operation with the biomass productivity attained at 910 mg/d. With the diverted flow to siphon part of NH_4^+-N loadings directly into the microalgal cultivation column of the SFB-AlgalBac photobioreactor, the microalgal biomass concentrations were noticed increasing from 270 mg/L up to 792 mg/L, corresponding to the increase in NH_4^+-N loadings from DW-1 to DW-3. At DW-1 operation where approximately 23-24 mg/L of NH_4^+-N concentrations were loaded from nutrient-rich wastewater, the microalgal biomass concentration had recorded a slight increase to 270 mg/L from 182 mg/L for the controlled operation in which the nitrogen source was presented only in the form of NO_3^--N supplied by the activated sludge effluent. Further increasing in NH_4^+-N loading with a reduced NO_3^--N loading from nutrient-rich wastewater and activated sludge effluent, respectively (Fig. 4.7), the DW-2.5 operation had demonstrated a huge leap in microalgal biomass concentration to 745 mg/L, which was about 2.75 times increase as opposed to DW-1 operation. Increasing the NH_4^+-N loading for the DW-3 operation saw a subtle increase in microalgal biomass concentrations to 792 mg/L. It was deduced that the microalgal biomasses under the DW-2.5 and DW-3 operations could fully utilize or uptake all the nitrogen species supplied as near complete total nitrogen removals (>94 %) were achieved. The microalgal biomass productivities were also not varied conspicuously during these two operations.

The effectiveness of blended nitrogen species, namely, NH_4^+-N and NO_3^--N, for growing microalgae was also determined in terms of amount of microalgal biomass produced per unit nitrogen assimilated (Table 4.2). As observed from Fig. 4.8, the microalgal biomass concentrations and productivities increased with increasing of NH_4^+-N loadings blended with the activated sludge effluent containing NO_3^--N,

inducing a more effective environment for nitrogenous nutrient uptake by the microalgal cells. Accordingly, higher NH_4^+-N loading had observed better nitrogen assimilation into the microalgal biomass, achieving a maximum microalgal growth of approximately 46 mg microalgal biomass/mg nitrogen. Beyond the DW-2.5 operation, the assimilated nitrogen could not further enhance the microalgal growth. While the specific growth rates derived from the microalgal biomass growth kinetics also increased with increasing of NH_4^+-N loadings, both the DW-2.5 and DW-3 operations had recorded the highest specific growth rate at 0.071 /d with similar values of maximum specific growth rate of 0.168 /d despite the 20% increase of NH_4^+-N loading in DW-3 operation. Under normal circumstance, the microalgal cells will uptake NH_4^+-N until the source is depleted before assimilating other nitrogen species due to the lesser energy requirement as compared with NO_3^--N where it has to undergone reduction into ammonium and conversion into amino acids, before being completely assimilated into the microalgal biomass [133]. However, the presence of various nitrogen species may induce different modes of nitrogen assimilation, resulting in more complex mechanisms between microalgal growth and nitrogen uptake [134], [135]. In this case, the threshold of NH_4^+-N concentrations may exceed the tolerance of the microalgae, i.e., *Chlorella vulgaris*, causing a shift over the nitrogen uptake mechanism from NH_4^+-N into NO_3^--N as the more preferred nitrogen species uptake. Similar studies had as well reported over the NO_3^--N preference as opposed to NH_4^+-N for nitrogen uptake into the microalgal cells of *Chlorella* sp. [78], [129]. Other studies also reported over the possibility of ammonium toxicity, causing the growth inhibition at certain NH_4^+-N concentration and nitrogenous nutrient availability [136], [137]. In the current study, all the NH_4^+-N concentrations were depleted amidst the DW-2.5 and DW-3 operations. The remaining traces of NO_3^--N concentrations were detected at merely 4 mg/L and 6 mg/L for DW-2.5 and DW-3 operations, respectively, signifying slightly better of NO_3^--N uptake by DW-2.5 as compared with DW-3 operations. This was also underpinned by the comparable if not better overall nitrogen assimilation rates of 0.0015 /d in DW-2.5 and 0.0014 /d in DW-3. Nevertheless, the mechanism between the microalgal

growth and nitrogen uptake is rather complex to be understood and the difference in nitrogen species availabilities may cause changes to the nitrogen assimilation process to be directed to the other metabolic pathways such as lipid accumulation and protein synthesis.

Table 4.2: Microalgal biomass growth kinetics determined from various diverted flows of blending nutrient-rich wastewater and activated sludge effluent serving as nitrogen sources for the SFB-AlgalBac photobioreactor.

Flow operation	**Microalgal biomass, X (mg/L)**	**Microalgal biomass per unit of nitrogen assimilated, Y (mg/mg)**	**Maximum specific growth rate, μ_{max} (/d)**	**Specific growth rate, μ (/d)**
Control	182	11.25	0.121	0.023
DW-1	270	16.69	0.156	0.059
DW-2.5	745	46.04	0.168	0.071
DW-3	792	48.95	0.168	0.071

4.3.3 Microalgal biomass compositions derived from various diverted wastewater loadings into SFB-AlgalBac photobioreactor

The cell compositions of harvested microalgal biomasses having been grown under various diverted wastewater flows from blending nutrient-rich wastewater with activated sludge effluent are shown in Fig. 4.9. For the controlled operation in which the sole nitrogen source was only NO_3^--N from the activated sludge effluent, the microalgal biomass was constituting of about 51 % of carbohydrate, 26 % of protein and 23 % of lipid. Viswanathan [30] had reported that the typical compositions of microalgal biomass were carbohydrate, protein and lipid, in the ranges of 5 - 23 %, 6 - 52 % and 7 - 23 %, respectively. The microalgal carbohydrate composition obtained in

this study was much higher, whilst the protein composition on the other hand was in the middle range in comparing with the compositions as reported by Viswanathan [30]. This was likely due to the nitrogen source in the cultivation medium being not fully assimilated into the microalgal cells in which the total nitrogen removal was mere reaching at 40.91 % amidst this flow operation. Indeed, the protein metabolic pathway in microalgal cells relied dependently on the available nitrogen source being assimilated from the cultivation medium and subsequently, being reduced into ammonium and converted into amino acids, before being assimilated as protein constituents [133]. Next, the microalgal carbohydrate and protein compositions were ratcheting down to 34.76 % and up to 40.63 %, respectively, amidst the DW-1 operation with the total nitrogen removal increasing to the tune of 66.85 % as opposed to the controlled operation. Therefore, the protein synthesis within the microalgal cells was correlating to the total nitrogen assimilated and could be as well altered based on the nitrogen species presented in the cultivation medium. Accordingly, when more NH_4^+-N source from nutrient-rich wastewater was loaded, resulting in higher NH_4^+-N than NO_3^--N concentrations in the cultivation mediums, the protein compositions in microalgal biomasses were seen decreasing to the 14 - 17 % range for DW-2.5 and DW-3 operations. While the carbohydrate compositions increased to the 52 - 54 % range, despite both similar operations had recorded the near complete removals of nitrogen (Fig. 4.7). During these two operations, the assimilated nitrogen source was primarily used to grow more microalgal biomass, instead of converting into protein and later being stored within the microalgal cells. This phenomenon was manifested in Fig. 4.8, showing higher biomass productivities in DW-2.5 and DW-3 operations than before. In addition, more microalgal biomasses per unit of nitrogen assimilated were also recorded in Table 3 for the DW-2.5 and DW-3 operations, signifying the rapid growth of microalgal biomasses. Thus, during the DW-2.5 and DW-3 operations, the assimilated nitrogen was channelled to grow more microalgal cells in increasing the carbohydrate contents rather than being accumulated in the form of cell proteins. Moreover, since the SFB-AlgalBac photobioreactor was adopting a continuous flow mode where there

would the continuous and simultaneous influx of nutrients and efflux of microalgal suspended biomass, the constant induced stress would be inevitably exerted towards the microalgal cultivation. In the case of DW-2.5 and DW-3 operations, the continuous influx of nitrogen nutrients coupled with the high amount of NH_4^+-N source being loaded into the cultivation medium would build up the nitrogen stress environment, resulting in the carbon partitioning. Thereby, triggering the high starch accumulation and eventually into triacylglycerol (TAG) within the microalgal cells [139], [140]. While many reported studies showcased on lipid accumulation in microalgal cells under nitrogen deprivation or limitation environments [141], [142], this study provided an exemption in which the nitrogen sources could affect the lipid accumulation in microalgal cells instead. Another similar study also suggested that nitrogen sources could influence lipid accumulation in microalgal cells in which higher lipid productivity was reported in microalgae grown in cultures supplied with various nitrogen sources, instead of those with sole nitrogen source [133]. Accordingly, the higher lipid compositions (28 - 29 %) were observed during the DW-2.5 and DW-3 operations as opposed to the 24% in the DW-1 operation. This had further supported the carbon partitioning phenomenon, leading to the TAG accumulation which was indeed desirable for biodiesel production from harvested microalgal biomass. Meanwhile, the residual microalgal biomass after lipid extraction could be further utilized for possible value-added bioproducts production. The residual biomass rich in metabolites such as carbohydrates, proteins, bioactive compounds and biomaterials could be further processed and refined into other bioenergy sources or even as animal-feed supplements and biofertilizers as part of biorefinery paradigms [143], [144].

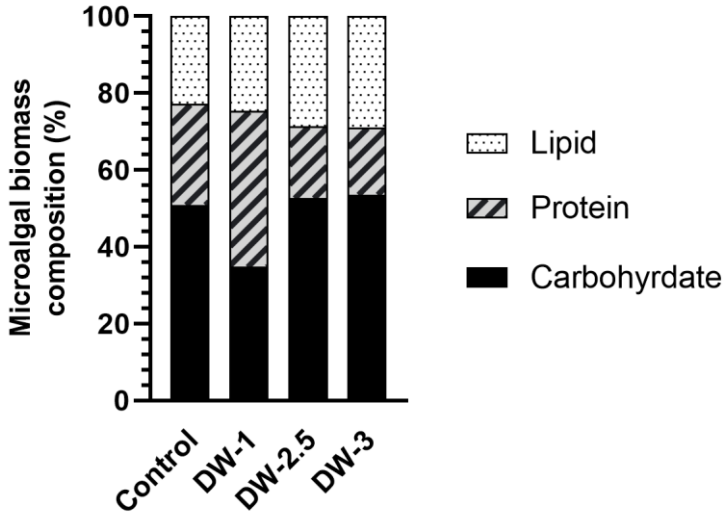


Figure 4.9: Variation of microalgal biomass compositions derived from the SFB-AlgalBac photobioreactor under various diverted flows from blending nutrient-rich wastewater and activated sludge effluent.

4.3.4 Microalgal biodiesel compositions derived from various diverted wastewater loadings

The biodiesels derived from harvested microalgal biomasses cultivated in the SFB-AlgalBac photobioreactor under various diverted flows from blending nutrient-rich wastewater and activated sludge effluent were quantified in terms of FAME profiles as demonstrated in Table 4.3. In this regard, the FAME profiles were further classified into the saturated fatty acids (SFA), unsaturated fatty acids (mono- and poly-FAME species) (USFA) and others. The overall microalgal SFA were observed decreasing with the increase of NH_4^+-N loading blended with the activated sludge effluent, i.e., from 33.36 % in the controlled operation to 20.78 % in the DW-3 operation. While the microalgal USFA were in vice-versa instead, i.e., from 66.64 % up to 76.36 % when the NH_4^+-N loading was correspondingly increasing from the controlled to DW-3 operations. Therefore, it could be deduced that the presence of different nitrogen species in the cultivation medium could alter the composition of SFA and USFA

produced from the harvested microalgal biomass. By tuning the cultivation medium to contain higher concentration of NH_4^+-N would produce higher fraction of USFA; while more NO_3^--N would divert the microalgal FAME composition to contain more SFA instead. Also, the higher fraction of USFA may reflect the effectiveness of nitrogen assimilation by the microalgae as higher total nitrogen removal at increasing NH_4^+-N loading was recorded from DW-1 to DW-3 operations (Fig. 4.7). A study by Nordin et al. [31] reported that the *Chlorella vulgaris* cultivated under nitrogen-sufficient condition would obtain higher fraction of USFA as opposed to SFA. Thus, higher nitrogen assimilation into the microalgae could result in higher fraction of USFA in the microalgal FAME composition. Moreover, the FAME profiles derived from the harvested microalgal biomasses comprised mainly of C16:0 (palmitic), C18:0 (stearate), C18:1 (oleate), C18:2 (linoleate) and C18:3 (linolenate), accounting to approximately 97.16 - 100 %. Indeed, the major constituents of FAME species which were C16-C18 are as well conforming to the requirement of quality biodiesel [145]. These constituents of biodiesel would be favourable as it induces the kinetic viscosity and promotes the fuel-air mixing for efficient biodiesel combustion properties [146]. The presence of C18:1, C18:2 and C18:3 in the microalgal biodiesel was also lowering the biodiesel pour point in which easing the microalgal biodiesel consumption in the countries or regions with cold climate [133].

Table 4.3: FAME profiles derived from microalgal biomasses cultivated under various diverted flows from blending nutrient-rich wastewater and activated sludge effluent into the SFB-AlgalBac photobioreactor.

FAME species	**FAME Content (%)**			
	Control	**DW-1**	**DW-2.5**	**DW-3**
Saturated FAME				
C16:0	33.36	28.22	21.73	17.67
C18:0	-	-	3.55	3.11

Subtotal	33.36	28.22	25.28	20.78
Monounsaturated FAME				
C18:1	12.17	9.40	26.49	33.35
Subtotal	12.17	9.40	26.49	33.35
Polyunsaturated FAME				
C18:2	28.13	33.42	26.44	22.65
C18:3	26.34	28.96	21.79	20.36
Subtotal	54.47	62.38	48.23	43.01
Total	100.00	100.00	100.00	97.14
Others	-	-	-	2.86

4.4 Energy feasibility of microalgal biomass production via SFB-AlgalBac photobioreactor for commercial biodiesel application

4.4.1 Comparison of energy demands of microalgae-to-biodiesel between best-case and studied scenarios

The net energy ratio (NER) is a quantification metric used to assess the feasibility of an energy system, and defined as $NER = \frac{Primary\ Energy\ Output}{Non-renewable\ Energy\ Input}$ [112], [113]. The system is deemed energy deficit should the NER value is lesser than 1, while NER values of equal or more than 1 are correspondingly heralding net or positive energy return from the system which is desirable. The NER values derived from the microalgae-to-biomass/lipid/biodiesel value chains via SFB-AlgalBac photobioreactor under the best-case and base study scenarios were shown in Table 4.4, while the energy

demands for the respective process inputs in the microalgae-to-biodiesel value chain are tabulated in Table 4.5. In producing dry microalgal biomass (24 MJ/kg microalgae biomass) which constituted of two main processes, namely, microalgal cultivation and biomass harvesting and dewatering, the NER values obtained were >1, which were 4.95 under base study scenario, whereas a much higher NER value of 8.38 was obtained under best-case scenario. The higher NER value obtained under the best-case scenario was primarily due to the much lower energy inputs demanded by the microalgal cultivation process. Under the best-case scenario, it was assumed that ~52 h of operation were sufficient to produce the required dry microalgal biomass as compared with the base study operation which needed ~222 h of operation. During the microalgal cultivation, most of the energy inputs were from electricity which was required for aeration, water pumping and light irradiation in growing and maintaining the microalgal culture which consumed 0.236 MJ (best-case) to 7.296 MJ (base study) of energy. Of these energy inputs, the light irradiation was the most energy intensive input which constituted of about 57% of the total energy inputs for microalgal cultivation alone under base study. The second consecutive process which was the microalgal biomass harvesting and dewatering required about 10.36 MJ of energy inputs which were assumed to be equivalent across the best-case and base study scenarios due to the same amounts of dry microalgal biomass being acquired.

Table 4.4: NER values of various bioenergy products, namely, dry microalgal biomass, lipid and biodiesel, from microalgae-to-biodiesel system.

Scenario	Dry biomass	Lipid	Biodiesel
Best-case	$\frac{88.8\ MJ}{10.596\ MJ} = 8.38$	$\frac{38.5\ MJ}{162.596\ MJ} = 0.24$	$\frac{40\ MJ}{171.857\ MJ} = 0.23$
Base study	$\frac{88.8\ MJ}{17.956\ MJ} = 4.95$	$\frac{38.5\ MJ}{274.476\ MJ} = 0.14$	$\frac{40\ MJ}{283.737\ MJ} = 0.14$

Base energy values of dry microalgal biomass, lipid and biodiesel were 24 MJ/kg (3.7 kg) [147], 35 MJ/kg (1.1 kg) [82] and 40 MJ/kg [105], respectively.

Table 4.5: Direct process contributions to produce 1 kg of biodiesel.

Process	**Lab results and literature data**	**Best-case (~52 h)**		**Existing (~222 h)**	
		Amount	**MJ**	**Amount**	**MJ**
Microalgae growth cultivation (3.7 kg biomass):					
Electricity (a) Air compressor	0.29 MJ/d	2.17 d	0.628	9.25 d	2.683
(b) Influent water pumping	0.195 kW h/kg algae biomass	0.722 kW h	2.597	0.722 kW h	2.597
(c) Light irradiation	5.4 W	280.8 W h	1.011	1198.8 W h	4.316
Credit for treated effluent/discharge	0.004 MJ/L	1000 L	(-4)	500 L	(-2)
Harvesting and dewatering:					
Electricity (Drying)	0.0028 MJ/g of dried algal biomass	3.7 kg	10.36	3.7 kg	10.36
Sub-total			**10.596**		**17.956**
Lipid extraction:					
Energy (Extraction & evaporation)	152 MJ/kg biodiesel	-	152	-	-
Solvent – Methanol	35.1 MJ/kg	-	-	49.21 kg	1727.27
Solvent – Trichloromethane	33.8 MJ/kg	-	-	24.79 kg	837.91
Credit for recycled solvent	90% of used solvent	-	-	-	(-2308.66)
Sub-total			**162.596**		**274.476**
Biodiesel production:					
(a) Electricity	0.336 kW h/kg biodiesel	-	1.21	-	1.21
(b) Heating	4.05 MJ/kg biodiesel	-	4.05	-	4.05
(c) Solvent – Methanol	35.1 MJ/kg	0.114 kg	4.001	0.114 kg	4.001
Total Cumulative Energy Demand			**171.857**		**283.737**

Overall, the entire life cycle from microalgae-to-biodiesel via the SFB-AlgalBac photobioreactor had translated to a total energy input of 283.737 MJ to produce 1 kg of biodiesel (40 MJ) in which had subsequently resulted in NER value of 0.14. Under the best-case scenario, the NER value to produce 1 kg of biodiesel had increased to 0.23 where a total of 171.857 MJ of energy input was required from the entire microalgal-to-biodiesel value chain. While being operated under positive and optimum input parameters (best-case scenario), the cumulative energy demand from the microalgae-to-biodiesel value chain could be reduced up to 39 % as evidenced from the difference in obtained cumulative energy demands between best-case and base study scenarios. Overall, the most energy demand unit came from the lipid extraction process in which had totalled to 88 – 90 % of the cumulative energy demand for both scenarios, followed by harvesting and dewatering (3 - 6 %), biodiesel production (3 – 5 %) and lastly, microalgal cultivation (0 - 3 %) under both the best-case and base study scenarios. The most energy intensive process from lipid extraction was largely due to the usage of chemical solvents, namely, methanol (35.1 MJ/kg) and chloroform (33.8 MJ/kg), which had a high initial energy demand. Other reported studies also underpinned that the extraction process was the most energy intensive process involved in microalgae-to-bioproducts life cycles and was as well mainly due to the usage of chemical solvents [105], [112]. While the current study managed to reduce most of the energy demands (90 % of solvent recycling) incurred from the usage of chemical solvents, the rest of the chemical solvents was unable to be recovered (10 %). These lost solvents had given rise to the 256.52 MJ in which also had contributed to 90 % of the total cumulative energy demands. On the other hand, the best-case scenario which utilized an optimum reported energy value from lipid extraction had resulted in the 88 % of the total cumulative energy demands. Therefore, it was confirmed that the extraction process was the main challenge to be addressed in reducing the overall energy demand incurred in the microalgae-to-biodiesel value chain system in order to proffer a more practical and feasible production of biodiesel.

4.4.2 Case comparison among established life cycle systems for microalgal biofuels and future perspectives

Depending on the targeted microalgal biofuel end-products, the overall NER values from the life cycle system may differ due to the differences in cumulative energy inputs involved. The reported NER values from various life cycle systems for microalgal biofuels are compiled in Table 4.6. In evaluating the feasibility of SFB-AlgalBac photobioreactor to produce microalgal biomass in comparison with the conventional microalgal cultivation systems, this study obtained a positive yet high NER value of 8.38 under best-case scenario. In comparison with a study by Magalhaes et al. [148], the NER was merely recorded at 1.09 while using the conventional photobioreactor system. The huge disparity between the two NER values was mainly due to the energy inputs requirement in maintaining the microalgal culture. For microalgal cultivation, the inputs besides electricity such as CO_2 and nutrients often contribute to the additional energy input costs and demands. In this study, the SFB-AlgalBac photobioreactor exploited the microalgal-bacterial symbiosis where there was a gaseous exchange process (O_2 and CO_2) between the microalgal and bacterial consortia. Hence, eliminating the need for CO_2 sparging or supply in sustaining the microalgal culture. Also, the nutrients necessary for microalgal growth was supplied by wastewater medium, instead of chemical fertilizers which had greatly reduced the nutrient energy inputs required for microalgal cultivation. Other approaches that could further reduce the energy inputs for microalgal cultivation include the use of flue gasses as a substitute to CO_2 supply and microalgal cultivation under various nutrient-rich wastewaters. [149], [150]. These alternatives have been widely explored for making the microalgal cultivation to be more practical and feasible [151], [152].

Table 4.5: NER values and system boundaries of reported LCA studies for various microalgal biofuels.

LCA	**NER**	**Functional product**	**Microalgal species**	**$E_{operation}$**	**$E_{harvesting/dewatering}$**	**E_{co2}**	**$E_{nutrient}$**	**$E_{downstream}$**	**Remark**
This study	8.38	Biomass	*Chlorella vulgaris*	●	●	○	○	○	Algal-bacterial photobioreactor in wastewater
	0.23	Biodiesel		●	●	○	○	● (Solvent extraction + Transesterification)	
Razon and Tan (2011)	0.12	Biodiesel and biogas	*Nannochloropsis* sp.	●	●	●	●	● (Biogas generation + Solvent extraction + Transesterification)	Raceway pond
	0.40	Biodiesel and biogas	*Haematococcus pluvialis*	●	●	●	●	● (Biogas digestion + Solvent extraction + Transesterification)	Photobioreactor + Raceway pond
Nava-Bravo et al. (2021)	2.7	Biojet fuel	*Scenedesmus* sp.	●	●	○	○	● (HTL + biocrude conversion)	HRAP in wastewater and harvested by ozone-air flotation
Magalhães et al. (2022)	1.09	Biomass	*Scenedesmus* sp.	●	○	●	●	○	Photobioreactor

Siqueira et al. (2022)	2.0	Biogas	n.a	●	○	○	○	● (Anaerobic co-digestion)	HRAP in vinasse
Zhang et al. (2022)	3.1	Biojet fuel	*Trentepohlia*	●	●	○	○	● (HTL + biocrude conversion)	HRAP in wastewater and harvested by ozone-air flotation
Huang et al., (2022)	0.73	Biodiesel	*Chlorella vulgaris*	●	●	○	●	● (Solvent extraction after mild hydrothermal treatment + Transesterification)	Raceway pond with flue gas pumping

For a direct comparison among the NER values for microalgae-to-biodiesel system, this study had reported a NER value of 0.23 under best-case scenario which was in the range of expected NER values of 0.12 for *Nannochloropsis* sp. to 0.40 for *Haematococcus pluvialis* [153]. However, Razon and Tan [153] took the combination of biodiesel and biogas as the energy output in their study. In this case, it was deduced that the microalgal species may affect the yield of the targeted biofuel end-product, i.e., biodiesel, which were mostly influenced by a particular microalgal lipid composition. On the other hand, Huang et al. [150] reported a NER value of 0.73 for biodiesel production from *Chlorella vulgaris* which was much higher than this study despite the same microalgal strain being used. The higher NER value reported was attributed to the lower energy inputs during the downstream process. Instead of directly subjecting the microalgae biomass to lipid extraction, Huang et al. [150] applied a mild hydrothermal process prior to the lipid extraction which managed to reduce the amount of solvents used. Hence, reducing the energy inputs required for the downstream process in converting lipid into biodiesel. Nevertheless, the net or positive NER value ($\geq$1) is rather difficult to be achieved for microalgae-to-biodiesel systems as of now. As discussed previously, the energy inputs from the extraction process which had accounted to 88 % of the total energy demand (this study) were identified as a challenge to be addressed in lowering the energy demand while considering the feasibility of microalgal biofuel production. Greener or sustainable approaches had been studied for reducing the dependence on conventional solvents used for lipid extraction and conversion into biodiesel. Among the approaches are thermo-physical processes which are chemical solvent-free, e.g., hydrothermal liquefaction (HTL), subcritical water extraction, hydrotreatment, pyrolysis etc [154], [155]. These processes are either being utilized as the pre-treatment or direct conversion from biomass into biofuel. Also, the development of green solvents, i.e., ionic liquids, for catalytic conversion, lipid extraction and biodiesel production and purification have been of interest as a potential

clean and cost-effective technology in replacing the traditional modes of biofuel production [156].

Other microalgal biofuel end-products such as biogas and biojet fuel had reported positive NER values as opposed to microalgae-to-biodiesel systems. This was mainly due to the difference in energy outputs involved in the downstream processes for biomass conversion into biogas or bio-jet fuel. Microalgae-to-biojet fuel systems recorded higher NER values of 2.7 - 3.1 and was attributed to the much lesser energy inputs required in biocrude conversion into biojet fuel [155], [157]. The facilitation of hydro-based treatment technologies which converted the biomass into biocrude oil, namely, HTL or pyrolysis, had been reported requiring lower production costs, resulting in lower energy demand in the downstream process [155], [158]. On the other hand, Siqueira et al. [159] reported a NER value of 2.0 for microalgae-to-biogas system. The positive NER value was again attributed to the lesser energy inputs required in biomass conversion into biogas via anaerobic digestion. The energy and cost advantages from biogas technology lies in the simplicity which utilizes microbes to degrade any organic-rich biomass waste into methane with miniscule amounts of carbon dioxide and other gasses under anaerobic condition [160]. In this case, the NER value obtained from this study could be further increased by exploiting the residual biomass rich in carbohydrate content after lipid extraction for processing and converting into other potential microalgal biofuel products, e.g., biogas, biohydrogen, bioethanol, etc.

To summarize, the following approaches and considerations could be taken into account in reducing the overall energy demand for associated microalgal biofuel systems. Hence, making the production of microalgal biofuels to be more cost-competitive for commercial application.

- Microalgal cultivation – hybrid cultivation mode which involves initial cultivation in PBRs to induce high biomass specific growth, and later being

migrated to outdoor raceway ponds/HRAPs for a continuous biomass production with reduced input costs for supplying CO_2, nutrients and light; flue gas as a substitute to CO_2 sparging/supply; wastewater as a substitute to potable water and nutrient medium supplies.

- Harvesting and dewatering – sustainable techniques such as flotation or ozone-induced flotation could potentially reduce the energy costs and environmental impacts associated to the traditional harvesting/dewatering techniques such as centrifugation, filtration, coagulation, etc.

- Downstream refinery – direct lipid/oil conversion using thermo-physical treatment processes, e.g., HTL, subcritical water extraction, hydrotreatment and pyrolysis, could also reduce the energy costs and environmental impacts associated to the usage of chemical/organic solvents; utilization of residual biomass after lipid/oil extraction for conversion into other valuable bio-products, e.g., biogas, biohydrogen, bioethanol, biofertilizer, supplements, etc. (cradle-to-gate approach).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

A novel photobioreactor was designed to exploit the synergistic relationships between microalgae and bacteria in the form of activated sludge in bioremediating nutrient-rich wastewater while generating microalgal biomass in a continuous flow mode. The synergistic associations between microalgae and bacterial activated sludge consortia stemming from the simultaneous nitrification and assimilation mechanisms in reducing the NH_4^+-N species abundant in wastewater into oxidized nitrogen species (NO_2^--N and NO_3^+-N) which were subsequently assimilated by the microalgae cells at a higher uptake rate. Hence, promoting microalgae biomass growth while diminishing the occurrence of free ammonia toxicity which could be detrimental to microalgae growth. The performance of the SFB-AlgalBac photobioreactor was studied along the increase in influent flow rates. The 5.0 L/d flow rate was recognized to be the most optimum with the highest microalgal NO_3^--N assimilation rate of 0.0271 /d recorded, followed by the highest microalgal biomass productivity achieved at 1350 mg/d during the steady state. Further increase in influent flow rate to 10.0 L/d had led to the descent of microalgal biomass productivity rendered by the ineffective NO_3^--N assimilation as excessive microalgal biomass was washout. The kinetics of microalgal biomass growth model for SFB-AlgalBac photobioreactor construed this occurrence was stemming from the ineffective nitrogen assimilation by microalgae due to the excessive immature cells washout at higher influent flow rate (10.0 L/d). In this regard, the microalgal

biomass per unit of nitrogen assimilated values were attained at 16.69 mg/mg for 5.0 L/d flow rate as opposed to merely 7.73 mg/mg for 10.0 L/d flow rate, despite both having comparable specific growth rates for microalgal biomass. Moreover, the new photobioreactor also permitted the influent to be treated by the activated sludge process prior to the secondary treatment by microalgae. This had improved the microalgal biomass settleability up to 37 % with increasing bio-floc sizes had been observed at increasing flow rates. The flocculation of microalgal biomass could be attributed by two factors with the primary factor rising from the presence of extracellular polymeric substances derived from the activated sludge culture; and the latter was induced by the stress exerted to the microalgal culture by the increasing hydraulic washing out strengths.

Further optimization of the SFB-AlgalBac photobioreactor was achieved under a dual nutrient heterogeneity mode consisting of a blended mixture of nutrient-rich wastewater (NH_4^+-N source) and activated sludge effluent (NO_3^--N source), stemming from effective nitrogen nutrient management. This was accompanied by a significant increase in microalgal biomass production at the optimum NH_4^+-N and NO_3^--N loadings of 60 and 58 mg/d, respectively. In this regard, about 94 % of total nitrogen was removed from assimilation process for growing 745 - 792 mg/L of microalgal biomass. The shift in nitrogen preference to NH_4^+-N over NO_3^--N had resulted in a more effective nitrogen assimilation by the microalgae, prompting the accumulation of carbohydrate and lipid over protein in the biomass compositions which were in favour of microalgal biofuel production. The extracted microalgal lipid had also recorded higher USFA fraction as opposed to SFA. In fact, the major constituent of FAME species in the biodiesel was comprising of C16-C18, satisfying the quality biodiesel

requirements. In short, the unveiled mechanism of nitrogen-assimilation executed by activated sludge and microalgae had assuage the NH_4^+-N toxicity, whilst enhancing the total nitrogen removal and microalgal biodiesel production via balancing the NH_4^+-N and NO_3^--N sources introduced into dual heterogeneity mode photobioreactor.

The techno-economic feasibility of the developed SFB-AlgalBac photobioreactor was evaluated in terms of energy feasibility for producing microalgal biomass and biofuel systems. The life cycle studies revealed that the photobioreactor recorded a NER value of 8.38 under the best-case scenario in producing microalgal biomass which was superior as opposed to conventional microalgal bioreactor systems. However, when the life cycle boundary was extended from microalgal-to-biodiesel system, the NER value dropped to 0.23 which had resulted in a negative energy return. The huge disparity in the NER value was identified to be from the high energy demand incurred in the downstream processes required in converting the microalgal biomass into lipid and then biodiesel. The high energy demand was mainly from the energy inputs from chemical-driven reaction processes for conversion into biofuels; accounting for 88 % of the total energy demand in the microalgae-to-biodiesel system. Nevertheless, the NER value recorded in the study was concurrent with other reported microalgae-to-biodiesel systems (0.12 - 0.40) which suggested that achieving net or positive NER value ($\geq$1) was rather difficult to be achieved for microalgae-to-biodiesel systems as of now.

While this study had improved the energy feasibility for the upstream process in cultivating microalgal biomass via the SFB-AlgalBac photobioreactor design, sustainable approaches to address the high energy demand associated with the downstream refinery processes for microalgal-to-biofuel energy systems in making the

production of microalgal biofuels more cost-competitive and practical for commercialization. Among the approaches and considerations which could be adopted included utilization of solvent-free treatment/conversion processes such as thermo-physical based treatment methods, utilization of residual biomass after lipid/oil extraction into other bio-products and sustainable harvesting and dewatering methods which could reduce the energy demand stemming from conventional techniques, e.g., oven-drying, centrifugation and coagulation which consumed electrical energy.

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APPENDIX A

LIST OF PUBLICATIONS

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Research article

Impact of various microalgal-bacterial populations on municipal wastewater bioremediation and its energy feasibility for lipid-based biofuel production

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ABSTRACT

The microalgal-bacterial co-cultivation was adopted as an alternative in making microbial-based biofuel production to be more feasible in considering the economic and environmental prospects. Accordingly, the microalgal-bacterial symbiotic relationship was exploited to enhance the microbial biomass yield, while bioremediating the nitrogen-rich municipal wastewater. An optimized inoculation ratio of microalgae and activated sludge (AS:MA) was predetermined and further optimization was performed in terms of different increment ratios to enhance the bioremediation process. The nitrogen removal was found accelerating with the increase of the increment ratios of inoculated AS:MA, though all the increment ratios had recorded a near complete total nitrogen removal (94–95%). In light of treatment efficiency and lipid production, the increment ratio of 0.5 was hailed as the best microbial population size in accounting the total nitrogen removal efficiency of 94.45%, while not compromising the lipid production of 0.241 g/L. Moreover, the cultures in municipal wastewater had attained higher biomass and lipid productions of 1.42 g/L and 0.242 g/L, respectively, as compared with the synthetic wastewater which were only 1.12 g/L (biomass yield) and 0.175 g/L (lipid yield). This was possibly due to the presence of trace elements which had contributed to the increase of biomass yield; thus, higher lipid attainability from the microalgal-bacterial culture. This synergistic microalgal-bacterial approach had been proven to be effective in treating wastewater, while also producing useful biomass for eventual lipid production with comparable net energy ratio (NER) value of 0.27, obtained from the life-cycle analysis (LCA) studies. Thereby, contributing towards long-term sustainability and possible commercialization of microbial-based biofuel production.

processes

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Article

Comparative Performances of Microalgal-Bacterial Co-Cultivation to Bioremediate Synthetic and Municipal Wastewaters Whilst Producing Biodiesel Sustainably

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Abstract: The potentiality of a microalgal-bacterial culture system was explored in bioremediating wastewater while generating biomass for biodiesel production. A pre-determined optimal activated sludge and microalgal ratio was adopted and cultivation performance was evaluated in both synthetic and municipal wastewater media for nitrogen removal along with biomass and lipid generation for biodiesel production. The microalgal-bacterial consortium grown in the municipal wastewater medium produced higher biomass and lipid yields than those in the synthetic wastewater medium. The presence of trace elements in the municipal wastewater medium, e.g., iron and copper, contributed to the upsurge of biomass, thereby leading to higher lipid productivity. Both the microbial cultures in the synthetic and municipal wastewater media demonstrated similar total nitrogen removal efficiencies above 97%. However, the nitrification and assimilation rates were relatively higher for the microbial culture in the municipal wastewater medium, corresponding to the higher microbial biomass growth. Accordingly, the feasibility of the microalgal-bacterial consortium for bioremediating real municipal wastewaters was attested in this study by virtue of higher biomass and lipid production. The assessment of fatty acid methylesters (FAME) composition showed the mixed microbial biomasses comprised 80–93% C16 to C18 FAME species, signifying efficient fuel combustion properties for quality biodiesel requirements.

Keywords: microalgal-bacterial cultures; wastewater treatment; biomass; lipid; biodiesel

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Novel sequential flow baffled microalgal-bacterial photobioreactor for enhancing nitrogen assimilation into microalgal biomass whilst bioremediating nutrient-rich wastewater simultaneously

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ABSTRACT

A novel sequential flow baffled microalgal-bacterial (SFB-AlgalBac) photobioreactor was designed to cater for the synergistic interactions between microalgal and bacterial consortia to enhance nitrogen assimilation into microalgal biomass from nutrient-rich wastewater medium. The performance of the SFB-AlgalBac photobioreactor was found to be optimum at the influent flow rate of 5.0 L/d, equivalent to 20 days of hydraulic retention time (HRT). The highest microalgal nitrogen assimilation rate (0.0271 /d) and biomass productivity (1350 mg/d) were recorded amidst this flow rate. Further increase to the 10.0 L/d flow rate reduced the photobioreactor performance, as evidenced by a reduction in microalgal biomass productivity (>10%). The microalgal biomass per unit of nitrogen assimilated values were attained at 16.69 mg/mg for the 5.0 L/d flow rate as opposed to 7.73 mg/mg for the 10.0 L/d flow rate, despite both having comparable specific growth rates. Also, the prior influent treatment by activated sludge was found to exude extracellular polymeric substances which significantly improved the microalgal biomass settleability up to 37%. The employment of SFB-AlgalBac photobioreactor is anticipated could exploit the low-cost nitrogen sources from nutrient-rich wastewaters via bioconversion into valuable microalgal biomass while fulfilling the requirements of sustainable wastewater treatment technologies.

sustainability

MDPI

Review

Assuaging Microalgal Harvesting Woes via Attached Growth: A Critical Review to Produce Sustainable Microalgal Feedstock

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Abstract: Third-generation biofuels that are derived from microalgal biomass have gained momentum as a way forward in the sustainable production of biodiesel. Such efforts are propelled by the intention to reduce our dependence on fossil fuels as the primary source of energy. Accordingly, growing microalgal biomass in the form of suspended cultivation has been a conventional technique for the past few decades. To overcome the inevitable harvesting shortcomings arising from the excessive energy and time needed to separate the planktonic microalgal cells from water medium, researchers have started to explore attached microalgal cultivation systems. This cultivation mode permits the ease of harvesting mature microalgal biomass, circumventing the need to employ complex harvesting techniques to single out the cells, and is economically attractive. However, the main bottleneck associated with attached microalgal growth is low biomass production due to the difficulties the microalgal cells have in forming attachment and populating thereafter. In this regard, the current review encompasses the novel techniques adopted to promote attached microalgal growth. The physicochemical effects such as the pH of the culture medium, hydrophobicity, as well as the substratum surface properties and abiotic factors that can determine the fate of exponential growth of attached microalgal cells, are critically reviewed. This review aims to unveil the benefits of an attached microalgal cultivation system as a promising harvesting technique to produce sustainable biodiesel for lasting applications.

Keywords: biodiesel; microalgae; attached growth; suspended growth; support material; harvest

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Dual nutrient heterogeneity modes in a continuous flow photobioreactor for optimum nitrogen assimilation to produce microalgal biodiesel

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ABSTRACT

The impact of different nitrogen sources on the microalga, *Chlorella vulgaris*, was studied in a newly developed microalgal-bacterial photobioreactor via a dual nutrient heterogeneity mode. The mechanisms of nitrogen transformation and valorisation were unveiled, and subsequently, optimized via dual nutrient heterogeneity feeding modes comprising of various NH_4^+-N and NO_3^--N concentrations. The nitrogen-assimilation mechanism from the microalgal-bacterial consortium was found to reduce microalgal growth inhibition, stemming at high NH_4^+-N concentrations. Accordingly, the total nitrogen removal efficiency was enhanced from 40.91% to 96.38% accompanied with maximum microalgal biomass production of up to 792 mg/L at a balanced or higher NH_4^+-N loading from mixed nitrogen environment. The harvested microalgal biomass contained high lipid accumulation of approximately 30% when fed with optimum NH_4^+-N and NO_3^--N loadings at 60 and 58 mg/d, respectively. At this mixed nitrogen species loadings, high unsaturated fatty acid methyl esters (FAME) were attained. Indeed, the major FAME species (97%–100%) fell within the C16-18 range, signifying biodiesel characteristics conformity to the requirements for quality biodiesel application. Therefore, the dual heterogeneity modes of balanced NH_4^+-N and NO_3^--N loadings into photobioreactor could offer effective microalgal nitrogen assimilation for sustainable wastewater treatment and microalgae-based biodiesel production simultaneously.

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Photoperiod-induced mixotrophic metabolism in *Chlorella vulgaris* for high biomass and lipid to biodiesel productions using municipal wastewater medium

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ABSTRACT

The performances of autotrophic and heterotrophic metabolisms in *Chlorella vulgaris* were assessed from various photoperiod regimes for the sustainable microalgal biodiesel production. Accordingly, the cellular biomass growth and lipid content in relation to the organic nutrient removals from municipal wastewater medium were evaluated from the various light/dark photoperiod cycles amidst mixotrophic metabolism. The mixotrophic treatment had been proven effective in producing high microalgal biomass and lipid while alternating its autotrophic and heterotrophic metabolisms. Thus, exploiting the optimum cellular biosynthesis pathways in inducing both biomass growth and lipid accumulation. Amidst mixotrophy, prolonging light condition would favour biomass growth, whilst extending dark condition had elevated cell lipid content. Accordingly, the 16:8 (light:dark) hours/cycle photoperiod was found adequate in yielding high biomass and lipid productions (0.89 and 0.16 g/L, respectively), while achieving near complete removal (>94%) of chemical oxygen demand and ammonium-nitrogen from municipal wastewater. The dominant FAME constituents of C16 and C18 comprised of 90.65%-100% of total FAME with the high unsaturation degree could proffer an excellent quality of microalgal biodiesel with low pour point, suitable for application in cold climate regions.