

EVALUATION OF CARBON DIOXIDE AND NITRATE
UTILIZATION FROM RECIRCULATING AQUACULTURE
SYSTEM FOR MICROALGAL PRODUCTION

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การประเมินผลการใช้คาร์บอนไดออกไซด์และไนโตรเจนจากระบบเพาะเลี้ยงสัตว์น้ำแบบหมุนเวียน
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งานวิจัยนี้ศึกษาการใช้คาร์บอนไดออกไซด์และไนเตรดจากระบบเพาะเลี้ยงปลาไนล์เพื่อเพาะเลี้ยงจุลสาหร่าย ส่วนแรกของงานวิจัยศึกษาความเป็นไปได้ในการใช้น้ำเสียจากระบบเพาะเลี้ยงปลาไนล์แบบหมุนเวียนที่มีไนเตรดสูงมาเพาะเลี้ยงจุลสาหร่าย *Scenedesmus armatus* โดยเปรียบเทียบกับการเพาะเลี้ยงจุลสาหร่ายด้วยอาหารเพาะเลี้ยงสูตร BG-11 ซึ่งพบว่าทั้ง 2 ชุดการทดลองให้ผลการผลิตชีวมวลและปริมาณรงควัตถุในเซลล์ *S. armatus* ไม่แตกต่างกัน แสดงให้เห็นว่าสามารถใช้น้ำเสียที่มี ไนเตรดสูงจากระบบเพาะเลี้ยงปลาไนล์แบบหมุนเวียนมาใช้เพาะเลี้ยง *S. armatus* ทดแทนอาหาร BG-11 ที่มีค่าใช้จ่ายสูงกว่าได้ ส่วนที่สองของการวิจัยศึกษาความเป็นไปได้ของการใช้อากาศจากถังเลี้ยงสัตว์น้ำที่มีคาร์บอนไดออกไซด์ความเข้มข้นสูง (935 ppm) มาเพาะเลี้ยงจุลสาหร่าย ผลการทดลองพบว่าจุลสาหร่าย *S. armatus* สามารถเติบโตได้ดีที่สุดเมื่อใช้อาหารสูตร BG-11 และอากาศที่มีคาร์บอนไดออกไซด์สูงจากถังเลี้ยงปลาไนล์ โดยให้อัตราการผลิตชีวมวล 110 มก./ล./วัน อย่างไรก็ตามสถานะดังกล่าวได้รับปริมาณน้ำหนักเซลล์แห้งและอัตราการเติบโตสูงสุดไม่แตกต่างกันอย่างมีนัยสำคัญเมื่อเปรียบเทียบกับสถานะอื่น ในการทดลองส่วนสุดท้ายได้ศึกษาผลของความหนาแน่นของปลาไนล์ต่อการผลิตคาร์บอนไดออกไซด์และไนเตรด รวมทั้งการนำไปใช้เพื่อเพาะเลี้ยงจุลสาหร่าย *S. armatus* โดยเพาะเลี้ยงปลาไนล์ที่ความหนาแน่น 3, 5 และ 10 กก./ลบ.ม. ในระบบเพาะเลี้ยงสัตว์น้ำแบบหมุนเวียนเป็นเวลา 8 วัน พบว่าความเข้มข้นของไนเตรดตลอดการเพาะเลี้ยงมีค่าค่อนข้างคงที่ประมาณ 45.3 มก. ไนโตรเจน/ล. และพบการผลิตคาร์บอนไดออกไซด์สูงสุดในการเพาะเลี้ยงปลาไนล์ที่ความหนาแน่น 10 กก./ลบ.ม. ในส่วนของการใช้คาร์บอนไดออกไซด์และไนเตรดเพื่อเพาะเลี้ยงจุลสาหร่ายพบว่ามีเพียงอากาศจากถังเลี้ยงปลาที่ความหนาแน่น 10 กก./ลบ.ม. เท่านั้นที่มีอัตราการผลิตชีวมวลจุลสาหร่าย *S. armatus* มากกว่าการเพาะเลี้ยงด้วยอากาศปกติอย่างมีนัยสำคัญทางสถิติ นอกจากนี้การประเมินสมดุลมวลคาร์บอนและไนโตรเจนจากการเพาะเลี้ยงปลาไนล์พบว่าคาร์บอนร้อยละ 7.99 ถึง 16.42 และไนโตรเจนร้อยละ 7.58 ถึง 15.58 ในอาหารปลาถูกเปลี่ยนไปเป็นชีวมวลของปลาไนล์ ขณะที่ร้อยละ 50.67 ถึง 73.46 ของคาร์บอนในอาหารปลาถูกเปลี่ยนไปเป็นคาร์บอนไดออกไซด์ซึ่งสามารถนำไปใช้ในการเพาะเลี้ยงจุลสาหร่ายต่อไป ผลการประเมินสมดุลมวลคาร์บอนและไนโตรเจนจากการเพาะเลี้ยง *S. armatus* พบว่าประมาณร้อยละ 2.2 ถึง 3.4 ของคาร์บอนไดออกไซด์ที่เข้าสู่ระบบและร้อยละ 33.40 ถึง 74.63 ของไนเตรดในน้ำเสียถูกเปลี่ยนไปเป็นชีวมวลของจุลสาหร่าย ผลการทดลองที่ได้รับแสดงให้เห็นว่าการเพาะเลี้ยงจุลสาหร่ายสามารถใช้ของเสียจากระบบเพาะเลี้ยงปลาไนล์แบบหมุนเวียน ได้แก่ คาร์บอนไดออกไซด์และไนเตรด มาผลิตชีวมวลของสาหร่ายหรือผลิตภัณฑ์อื่นๆ ซึ่งเป็นการเพิ่มมูลค่าของกระบวนการ อีกทั้งลดการปล่อยของเสียเหล่านี้ออกสู่สิ่งแวดล้อม ในภาพรวมจะเห็นว่างานวิจัยชิ้นนี้สอดคล้องกับการพัฒนาเศรษฐกิจฐานชีวภาพ เศรษฐกิจหมุนเวียน และเศรษฐกิจสีเขียว (BCG Economy) ซึ่งเป็นนโยบายหลักของรัฐบาลในการพัฒนาประเทศไทยให้หลุดพ้นจากประเทศกับดักรายได้ปานกลาง

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Kittikoon Sucunthowong : EVALUATION OF CARBON DIOXIDE AND NITRATE UTILIZATION FROM RECIRCULATING AQUACULTURE SYSTEM FOR MICROALGAL PRODUCTION. Advisor: Assoc. Prof. KASIDIT NOOTONG, Ph.D. Co-advisor: Sorawit Powtongsook, Ph.D.

This study evaluated carbon dioxide and nitrate utilization from tilapia cultivating system by means of microalgal cultivation. The first part of this study evaluated the feasibility of using nitrate-rich effluent from aquaculture to cultivate *Scenedesmus armatus* in comparison with the cultivation using BG-11 media. Comparable biomass productivity and pigment content of *S. armatus* from the cultivation indicated that nitrate-rich effluent from aquaculture could be used as substitute for a more expensive BG-11 growth media. The second part of this study evaluated the feasibility of utilizing carbon dioxide concentrated air from roughly 3 kg/m³ tilapia cultured tank to grow *S. armatus*. It was found that the maximum productivity of *S. armatus* at 110 mg/L-day was associated with using BG-11 media and carbon dioxide from fish tank although biomass dried weights and maximum specific growth rates were insignificant different as compared to other treatments using ambient air and BG-11 media without Na₂CO₃. The final part of the study examined the effect of fish biomass on carbon dioxide and nitrate production and their utilization by means of microalgal cultivation. Approximately 3, 5 and 10 kg/m³ of fish (tilapia) biomass were introduced into the recirculating aquaculture system and cultivated for 8 days and measured for carbon dioxide and nitrate production. Nitrate concentrations were relatively constant at 45.3 mg N/L for the range of fish biomass considered. The highest carbon dioxide production was found in 10 kg/m³ fish cultivation, measured at 1,605 and 915 ppm from outlet of fish tank and solid separating unit, respectively. Outlet air from this particular fish cultivation (i.e., 10 kg/m³) also yielded significantly higher biomass of *S. armatus* as compared to those using ambient air. Carbon and nitrogen mass balance calculation from tilapia cultivation indicated that 7.99% to 16.42% of carbon and 7.58% to 15.58% of nitrogen from feed were accounted in fish biomass while significantly larger fraction of carbon in feed from 50.67% to 73.46% was converted to carbon dioxide. Only 2.2% to 3.4% of carbon dioxide produced were accounted into the biomass of *S. armatus*, whereas 33.40% to 74.63% of input nitrogen, mostly nitrate, from solid separating unit were transferred into microalgal biomass. The results from this work clearly demonstrated that microalgal cultivation was able to utilize wastes, namely carbon dioxide and nitrate, from recirculating aquaculture system to produce valuable products, and moreover, reduced adverse environmental impacts. In the broader picture, this work followed closely the concept of bio-, circular- and green (i.e., BCG) economy, which is one of the flagship policies of Thai government to propel the country from middle-income trapped status.

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CHAPTER I

INTRODUCTION

1.1 Background and significance of research

Aquaculture is a sustainable food production that has a potential to support global demand for increasing protein consumption. Unlike fishery, aquaculture reduces the use of natural fish stocks in the oceans [1]. Aquaculture is moving towards an intensive cultivation in a closed system, which employed technology to facilitate high biomass cultures, effective water utilization and less wastewater discharge [2, 3]. Various wastes are generated during intensive aquaculture and requires proper treatment to ensure suitable conditions for cultured animals and to satisfy more stringent water regulations. Majority of aquaculture wastes include inorganic nitrogenous compounds (e.g., ammonia, nitrite and nitrate), organic solids and carbon dioxide, which are originated from unconsumed feeds, microbial growth within the cultivating system and animal excretion [4]. For inorganic nitrogen compounds, they are generally treated by nitrifying biofilter to convert ammonia to nitrate, which is significantly less toxic than ammonia and nitrite. Ammonia can also be controlled by assimilating directly into bacterial biomass in a process called biofloc technology [5, 6]. Despite lower toxicity as compared to ammonia and nitrite, nitrate is rapidly accumulated in nitrifying biofilter unit and cultured tanks and the discharge of nitrate-rich effluent into natural water resources can accelerate eutrophication. The conventional nitrate treatment for intensive aquaculture system is heterotrophic denitrification, a process that reduces nitrate to nitrogen gas under anaerobic condition. Moreover, there are reports citing the use of photosynthetic systems such as aquaponics, planting in greenhouse, or microalgal process to reduce nitrate in wastewater [7, 8]. For organic solids, they are usually removed from water by filtration, centrifugation or sedimentation, and then used for landfill, fertilization or biogas production [9].

Besides inorganic nitrogen compounds and solids, carbon dioxide is also produced during aquaculture cultivation as a result of respiration from aquatic animals and microorganisms in water. It was estimated that carbon dioxide production, ranged from 1.1 to 1.7 kg CO₂/kg-feed, was generated from aquaculture cultivating systems [10, 11]. The presence of excess carbon dioxide in water can be detrimental, especially during the intensive aquaculture cultivation in which significant amount of carbon dioxide is produced, leading to substantial decrease of pH in water unless adequate control system is available [10]. Moreover, carbon dioxide can diffuse in water, through water-air interface and finally enter the atmosphere. Results of carbon mass balance from the recirculating system for catfish (*Clarias gariepinus*) revealed that approximately 47% of carbon in feeds were converted to carbon dioxide as compared to roughly 30% that was converted into fish biomass [12]. The same work also indicated that the amount of carbon dioxide emitted into the atmosphere from aquaculture cultivating systems depended on feeding rate, which increased with fish production. If aquaculture production worldwide still expands at the current rate, it would be inevitable that significant amount of carbon dioxide will be released into the atmosphere. Based on the literature survey, the process that directly controls or captures carbon dioxide from aquaculture cultivating system is not available, and therefore it is necessary to introduce the novel concept of carbon dioxide capture from intensive aquaculture cultivation by creating carbon dioxide storage above the water surface and then utilizing the captured carbon dioxide in downstream processes. Specifically, carbon dioxide from aquaculture cultivation is concentrated and stored in an air locked system with only one outlet to allow transportation to photosynthetic systems such as aquaponics or microalgae processes, where the stored carbon dioxide is utilized together with nitrogenous compounds (i.e., nitrate) from aquaculture effluent to generate biomass and valuable byproducts, which can be sold to improve the economic feasibility of process, while oxygen production as a result of photosynthesis can be recycled to animal cultured tanks to lower oxygen and energy consumption [13]. Although aquaponics is simple to build and requires lower capital and operational costs, microalgal process was reported to consume carbon dioxide per unit area approximately 7 to 8 times higher and produced wider range of useful products that increase the economic feasibility of the process [14, 15].

Based on the information presented, this research introduced the concept of sustainable aquaculture that integrated the recirculating aquaculture system (RAS) to minimize wastewater discharge, along with carbon dioxide storage and microalgae cultivating unit to utilize carbon dioxide from aquaculture production and nutrients from the effluent. As illustrated in Fig. 1.1, this integrated system consists of the following units: (1) fish tank, which accumulates carbon dioxide in the headspace above water surface; (2) solid separating unit to maintain acceptable suspended solid concentration in cultured tank and to convert nitrogenous waste from aquaculture tank to less toxic nitrate; and (3) microalgal cultivation to utilize carbon dioxide from aquaculture tank as carbon source and nitrate-rich effluent from nitrification system (i.e., solid separating unit, biofilter tank) as nitrogen source to produce microalgal biomass and other valuable byproducts. High oxygen air as photosynthetic product from microalgal production unit, continuously stripped from liquid in photobioreactors, can be reused in aquaculture tanks or release directly into the atmosphere. Microalgal biomass can be utilized in many ways to improve economic values of the integrated system, for examples, feeding microalgal biomass containing high carotenoids content directly as live feeds for zooplanktons, which in turn was supplied as protein and carotenoids sources during the hatchery of economically important aquacultures or extracting microalgal biomass for pharmaceutical value-added compounds such as carotenoids and fatty acids and biofuels [16, 17]. It should also be pointed out that additional carbon dioxide production is possible in the solid separating unit via microbial respiration and can be stored together with carbon dioxide from aquaculture tanks [18]. Based on the information presented, it was reasonable to conduct the feasibility study to investigate many aspects of the process, for instance carbon dioxide production from aquaculture cultivation at different fish biomass and the possibility of utilizing nitrate from nitrifying biofilter treating aquaculture water and carbon dioxide to produce microalgal biomass.

1.2 Research objectives

1.2.1 To proof the concept of utilizing carbon dioxide and nitrogenous wastes from aquaculture cultivating system by photosynthetic process.

1.2.2 To evaluate the performance of recirculating aquaculture system equipped with carbon dioxide storage and microalgal cultivation.

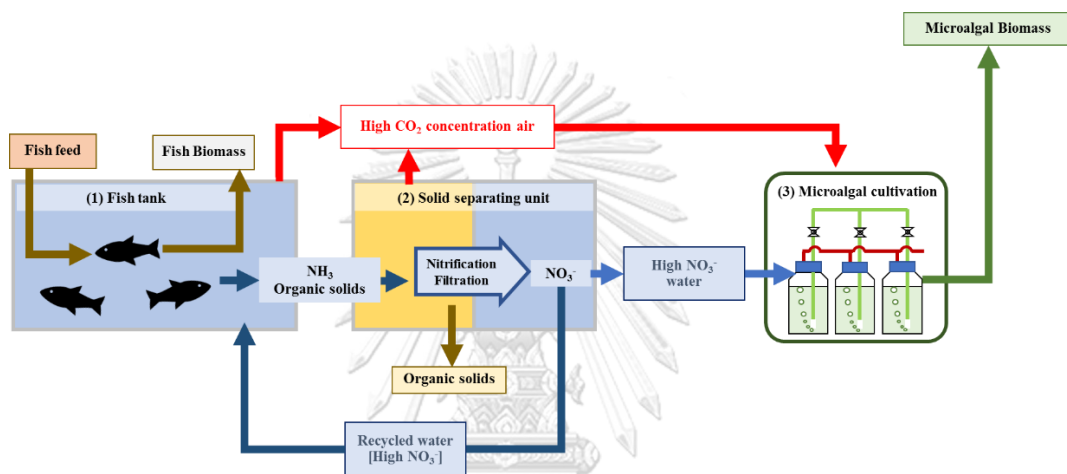


Figure 1.1. Overview of recirculating aquaculture system with carbon dioxide storage and solid separating unit and microalgal cultivation.

1.3 Hypothesis

Microalgal cultivation could utilize carbon dioxide and nitrogenous wastes from recirculating aquaculture system by conversion into microalgal biomass.

1.4 Scopes

1.4.1 Recirculating aquaculture system consisted of the following: (1) fish tank (FT) for aquaculture production and (2) solid separating unit (SU) for simultaneous solids-liquid separation and nitrification. Fish tank and solid separating unit were completely sealed to prevent air leakage. Microalgal cultivation (MC) was performed in Duran bottles or bubble column photobioreactors.

1.4.2 Nile tilapia (*Oreochromis niloticus*), with initial weight from 30 to 50 g, were used as model species. Fish were released into fish tank to attain the initial biomass from 3 to 10 kg/m³ to study the examine the effect of fish biomass density on carbon dioxide and nitrate production. *Scenedesmus armatus* was selected for photoautotrophic microalgal cultivation.

1.4.3 Recirculating aquaculture system (i.e., FT and SU) was operated until attaining mature state condition for a given fish biomass. Nitrate and carbon dioxide at mature state condition were sent to microalgal cultivation. Results, which included inorganic nitrogen (i.e., ammonium, nitrite and nitrate) treatment, carbon dioxide production and microalgal growth at different fish stocking densities, were compared.

1.4.4 The following variables were routinely measured during the experiment: (1) carbon dioxide concentration in overhead space of fish tank and outlet of solid separating unit, (2) concentration of ammonium, nitrite and nitrate in fish tank, effluent from solid separating unit and liquid in photobioreactors for microalgal cultivation (3) cell number density and dried weight of *S. armatus* in photobioreactors for microalgal cultivation and (4) fish weight and length.

1.5. Benefits

This research produced prototype technology for integrated aquaculture cultivating system and photosynthetic process that were capable of reducing carbon dioxide emission and lowered nitrate concentration in the discharged stream by converting these wastes into valuable biomass. The results obtained also paved the way for the development of larger sustainable aquaculture systems, which were important in protecting the environment and improving the standard of living for local farmers. Moreover, with the appropriate strain of microalgae, this technology could be employed to produce valuable biorefinery products at reasonable cost, hence serving the government policy of bio- circular- and green (BCG) economic platform.

CHAPTER II

LITERATURE REVIEW

2.1 Aquaculture

Aquaculture is a farming of aquatic species including fish, crustaceans (e.g., shrimps, prawns and crabs), shellfish and aquatic plants for human consumption [5]. Aquaculture can be classified into 3 types based on water utilization and wastewater management, namely opened aquaculture systems, closed aquaculture systems and semi-closed aquaculture systems. Table 2.1 compares advantages and disadvantages between each aquaculture system.

2.1.1 Opened aquaculture systems

Opened aquaculture systems are the basic and simplest aquaculture system, generally referred to as farming in natural water resources. Natural water is continuously flowed through the production system to keep good water quality. Wastes from cultured species are discharged directly into natural water resources and cause negative environmental impacts such as toxicity of nitrogenous compounds towards fish and eutrophication [5]. Cultured animals in opened aquaculture system is susceptible to contamination with pathogen from upstream [5].

2.1.2 Closed aquaculture systems

Closed system aquacultures are farming of aquatic animals that is separated from natural water resources. These systems required wastewater treatment processes (e.g. filtration, nitrification and denitrification) to ensure acceptable water quality for reuse [10]. Closed system aquaculture is complex and requires skilled labors, thereby incurring high cost of investment and operation.

2.1.3 Semi-closed aquaculture systems

Semi-closed aquaculture systems are similar to closed aquaculture system but required periodic water exchange between cultured tank and natural water resource to prevent water quality reaching critical condition that could be danger to cultured animals.

Table 2.1. Advantages and disadvantages of opened, closed and semi-closed aquaculture systems [3, 5, 10]

Type of system	Advantages	Disadvantages
Opened system	<ul style="list-style-type: none"> - Low investment cost 	<ul style="list-style-type: none"> - Difficulty to control water quality - Unsteady production - Low production yield
Semi-closed system	<ul style="list-style-type: none"> - Able to control aquaculture condition to a certain extent - Higher production yield than opened system 	<ul style="list-style-type: none"> - Higher investment cost than opened system - Still require water exchange
Closed system	<ul style="list-style-type: none"> - Complete control of water quality and low risk from contamination - Required less water for cultivation - Highest production yield 	<ul style="list-style-type: none"> - Very high investment cost - Require skill labors and operators

2.2 Water quality parameter in aquaculture

Good water quality is the most important factor for successful aquaculture. Important water parameters to be considered in this thesis were dissolve oxygen (DO), temperature, inorganic nitrogen compounds, alkalinity and pH.

2.2.1 Dissolved oxygen (DO)

Dissolved oxygen is the most important and most critical parameter because oxygen is required for respiration of cultured animals. As showed in Table 2.2, dissolved oxygen concentration should exceed 5 and 6 mg/L for tropical zone fish and temperate zone fish to obtain well growth [5, 19, 20]. Dissolving ambient air to aquaculture system as oxygen source by air pump increases dissolved oxygen concentration to saturation point that should be adequate for low density aquaculture, while use of pure oxygen or pressurized oxygen is suited for intensive or super-intensive aquaculture [21, 22]. Table 2.2 displays the effect of dissolved oxygen concentration on animal health.

Table 2.2 Effect of dissolved oxygen on aquatic animal health

Dissolved oxygen (mg/L)	Influence on aquatic animals
< 1 mg/L	Acute toxicity to aquatic animal
1-5 mg/L	Aquatic animals can live but the growth rate is decreased.
> 5 mg/L	Optimum condition for warm water fish
> 6 mg/L	Optimum condition for cool water fish

2.2.2 Temperature

Water temperature in aquaculture system depends on the amount of sunlight and ambient temperature. Water temperature affects the physiological processes of aquatic animal, such as respiration, growth and reproduction [5]. Fishes are classified as endotherm, which means their body temperature is equal to their surrounding environment and their metabolism processes depend on water temperature. Rapid changing of temperature will make their metabolism processes abnormal. However, aquatic animals can still tolerance the seasonal changing of water temperature [10]. Moreover, dissolved oxygen depends on water temperature. Lower dissolved oxygen is associated with increasing water temperature [23].

2.2.3 Inorganic nitrogen compounds

Nitrogen is an essential element for all living organisms as it is the basic element in proteins, and nucleic acids. Uneaten feed along with animal urine and feces will degrade into nitrogenous wastes such as ammonia nitrite and nitrate, which are known to be toxic towards aquacultures.

Ammonia

Ammonia in aquaculture system is generated from microbial degradation of unconsumed proteins in feed and animal excretion. Toxicity of ammonia is dependent on the percentage of un-ionized ammonia, which is the most toxic form. Increasing of pH, salinity or temperature increase the proportion of un-ionized ammonia. Un-ionized ammonia should be below 0.05 mg N/L while the total ammonia concentration should be less than 1.0 mg N/L for long term exposure.

Nitrite

Nitrite is the intermediate in nitrification process or produced during the reduction process from nitrate to nitrogen gas under anaerobic condition (i.e., denitrification). Nitrite accumulation can be found during incomplete nitrification. When nitrite enters bloodstream, it changes hemoglobin to methemoglobin by oxidizing iron in hemoglobin molecule from ferrous state (Fe^{2+}) to ferric state (Fe^{3+}). Methemoglobin has low ability to carry oxygen as compared to hemoglobin, thus making the bloodstream lack of oxygen (i.e., hypoxia), hence the common name “brown-blood disease” [24, 25]. Toxicity of nitrite increases when dissolved oxygen concentration decreases especially when the temperature is high. Nitrite concentration should be below 1 mg N/L.

Nitrate

Nitrate is the final product of nitrification [26]. Nitrate is the least toxic inorganic nitrogen compounds as compared to ammonia and nitrite, thereby it is common to find recirculating aquaculture system operated until reaching high nitrate concentration. However, it should be caution that nitrate can be partially reduced to toxic nitrite when aquaculture system has low dissolved oxygen concentration and

high temperature. In recirculation aquaculture system, nitrate levels are usually controlled by water exchanges or performing denitrification that reduces nitrate to nitrogen gas before releasing into the atmosphere [10, 27].

2.2.4 pH (Acid-base of water)

pH is a negative logarithm of hydrogen ion (H^+) concentration. Water in recirculating aquaculture systems should be maintained pH in the range from 6.5 to 9.0, which are the optimal range for many aquatic species. Hydrogen sulfide is more toxic when pH decreases. Nitrite is reduced under acidic condition to nitrous acid, which inhibited nitrite oxidizing bacteria, resulting in incomplete nitrification. When the pH of water increases more than 9, ammonia will be present in the more toxic un-ionized form [10]. Under intensive cultivation, carbon dioxide from respiration of cultured animals will react with water to form carbonic acid, hence decreasing the water pH of the cultivating system. In order to avoid significant pH reduction, increase aeration and mixing are needed to strip carbon dioxide from water and alkalinity must be maintained above 150 mg $CaCO_3/L$ [10]. The effect of pH on aquatic animals is summarized in Table 2.3.

Table 2.3. Effect of pH on aquatic animals [10]

pH	Effect on aquatic animals
pH below 4.0	Death likely
pH 4.0 - 5.0	Inhibit the reproductive system
pH 4.0 - 6.0	Slow growth
pH 6.5 - 9.0	Optimal range for growth
pH 9.0 - 11.0	Slow growth
pH 9.5 - 11.0	Inhibit the reproductive system
pH above 11.0	Death likely

2.2.5 Alkalinity

Alkalinity is a chemical measurement of pH-buffering capacity, which is reported as mg/L $CaCO_3$. Bicarbonates represent the major form of alkalinity in

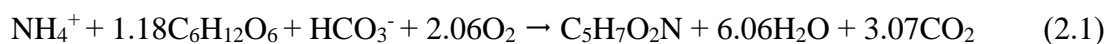
natural waters; its source being the partitioning of CO₂ from the atmosphere and the weathering of carbonate minerals in rocks and soil. Alkalinity is necessary for nitrification, which requires alkalinity at 7.14 g CaCO₃ for 1 g ammonia nitrified. Alkalinity should be adjusted by adding of sodium bicarbonate (NaHCO₃). The recommended range of alkalinity for aquaculture is between 50 and 300 mg CaCO₃/L [10].

2.3 Biological process for nitrogenous waste treatment

Biological processes for nitrogenous waste treatment are based on nitrogen cycle [28]. Important process to be considered here includes ammonia assimilation, nitrification, and heterotrophic denitrification.

2.3.1 Ammonia assimilation

Ammonia is assimilated directly into cells of heterotrophic bacteria or microalgae. Assimilated ammonia is utilized as nitrogen source for synthesis of essential compounds such as amino acids, proteins and nucleic acids. The final products from ammonia assimilation process by heterotrophic bacteria are bacterial biomass, water and carbon dioxide as showed in equation 2.1 [29]. Unlike bacteria, ammonia assimilation process by microalgae produces biomass and oxygen as showed in equation 2.2 [29]. Biomass of bacteria and microalgae in the reactions are represented as C₅H₇O₂N and C₁₀₆H₂₆₃O₁₁₀N₁₆P, respectively.

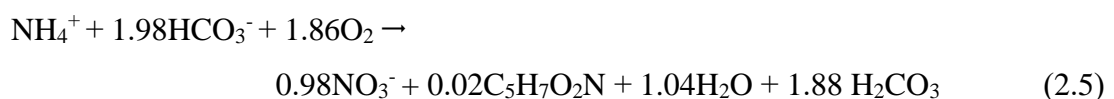
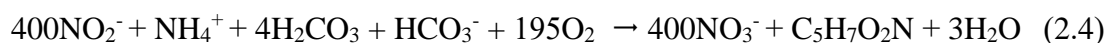
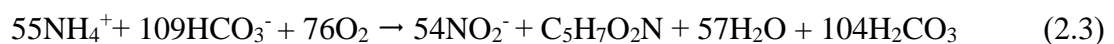


The concept of biofloc technology system is also based on assimilation of ammonia directly into heterotrophic bacterial cells. In order to accelerate the assimilating process, addition of organic carbon into the treatment tanks or ponds should be carried out at the weight C : N > 10 [6]. Aerobic condition and well mixing must be maintained in order to avoid the formation of anaerobic metabolites. However, work by Nootong et al. (2013) [30] also pointed out the significant

contribution from nitrification on inorganic nitrogen treatment in biofloc technology system after the complete nitrification was established. The advantage of biofloc system as pointed out by Avnimelech (1999) [6] was the ability to recycle unconsumed feed proteins by means of biofloc consumption by important economical aquatic animals such as tilapia, carp or shrimp. The main setback of heterotrophic biofloc system is high energy requirement for oxygenation and mixing as well as the difficulty in maintaining suitable range of biofloc (i.e., suspended solids) in cultured tanks [4].

2.3.2 Nitrification

Nitrification is the two-step process that converts ammonia successively to nitrite and nitrate under aerobic condition. Ammonia oxidizing bacteria (AOB) (e.g. *Nitrosomonas*) are responsible for the oxidation of ammonia to nitrite (Equation 2.3) while nitrite oxidizing bacteria (NOB) (e.g. *Nitrospira*, *Nitrobacter*) convert nitrite to nitrate (Equation 2.4). Nitrifying treatment system can be carried out in suspended-growth or attach-growth configuration. For attach-growth, material with high specific surface area and high void ratio, such as sand particles or complex plastic particles can be used [10, 11]. The disadvantage associated with nitrification is slow bacterial growth, thereby leading to long startup period approximately 30 to 40 days. Suitable pH for nitrification ranges from 7.0 to 8.2, while DO should be greater than 2.0 mg/L. The overall reaction for nitrification is displayed in equation 2.5 with $C_5H_7O_2N$ represents bacterial biomass.



2.3.3 Heterotrophic denitrification

Heterotrophic denitrification is the process that reduces nitrate into nitric oxide, nitrous oxide and nitrogen gas as the final product under anaerobic condition. Organic carbon compounds (e.g., methanol, acetate, formic acid) must be added during the process because denitrification is carried out by heterotrophic bacterial genus such as *Pseudomonas*, *Thiobacillus* and *Bacillus* [31] that require external organic carbon for growth. Methanol is the most common organic carbon for denitrification due to its price. Suitable pH for denitrification ranges from 7.0 to 8.2, while DO should be less than 0.5 mg/L. The overall reaction of heterotrophic denitrification is showed in equation 2.6.



2.4 Nile Tilapia



Figure 2.1. Nile tilapia (*Oreochromis niloticus*)

Nile tilapia (*Oreochromis niloticus*) is a tropical fish, which have deep bodied with cycloid scales that prefers to live in shallow water. Its body has silver color with olive, grey or black stripes on body. This fish often flushes into red color during the breeding season [32]. Nile tilapia can live in water that have temperature from 11 to 42 °C but prefer temperature ranges from 31 to 36 °C. The fish is an omnivorous grazer that can feed phytoplankton, aquatic plants, invertebrates, benthic fauna as its food [33]. Nile Tilapia is widely introduced for aquaculture cultivation because of it has wide range of ecological adaptations [34].

2.5 Wastes from nitrifying aquaculture system and treatment methods

2.5.1 Suspended solids

Suspended solids in aquaculture system are generated from uneaten feed, animal feces and microbial biomass proliferated in aquaculture system [6]. The size of suspended solids in aquaculture system ranges from 30 to 250 μm [30, 35]. Suspended solids should be maintained below 80 mg/L [10]. Many methods can be used to separate suspended solids from water including gravitational sedimentation, filtration (e.g., conventional and crossflow), flotation and centrifugation [36]. Separated solids are usually sent to landfill or used for biogas production depending on their composition [9].

2.5.2 Nitrogenous wastes

Nitrogenous wastes in recirculating aquaculture system are generated from uneaten feed, animal excretion, and microbial conversions from different biological pathways. For nitrifying aquaculture system, nitrate accumulation will follow after long period of cultivation. If effluent containing high nitrate concentration is discharged into environment, it will accelerate eutrophication and cause oxygen depletion at night [37, 38]. Drinking water containing nitrate can be harmful especially with enfant less than 1 month old, as nitrate can cause “blue-baby syndrome”, a symptom caused by nitrate binding with hemoglobin and consequently hinder oxygen transport to in blood stream. Heterotrophic denitrification can be used to remove nitrate from cultured tank before final discharge. Another method for nitrate removal is employing photosynthesis process (e.g., aquaponic, microalgal cultivation) to convert nitrate into biomass [7, 8].

2.5.3 Carbon dioxide

Carbon dioxide is introduced into water through respiration of aquatic species and microorganisms. The problem of carbon dioxide is apparent in intensive aquacultures, where carbon dioxide from respiration is dissolved in water to form carbonic acid, hence decreasing pH of cultured tank. Carbon dioxide is inorganic carbon which can react with water in aquaculture tank to form carbonic acid (H_2CO_3)

and change its form into Bicarbonate ion (HCO_3^-) and carbonate ion (CO_3^{2-}) depending on water pH. Dissolved carbon dioxide in water can diffuse to atmosphere through water-air interface (Deacon, 1977). Carbon dioxide is also an important greenhouse gas that cause global concern such as global warming [39]. Base on literature review, carbon dioxide removal via photosynthetic process (e.g., tree plantation, microalgal process) is considered as sustainable option as carbon dioxide is used to produce biomass and oxygen as well as other valuable byproducts [13].

2.6 *Scenedesmus*

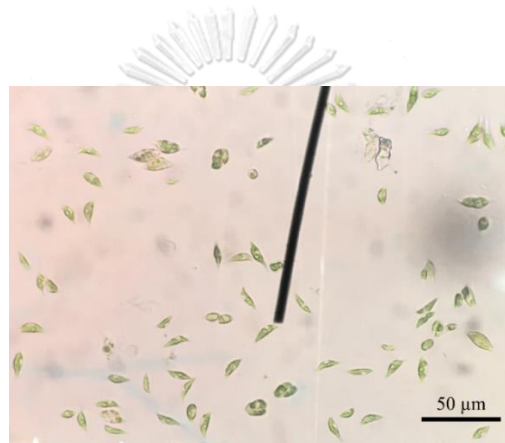


Figure 2.2. *Scenedesmus armatus*.

Scenedesmus sp. is single cell or flat colonial microalgae. The colonies mostly have two or four cells but occasionally have 8, 16 or 32 cells attached in two or three rows. *Scenedesmus* are found in various shapes (e.g., crescent, ovoid, spindle-shape, cylindrical). Cell wall of *Scenedesmus* sp. is smooth or granulated, with lateral ridges, teeth or spines [40]. *Scenedesmus* sp. is used to produce high valued products and biofuel or used in wastewater treatment [41-43]. Figure 2.2 illustrate the photo of microalgal *Scenedesmus armatus*.

2.7 Factors affecting growth of *Scenedesmus* sp.

2.7.1 Nitrogen

Scenedesmus sp. can use ammonia, nitrate and urea as nitrogen source for growth, but different growth rates were observed depending on types of nitrogen [44, 45]. Nitrogen concentration is another factor that affects growth of *Scenedesmus*. The effect of nitrogen source and nitrogen concentration on *Scenedesmus* cultivation are summarized in Table 2.4.

Table 2.4. Effect of nitrogen source and nitrogen concentration on the productivity of *Scenedesmus obliquus* FSP-3 when cultivating in 1 L photobioreactor with starter biomass of 40 mg/L, air flow rate of 0.4 vvm, 2.5% CO₂ v/v and light intensity of 300 $\mu\text{mol}/\text{m}^2/\text{s}$ light intensity [46].

Nitrogen source	Nitrogen concentration (mg N/L)	Biomass productivity (mg/L/day)
Calcium nitrate Ca(NO ₃) ₂	112	942.0 \pm 14.1
Ammonium sulfate (NH ₄) ₂ SO ₄	112	286.3 \pm 6.1
Urea NH ₂ CONH ₂	112	477.8 \pm 8.4
Calcium nitrate Ca(NO ₃) ₂	224	872.1 \pm 10.9
Calcium nitrate Ca(NO ₃) ₂	336	859.1 \pm 11.3

2.7.2 Phosphorus

Scenedesmus sp. can only use phosphorus in the form of orthophosphate for growth. Microalgae use three different processes to transform phosphorus into high energy organic compounds (i.e., ATP) including phosphorylation at the substrate level, oxidative phosphorylation and photophosphorylation. The general reaction for

these processes can be represented in equation 2.7. In the first two processes, energy comes from either the oxidation of the respiratory substrates or the electron transport system of the mitochondria. In the third process, light energy is transformed and incorporated into ATP [47]. Phosphate concentration is the only factor affecting growth of *Scenedesmus* as demonstrated in Table 2.5.



Table 2.5. Effect of phosphate concentration on biomass productivity of *Scenedesmus abundans* when cultivating with BG-11 growth medium, temperature at 27 °C, light intensity at 3,000 lux and light/dark cycle of 14:10 hr. [48].

Phosphate concentration (mg P/L)	Biomass productivity (mg/L/day)
20	39.5 ± 1.6
40	73.5 ± 1.2
60	134 ± 1.4
80	110.5 ± 2.1

2.7.3 Light intensity

Scenedesmus sp. is photosynthetic organism, which has chloroplasts that are able to collect energy from light and synthesize the organic carbon by using carbon dioxide as inorganic carbon source. Growth of *Scenedesmus* sp. is affected by light intensity during the cultivation. Photoperiod is another factor that affects the growth. Increasing light intensity and exposure time generally results in higher growth. However, excessive light intensity may cause photoinhibition and consequently decreases growth. [49]. The effect of light intensity and exposure time on *Scenedesmus* sp. cultivation are displayed in Table 2.6.

Table 2.6. Effect of light intensity and exposure time on biomass productivity of *Scenedesmus* sp.

Microalgal species	Medium	Light intensity/ Exposure time	Productivity (mg/L/day)	Reference
<i>Scenedesmus</i> sp.	tannery wastewater (NH ₃ =206 mg N/L)	80 $\mu\text{mol}/\text{m}^2/\text{s}$ with cycle light/dark 12:12 hr.	96.67	[50]
		140 $\mu\text{mol}/\text{m}^2/\text{s}$ with cycle light/dark 12:12 hr.	165.11	
		200 $\mu\text{mol}/\text{m}^2/\text{s}$ with cycle light/dark 12:12 hr.	210.52	
		3,000 Lux with cycle light/dark 14:10 hr.	104.5 \pm 2.1	
<i>Scenedesmus</i> <i>abundans</i>	BG-11 medium (NO ₃ = 247 mg N/L)	4,000 Lux with cycle light/dark 14:10 hr.	109 \pm 1.7	[48]
		6,000 Lux with cycle light/dark 14:10 hr.	119.5 \pm 1.3	

2.8 Related researches

2.8.1 Inorganic nitrogen control by biofloc technology

There are reports of employing biofloc technology to control inorganic nitrogen concentration during aquacultures [6, 30]. Sittplangkoon (2003) [51] investigated the ability of biological solids (i.e., bioflocs) in controlling inorganic

nitrogen concentration in 500-L tilapia cultivating tanks for 60 days without water exchange. The results showed that maintaining suspended solids concentration in the range from 200 to 300 mg SS/L was able to control ammonia and nitrite below 1.0 mg N/L given tilapia weight in tank was below 11 kg/m³, which corresponded to the nitrogen loading from feed at 2.3 mg N/L/day. Separated experiment indicated that the rate of ammonium degradation by biological solids from tilapia cultivation was 14.43 ± 3.45 mg-N/g-SS/day when the initial ammonia concentration was at 1 mg N/L [51]. Ammonium degradation rates described earlier were significantly higher than those (7.49 ± 0.36 mg-N/g-SS/day at 1 mg N/L) reported by Kunwong (2016) [52]. These data indicated that aquaculture system with biofloc technology can support nitrogen loading from feed ranged from 1.50 to 4.33 mg N/L/day.

The solid-liquid separation by filtration or sedimentation is necessary for maintaining acceptable solid concentration in recirculating aquaculture system. Nurit (2012) [53] employed the upflow solid separator based on gravitational sedimentation to obtain solid removal efficiency about 71% to 73% when the upflow volumetric flow rate of water were 2,640 L/day. Later work by Kunwong (2016) [52] built the filtration system with 100 µm pored size filter subjected to the optimal flow rate of 3,000 L/day (i.e., 1,000% of total water volume) to obtain the removal efficiency about 89%.

2.8.2 Carbon and nitrogen mass balance in recirculating aquaculture system

The amount of wastes generated from aquaculture can be calculated through carbon and nitrogen mass balance. The carbon mass balance analysis from the cultivation of Gilthead seabream (*Sparus aurata*) in the 10 m³ basin with wastewater treatment found that approximately 18.3% of carbon in feed was converted to fish biomass, 11% to biological sludge while the remaining (63.2%) was assumed to be used in respiration by fish and microorganisms in cultivating system [54]. Yogeve et al. (2017) [12] conducted a super-intensive cultivation African sharp-tooth catfish (*Clarias gariepinus*) in 1 m³ fish tank of pilot-scale recirculating aquaculture system consisting of 220 L upflow solid filter and 1.84 m³ nitrification unit. Fish were fed daily with 45% protein feed at 1% to 2% of total fish weight per day. After 147 days of cultivation, the survival rate of 95% and feed conversion ratio

of 1.47 were obtained. Results of carbon and nitrogen mass balance calculation found that 27% of carbon and 28.4% of nitrogen from fish feed were converted to fish biomass; 37% of carbon and 39.2% of nitrogen in feed were converted to organic solids; and 36% of carbon from feed were converted to carbon dioxide. Clearly, significant amount of carbon in feed was converted to carbon dioxide during aquaculture cultivation. For the nitrogen mass balance, Neori et al. (2007) [54] indicated that approximately 15.4% of nitrogen in feed was converted to fish biomass while 14.3% of nitrogen in feed was converted to organic sludge. Significant fraction (70.3%) of nitrogen in feed of was in dissolved inorganic nitrogen (i.e., ammonia, nitrite and nitrate) while Yogeve et al. (2017) [12] reported that significant amount of nitrogen in feed was presented in inorganic nitrogen form and need to be treated, and nitrification exerted important role in the treatment process. It was estimated that approximately 32.4% of nitrogen in feed were converted to inorganic nitrogenous wastes [12].

2.8.3 Microalgal cultivation with aquatic wastewater

Using aquatic wastewater for microalgal cultivation is an alternative method for reducing inorganic nitrogen and phosphorus from aquaculture effluent. The advantage of this approach is gaining microalgal biomass and other valuable products. Ansari et al. (2017) [8] investigated the nutrient removal (e.g., nitrogen and phosphorus) by cultivating *Scenedesmus obliquus*, *Chlorella sorokiniana* and *Ankistrodesmus falcatus* with aquatic wastewater containing ammonia, nitrite, nitrate and phosphate concentration at 5.32 ± 0.45 mg N/L, 5.52 ± 0.18 mg N/L, 40.67 ± 0.84 mg N/L and 8.82 ± 0.02 mg P/L respectively. Microalgae were cultured in 1-L conical flask subjected to temperature of 25 °C, light intensity of 120 $\mu\text{mol}/\text{m}^2/\text{s}$ and light/dark cycle of 16:8 hours, and found that nitrate and phosphate removal efficiencies varied from 75.76% to 80.85% and from 98.52% to 100%, respectively for the described microalgal strains. Similar results were obtained by Kuo et al. (2016) [55], who reported the cultivation of *Chlorella* sp. GD with aquatic wastewater containing ammonia, nitrate and phosphate at 5.6 ± 0.3 mg N/L, 12 ± 0.6 mg N/L and 6.8 ± 0.3 mg P/L, respectively. Microalgae were cultured in 1-L photobioreactors for 7 days under continuous illumination of 300 $\mu\text{mol}/\text{m}^2/\text{s}$ light and 2% CO₂ v/v, and

found that *Chlorella* cultivation subjected to 2% CO₂ v/v yielded ammonia, nitrate and phosphate removal efficiencies of 77%, 83% and 99%, respectively, higher than the cultivation using ambient air that resulted in the removal efficiency of ammonia, nitrate and phosphate at 61%, 61% and 87%, respectively. In addition, the presence of elevated CO₂ concentration (i.e., 2% v/v) could enhance biomass productivity of *Chlorella* (i.e., 309 mg/L·day) as compared to only 76 mg/L·day when using ambient air. The positive effect of CO₂ on growth was also reported for *Chlorococcum* [56]. These results indicated that supplying higher carbon dioxide concentration can increase nutrients removal efficiency and biomass productivity.



CHAPTER III

MATERIAL AND METHODS

This work was conducted at the Center of Excellence in Marine Biotechnology, Department of Marine Science, Faculty of Science, Chulalongkorn University. The work was divided into three parts. The first part evaluated the feasibility of using nitrate from nitrifying biofilter used to treat water in commercial fish farming. The second part studied the feasibility of using carbon dioxide from aquaculture production as carbon source for microalgal cultivation, and the final part examined the effects of fish biomass in cultured tank on carbon dioxide and nitrate production and their utilization during microalgal cultivation in photobioreactors.

3.1 Feasibility of using high nitrate effluent from nitrifying biofilter of commercial aquaculture farm to cultivate microalgae

Freshwater green microalga, *Scenedesmus armatus*, obtained from the Center of Excellence in Marine Biotechnology, Chulalongkorn University, was selected as model microalgal species. Inoculation was prepared by mixing 100 mL of cultured samples with 900 mL of sterilized BG-11 media in 1-L photobioreactors (i.e., Duran bottle) [57]. Starting cell number density was 1.0×10^5 cells/mL. The cultivation was carried out at 25 °C with continuous lighting at 5,500 lux using white LEDs. Sterile air, obtained by passing ambient air through filter cartridge with average pore size of 0.45 µm, was supplied at 0.8 L/min. The source of nitrate was the effluent from nitrifying biofilter tank used for treating water from fish recirculating system of commercial tilapia farm (PC Farm, Chachoengsao Province, Thailand). Nitrate concentration in water samples were approximately 25 mg N/L at the time of collection. Two treatments were conducted. The first treatment was the batch cultivation of *S. armatus* in 1-L Duran bottle using nitrifying biofilter effluent, whereas the second treatment was the cultivation using BG-11 growth media. The cultivation proceeded until the stationary phase was established. Cultured samples (15 mL) from Duran

bottles were obtained daily and analyzed for cell number density, microalgal biomass, concentration of nitrate and phosphate. Contents of chlorophyll, total carotenoids, lutein, beta-carotene and astaxanthin from biomass samples on the final day of cultivation were also determined. Fig. 3.1 illustrates the facility at PC Farm, Chachoengsao Province, Thailand, and the cultivation of *S. armatus* in Duran bottles using nitrate from nitrifying biofilter effluent of commercial fish farm.

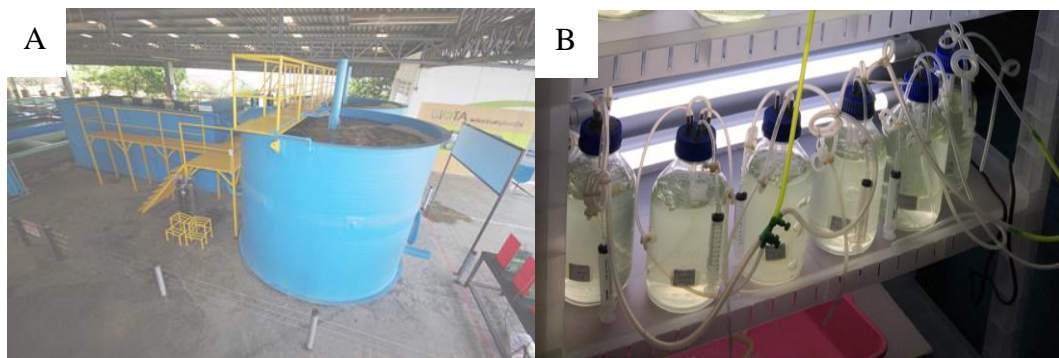


Figure 3.1. (A) Fish recirculating system of commercial tilapia farm at PC Farm, Chachoengsao Province, Thailand (B) Cultivation of *Scenedesmus armatus* in 1-L Duran bottles using high nitrate water from nitrifying biofilter of commercial fish farm.

3.2 Description of recirculating aquaculture system and microalgal cultivation

Recirculating aquaculture system and microalgal cultivation consisted of three units, namely (1) fish tank (FT), (2) solid separating unit (SU) and (3) microalgal cultivation in photobioreactors (MC). As illustrated in Fig. 3.2, water from fish tank was siphoned to the first chamber of solid separating unit, where suspended solids were retained inside by stainless steel screen with average pored size 150 μm . The first chamber was continuously aerated using air diffuser, which was located adjacent to stainless screen, to maintain aerobic condition. This also induced the vertical liquid flow over the stainless screen that reduced the rate of solids accumulation on stainless screen. Given the appropriate operating condition, complete oxidation of ammonia to

nitrate (i.e., nitrification) occurred in the first chamber. Additional solid filtration and oxygenation took place in the second chamber. Water containing high nitrate concentration as a result of nitrification from the solid separating unit could be returned to fish tank or directed to photobioreactors for microalgal cultivation. Carbon dioxide from the headspace of fish tank and solid separating unit were directed to the external air chamber that was equipped with carbon dioxide meter (Extech CO210) and electric fan inside, and subsequently pumped into photobioreactors for microalgal cultivation. Carbon dioxide utilization via photosynthesis and nitrate removal by microalgae led to cell growth and higher oxygen concentration in outlet air from photobioreactors. Fig. 3.3 and 3.4 illustrates the direction of air and liquid flow in recirculating aquaculture system and microalgal cultivation, respectively.

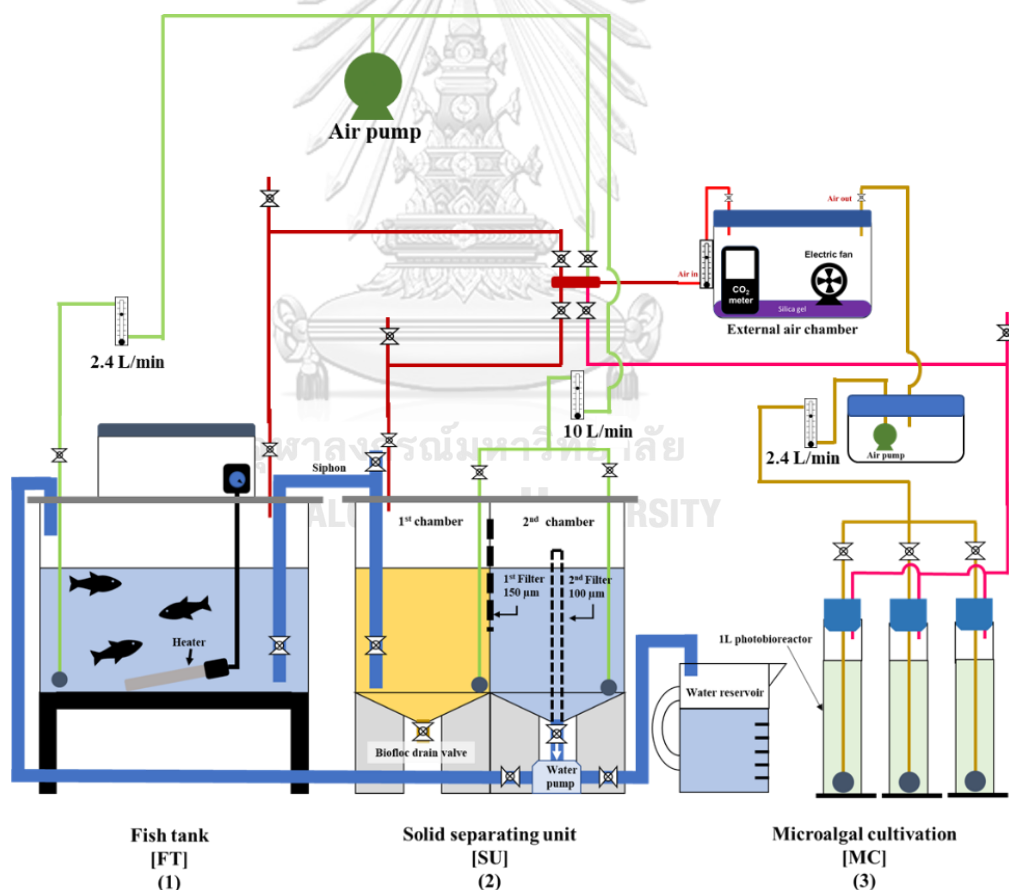


Figure 3.2. Schematic diagram of the integrated recirculating aquaculture system and microalgal cultivation

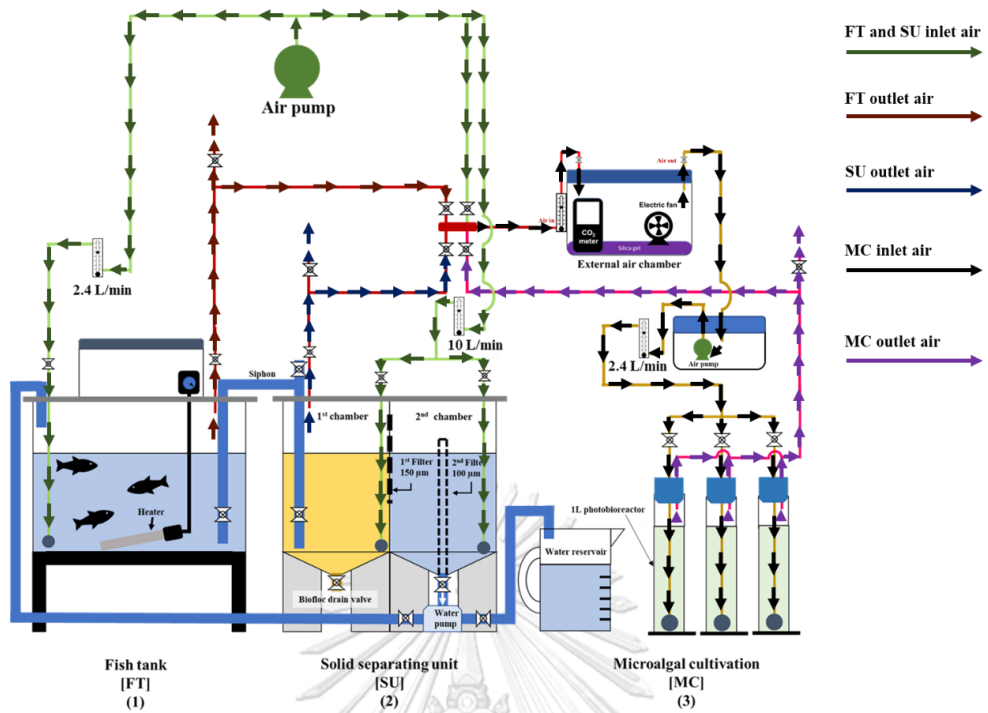


Figure 3.3. Air flow direction in the integrated recirculating aquaculture system and microalgal cultivation

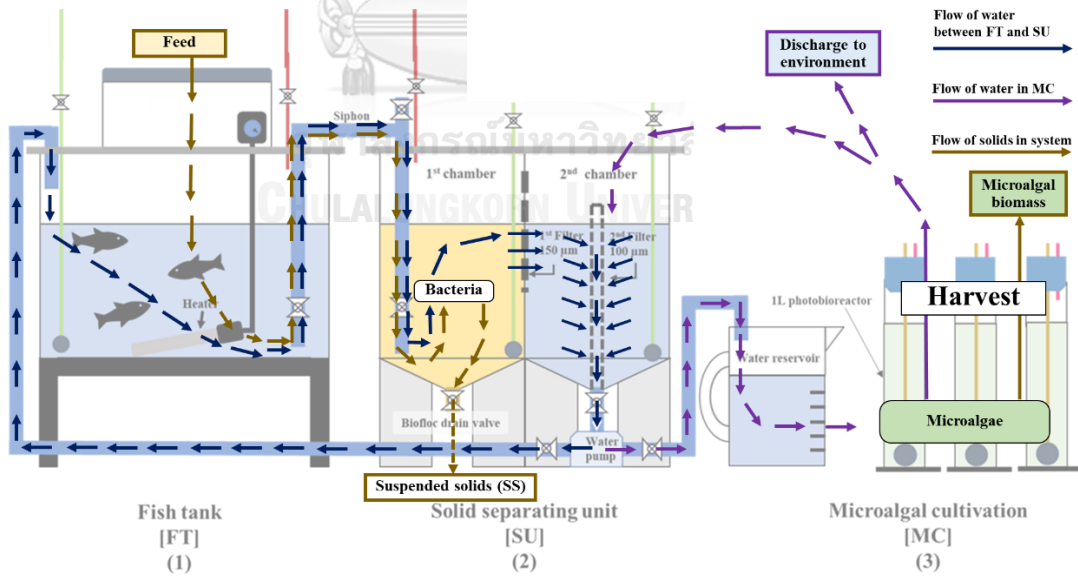


Figure 3.4. Water flow direction in the integrated recirculating aquaculture system and microalgal cultivation

3.2.1 Fish tank (FT)

Fish tank, used for aquaculture cultivation, was the rectangular glass tank (i.e., length 60 cm, width 30 cm and height 45 cm), which was entirely covered on top by PVC flange lid. The total working volume of the tank was 54 L. PVC lid was cut to make the rectangular hole, which was used to accommodate plastic box (i.e., width 34.5 cm; length 24.5 cm and height 15 cm) with plastic lid. Inside this plastic box, small heater was installed to maintain constant temperature. Air inlet and outlet ports were inserted through PVC lid by drilling two small openings (i.e., diameter 0.5 cm). PVC lid was also used to house PVC water pipe (i.e., inner diameter 0.5 inch) for siphoning water from fish tank to subsequent unit operations.

3.2.2 Solid separating unit (SU)

Solid separating unit was constructed from transparent rectangular acrylic tank (i.e., length 62.4 cm; width 31 cm; height 71 cm), which was divided into two chambers by plastic baffle, resulting in working volume of 27 L for each chamber. The baffle was cut to make the square opening (i.e., width 25 cm and length 25 cm), which was covered by stainless steel screen with average pored size of 150 μm . Air diffuser was placed adjacent to baffle to provide oxygen and create vertical liquid flow over screen surface. The bottom of both chambers was built as inverted pyramid to facilitate solid sedimentation and discharge through draining valves. The second chamber was installed with additional filters (i.e., stainless screen with average pored size of 100 μm), which were folded into cylindrical shape and connected to draining valve. Air diffuser in the second chamber was placed near the filter to provide additional oxygen for water before it was recycled to fish tank. Both chambers were covered with PVC plates.

3.2.3 Microalgal Cultivation (MC)

Microalgal cultivation was set up to utilize carbon dioxide from fish tank and nitrate from solid separating unit. Microalgal cultivation consisted of reservoir and 1-L bubble column photobioreactors. The plastic caps of photobioreactors were modified to accommodate air inlet and air outlet tubes and liquid sampling tube. Reservoir bottle (i.e., 5-L beaker) was used for temporary water storage before

passing water through filtration before entering photobioreactors. Inlet air tube was connected to air chamber, which received air containing elevated carbon dioxide concentration from the headspace of fish tank. White LED lamps were used to provide illumination at during microalgal cultivation. Fig. 3.5 illustrates the actual photo of photobioreactors for microalgal cultivation.

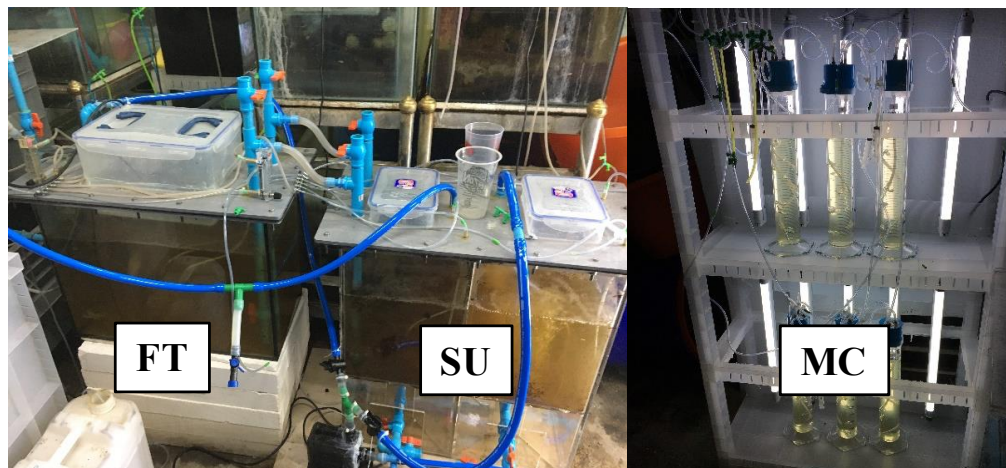


Figure 3.5. Recirculating aquaculture system (left) and microalgal cultivation (right)

3.3 Carbon dioxide production from fish tank and carbon dioxide utilization for microalgal cultivation

3.3.1 Carbon dioxide production from fish tank

This experiment investigated the production of carbon dioxide from aquaculture. Nile tilapia (*Oreochromis niloticus*) with average weight of 34.36 g/fish were released into fish tank to acclimate under new environment for 2 to 5 days before starting the experiment. The initial fish biomass in tank at the start of cultivation was about 3 kg/m³. Tilapia were fed daily with 15% protein commercial feed pellets at the rate of 3% of total fish weight per day. Dissolved oxygen (DO) concentration in fish tank were maintained above 5.0 mg/L by continuous aeration at 2.4 L/min while the temperature was maintained above 28 °C by using heater. Alkalinity and pH of water were kept within the range from 100 to 300 mg CaCO₃/L and from 7.5 to 8.5, respectively, by adding NaHCO₃ periodically. Tilapia cultivation

without water exchange was carried out for 8 days to minimize the effect of fish gaining weight. The solid separating unit was also operated during tilapia cultivation to control ammonia and nitrite concentration within the acceptable level of 1.0 mg N/L. Continuous aeration was provided in the solid separating unit at 10 L/min to maintain well mixed condition and aerobic environment suitable for nitrification. Constant liquid flow rate between fish tank and solid separating unit was maintained at 4,500 L/day throughout fish cultivation. Carbon dioxide concentration from inlet air to fish tank was measured twice daily before and after fish feeding while carbon dioxide concentration in outlet air from fish tank and solid separating unit were measured every hour using carbon dioxide meter (Extech CO-210). Water samples (15 mL) from fish tank and solid separating unit were collected every 2 days and analyzed for the concentration of ammonia, nitrite, nitrate pH and total alkalinity. Tilapia weight and length were also measured at the start and the end of fish cultivation. Two rounds of tilapia cultivation were carried out in this experiment.

3.3.2 Carbon dioxide utilization by microalgae

Inoculation of microalgae as described in section 3.1 was performed under high carbon dioxide concentration at 2% v/v prior to the fish cultivation in this section. Cultured samples of *S. armatus* were combined with sterile BG-11 growth media in bubble column photobioreactors (i.e., inner diameter 6.7 cm and height 35 cm), which were continuously illuminated with white light at 10,000 lux using LEDs. Starting cell number density was 1×10^5 cells/mL. Microalgal cultivation in bubble column photobioreactors and tilapia cultivation started on the same day and proceeded for 8 days. Four treatments (n = 3 for each treatment) were considered for microalgal cultivation. The first treatment (C) was the cultivation using ambient air and BG-11 media while the second treatment (T1) was cultivation using outlet air from fish tank and BG-11 media. Microalgal cultivation in the third treatment (T2) was supplied with ambient air and BG-11 media without Na₂CO₃ addition, and finally the fourth treatment (T3) was supplied with outlet air from fish tank and BG-11 media without Na₂CO₃ addition. Cultured samples (15 mL) from each treatment were obtained daily and analyzed for cell number density, cell dried weight and concentration of nitrate and phosphate. Cultured samples (10 mL) also were obtained

on the final day of cultivation from photobioreactors and analyzed for chlorophyll, total carotenoids, lutein, astaxanthin and beta-carotenoid. Fig. 3.6 illustrates graphical details of experimental set up for section 3.3.1 and 3.3.2.

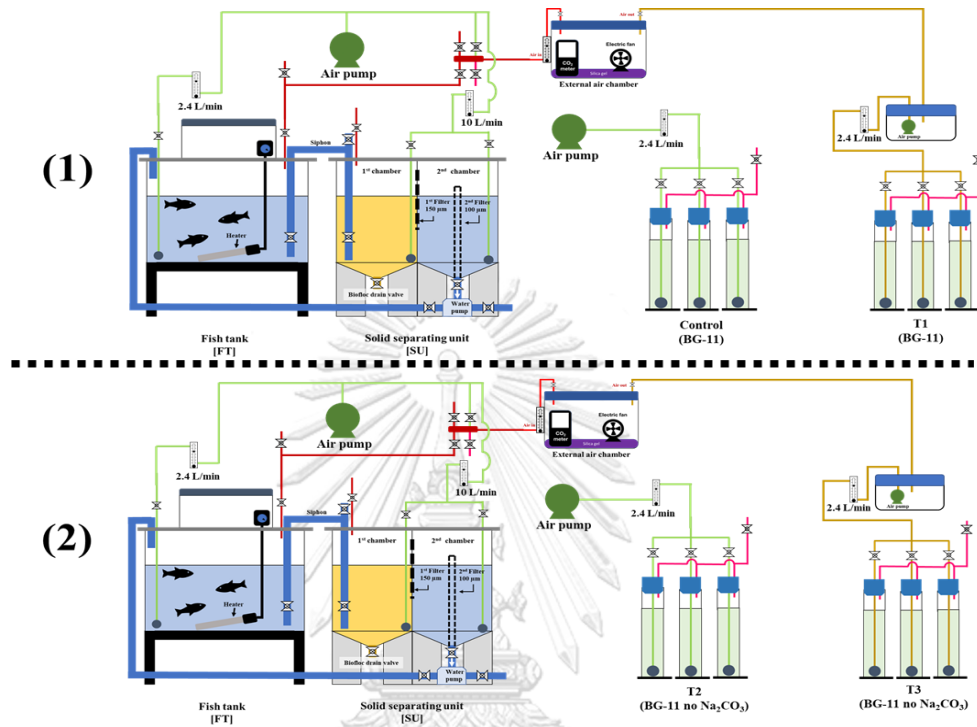


Figure 3.6. Graphical details of the experimental setup for experiment in section 3.3.1 and 3.3.2

3.4 Effects of fish biomass on carbon dioxide production and utilization of carbon dioxide and nitrate from recirculating aquaculture system for microalgal cultivation

This section studied the effects of varying fish biomass in fish tank on the production of carbon dioxide and nitrate, which were subsequently used for microalgal cultivation in photobioreactors. Fish biomass at 3, 5 and 10 kg/m³ were considered in this experiment. Tilapia were acclimated in fish tank for 2 to 5 days before starting the experiment. Fish cultivation lasted only 8 days to maintain relatively constant fish biomass. Tilapia were fed daily with 15% protein commercial feed pellets at the rate of 3% of total fish weight per day. The recirculating

aquaculture system, consisting of fish tank and solid separating unit as nitrifying unit, was operated in continuous mode. DO concentration in fish tank and solid separating unit were maintained above 5.0 mg/L to ensure suitable condition for tilapia and nitrification. Alkalinity and pH of water in the system were kept within the range from 120 to 180 mg CaCO₃/L and 7 to 8.5 by periodic addition of NaHCO₃. Suspended solid concentration in the first chamber of solid separating was controlled between 150 and 400 mg SS/L to ensure effective nitrification for the given fish biomass considered [51].

The experiment started when the recirculating aquaculture system reached mature state as indicated by the occurrence of complete nitrification (i.e., low ammonia and nitrite concentration and increasing nitrate concentration). At this point, effluent (900 mL) containing high nitrate concentration from the solid separating unit was transferred to 1-L bubble column (i.e., inner diameter 6.7 cm and height 35 cm) photobioreactors and mixed with cell cultures (100 mL) of *S. armatus*. Inoculation of *S. armatus* was carried out under high carbon dioxide environment at 2% v/v and light intensity of 10,000 lux. Starting cell number density in bubble column photobioreactor were 1.0×10^5 cells/mL. Two treatments (n = 3 for each treatment) of microalgal cultivation were considered for a given fish biomass. The first treatment was microalgal cultivation using ambient air while the second treatment was the cultivation using outlet air from fish tank. Fig. 3.7 illustrates the graphical detail of experimental set up for this section and Table 3.1 shows the frequency sample collection and analytical techniques used in this experiment.

On the last day of the experiment associated with 10 kg/m³ fish biomass, samples of inlet air of fish tank, outlet air of fish tank, outlet air of solid separating unit and outlet air of photobioreactors for both treatments were collected using air sampling bag and subsequently analyzed for carbon dioxide concentration using gas chromatography. Condition of gas chromatography is available in Appendix G. All tilapia in tank were measured for weight and length. Five tilapias were put to death by rapid cooling method [58], and put in temperature-controlled oven at 105 °C for at least 24 h to determine dried weight. For each fish biomass, approximately 3 g of dried tilapia biomass, dried fish feed, suspended solids from solid separating unit, and dried biomass of *S. armatus* were analyzed for carbon and

nitrogen contents using CHNS/O analyzer (Perkin-Elmer 2400 Series) to obtain data to be used during carbon and nitrogen mass balance calculation.

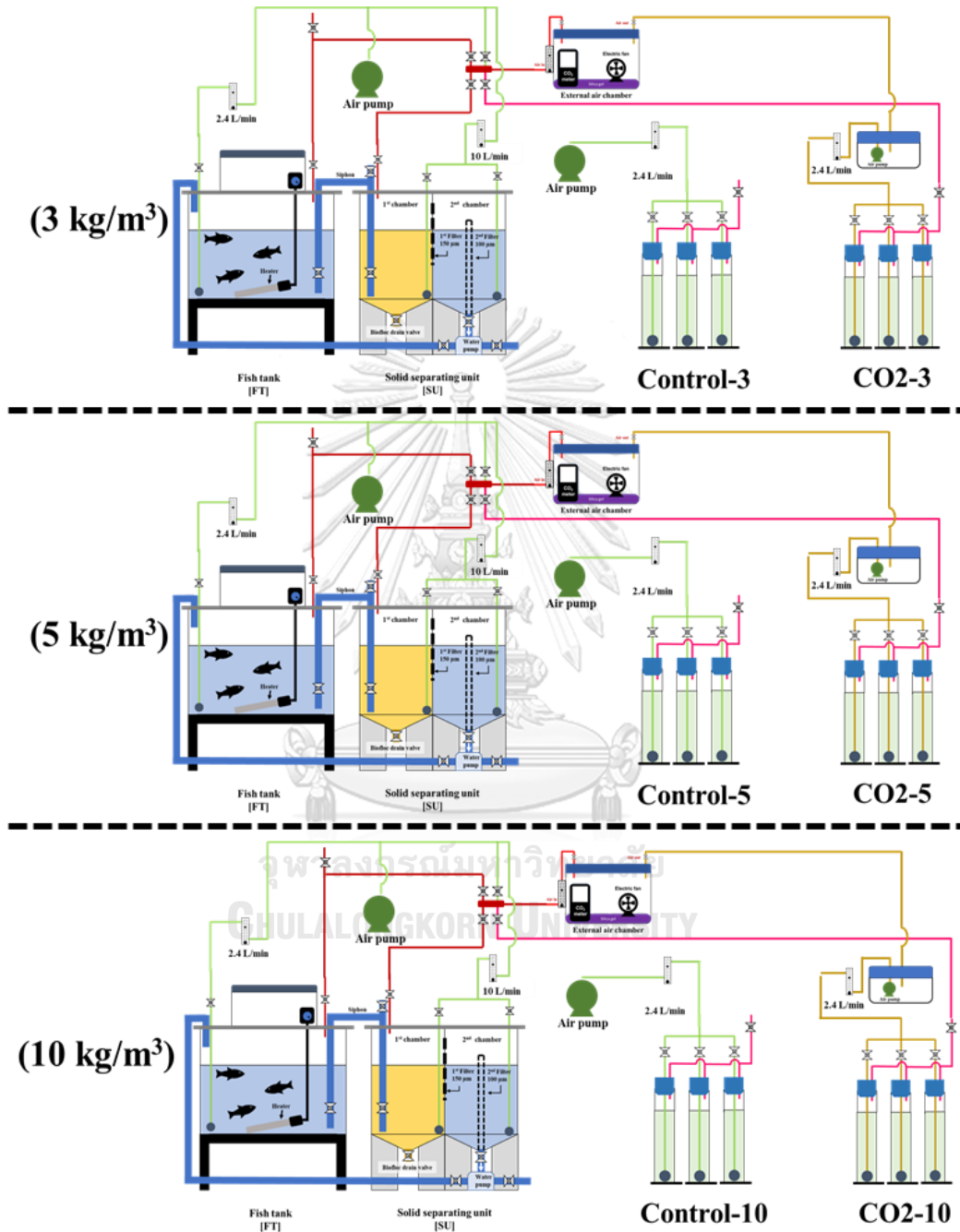


Figure 3.7. Graphical details of experimental setup for section 3.4

Table 3.1. Summary of parameters measured during the experiment in section 3.4

Sample	Sample size	Analyzed parameters	Frequency
Nile tilapia	-	Fish weight Fish length	At the start and the end of fish cultivation
Water from fish tank	15 mL	Total suspended solids TAN concentration NO ₂ ⁻ concentration NO ₃ ⁻ concentration pH, DO, temperature Total alkalinity	Every 2 days
Water from the 1 st chamber of solid separating unit	15 mL	Total suspended solids pH, DO, temperature	Every 2 days
Water from the 2 nd chamber of solid separating unit	15 mL	Total suspended solids TAN concentration NO ₂ ⁻ concentration NO ₃ ⁻ concentration pH, DO, temperature Total alkalinity	Every 2 days
Microalgal cultures from photobioreactors	1 mL	Cell number density	Everyday
Microalgal cultures from photobioreactors	15 mL	Microalgal biomass TAN concentration NO ₂ ⁻ concentration NO ₃ ⁻ concentration PO ₄ ³⁻ concentration pH, DO, temperature Total alkalinity	Every 2 days (TAN and NO ₂ ⁻ analyzed at the start and the end of the microalgal cultivation)

Sample	Sample size	Analyzed parameters	Frequency
Inlet air of fish tank	2.4 L/min	CO ₂ concentration measuring by carbon dioxide meter (Extech CO-210)	Twice a day before and after fish feeding
Inlet air of solid separating unit	10 L/min		
Outlet air of fish tank outlet (i.e., inlet air to microalgal cultivation)	2.4 L/min	CO ₂ concentration measuring by carbon dioxide meter (Extech CO-210)	Every hour
Outlet air of solid separating unit	10 L/min	CO ₂ concentration measuring by carbon dioxide meter (Extech CO-210)	Twice a day before and after fish feeding
Outlet air of microalgal cultivation	2.4 L/min	CO ₂ concentration measuring by carbon dioxide meter (Extech CO-210)	Twice a day before and after fish feeding

3.5 Carbon and nitrogen mass balance in recirculating aquaculture system calculation

Carbon and nitrogen mass balance calculation shows the distribution of carbon and nitrogen input to the recirculating aquaculture system into different forms namely suspended solids, fish biomass, dissolved inorganic carbon and carbon dioxide. The percentage of carbon in tilapia, suspended solids, feed and microalgal biomass from CHN analysis are displayed in Table 3.2. The amount of carbon and nitrogen in different forms can be calculated according to the following equations.

Table 3.2. Carbon and nitrogen contents in feed, fish biomass, suspended solids and microalgal biomass

Sources	% carbon	% nitrogen	Reference
Nile tilapia	49.90	6.76	Appendix G
Suspended solids	29.73	3.14	Appendix G
Feed	41.68	2.97	Appendix G
<i>S. armatus</i>	47.87	8.11	Appendix G

Mass of carbon in feed (C_F). The mass of carbon can be calculated according to equation 3.1

$$C_F = T_F \times f_{CF} \quad (3.1)$$

where C_F is the mass of carbon in feed (g C); T_F is mass of feed supplied in fish tank (g); and f_{CF} is the fraction of carbon in feed as shown in Table 3.2 (i.e., 0.4168).

Mass of carbon in fish (C_N). The mass of carbon in Nile tilapia can be calculated according to equation 3.2

$$C_N = T_N \times f_{CN} \quad (3.2)$$

where C_N is the mass of carbon in Nile tilapia (g C); T_N is the total fish dried weight (g); and f_{CN} is the fraction of carbon in fish as shown in Table 3.2 (i.e., 0.4990).

Mass of carbon in suspended solids (C_S). The mass carbon in suspended solids can be calculated according to equation 3.3

$$C_S = T_S \times f_{CS} \quad (3.3)$$

where C_s is the mass of carbon in suspended solids (g C) and T_s is the total suspended solids (g); and f_{CS} is the fraction of carbon in suspended solids as shown in Table 3.2 (i.e., 0.2973). The total suspended solid (T_s) can be calculated using equation 3.4

$$T_s = \frac{(SS_F \times 54) + (SS_{S1} \times 27) + (SS_{S2} \times 27)}{1,000} \quad (3.4)$$

where T_s is the total suspended solid (g); SS_F is suspended solid concentration in fish tank (mg SS/L); SS_{S1} is suspended solid concentration in the first chamber of solid separating unit (mg SS/L); and SS_{S2} is suspended solid concentration in the second chamber of solid separating unit (mg/L).

Mass of carbon in air stream (C_A). Mass of carbon in air stream input can be calculated according to equation 3.5

$$C_A = (TCO2_F + TCO2_S) \times f_{CC} \quad (3.5)$$

where C_A is the mass of carbon in air stream input (g C); $TCO2_F$ is the mass of carbon dioxide from inlet / outlet of fish tank (g), which can be calculated according to the procedure in Appendix C; $TCO2_S$ is the mass of carbon dioxide from inlet / outlet of solid separating unit (g), which can be calculated according to the procedure in Appendix C; and f_{CC} is the fraction of carbon in carbon dioxide (i.e., 0.2729).

Mass of inorganic carbon dissolved in water (C_D). Mass of carbon dissolved in water can be calculated according to equation 3.6

$$C_D = \frac{(DC_F + DC_S) \times 54 \times f_{CC}}{1,000} \quad (3.6)$$

where C_D is the mass of inorganic carbon dissolved in water (g C); DC_F is the mass of inorganic carbon dissolved in water in fish tank (mg CO₂/L); DC_S is the mass of inorganic carbon dissolved in water in solid separating unit (mg CO₂/L); and f_{CC} is the fraction of carbon in carbon dioxide (i.e., 0.2729).

Mass of nitrogen in feed (N_F). The mass of nitrogen in feed can be calculated according to equation 3.7

$$N_F = T_F \times f_{NF} \quad (3.7)$$

where N_F is the mass of nitrogen in feed (g N); T_F is the mass of feed supplied (g); and f_{NF} is the fraction of nitrogen in feed as shown in Table 3.2 (i.e., 0.0297)

Mass of nitrogen in fish (N_N). The mass of nitrogen in Nile tilapia can be calculated according to equation 3.8

$$N_N = T_N \times f_{NN} \quad (3.8)$$

where N_N is the mass of nitrogen in Nile tilapia (g N); T_N is total fish dried weight (g); and f_{NN} is the fraction of nitrogen in fish as shown in Table 3.2 (i.e., 0.0679).

Mass of nitrogen in suspended solid (N_S). The mass of nitrogen in suspended solid can be calculated according to equation 3.9

$$N_S = T_S \times f_{NS} \quad (3.9)$$

where N_S is the mass of nitrogen in suspended solid (g N); T_S is the total suspended solid, which can be calculated using equation 3.4; and f_{NS} is the fraction of nitrogen in suspended solid as shown in Table 3.2 (i.e., 0.2973).

Mass of inorganic nitrogen dissolved in water (N_D). The mass of inorganic nitrogen dissolved in water can be calculated according to equation 3.10

$$N_D = \frac{(TAN_F + NO2_F + NO3_F + TAN_S + NO2_S + NO3_S) \times 54}{1,000} \quad (3.10)$$

where N_D is the mass of inorganic nitrogen dissolved in water (g N); TAN_F is total ammonia nitrogen concentration in fish tank (mg N/L); TAN_S is total ammonia nitrogen concentration in solid separating unit mg N/L); $NO2_F$ is nitrite concentration in fish tank (mg N/L); $NO2_S$ is nitrite concentration in solid separating unit (mg N/L); $NO3_F$ is nitrate concentration in fish tank (mg N/L); and $NO3_S$ is nitrate concentration in solid separating unit (mg N/L). Equation 3.10 assumes that well mixed condition is established in recirculating aquaculture system so that the difference of TAN, nitrite and nitrate concentration between fish tank and solid separating unit was negligible.

3.6 Carbon mass balance and nitrogen mass balance in microalgal cultivating system calculation

Carbon and nitrogen mass balance calculation in microalgal cultivating system displays the distribution of input carbon (e.g., carbon dioxide) and nitrogen from feed into different forms namely microalgal biomass, dissolved inorganic carbon and nitrogen, and gases such as carbon dioxide and nitrogen gas. The amount of carbon and nitrogen in different forms during microalgal cultivation can be calculated according to the following equations.

Mass of carbon in microalgal biomass (CB). The mass of carbon in microalgal biomass can be calculated according to equation 3.11

$$C_B = \frac{B \times V \times f_{CB}}{1,000} \quad (3.11)$$

where C_B is the mass of carbon in microalgal biomass (g C); B is biomass concentration (mg/L); V is the total working volume of photobioreactor (L); f_{CB} is the fraction of carbon in microalgal biomass as shown in Table 3.2 (i.e., 0.4787).

Mass of inorganic carbon dissolved in water (C_{DC}). The mass of inorganic carbon dissolved in water can be calculated according to equation 3.12

$$C_{DC} = \frac{DC_M \times V \times f_{CC}}{1,000} \quad (3.12)$$

where C_{DC} is the mass of inorganic carbon dissolved in water (g C); DC_M is dissolved inorganic carbon concentration (mg CO₂/L), which can be calculated using Appendix C; V is the total working volume of photobioreactor (L); f_{CC} is the fraction of carbon in carbon dioxide (i.e., 0.2729).

Mass of carbon in air stream (C_C). The mass of carbon in air stream containing carbon dioxide can be calculated according to equation 3.13

$$C_C = TCO2_M \times f_{CC} \quad (3.13)$$

where C_C is the mass of carbon in air stream (g C); $TCO2_M$ is the mass carbon dioxide in air stream (g CO₂), which can be determined using procedure in Appendix C; and f_{CC} is the fraction of carbon in carbon dioxide (i.e., 0.2729).

Mass of nitrogen in microalgal biomass (N_B). The mass of nitrogen in microalgal biomass can be calculated according to equation 3.14

$$N_B = \frac{B \times V \times f_{NB}}{1,000} \quad (3.14)$$

where N_B is the mass of nitrogen in microalgal biomass (g N); B is microalgal biomass concentration (mg/L); V is the total working volume of photobioreactor (L); and f_{NB} is the fraction of nitrogen in microalgal biomass as shown in Table 3.2 (i.e., 0.0811).

Mass of inorganic nitrogen dissolved in water (N_{DN}). The mass of inorganic nitrogen dissolved in water can be calculated according to equation 3.15

$$N_{DN} = \frac{(TAN + NO_2 + NO_3) \times V}{1,000} \quad (3.15)$$

where N_{DN} is the mass of inorganic nitrogen dissolved in water (g N); TAN is total ammonia nitrogen concentration in photobioreactor (mg N/L); NO_2 is nitrite concentration in photobioreactor (mg N/L); NO_3 nitrate concentration in photobioreactor (mg N/L); and V is total working volume of photobioreactor (L).

3.7 Analytical techniques

All fish were measured their total length with caliper and weight with 1-digit scientific balance (Fig 3.8) at the beginning and the end of tilapia cultivation. Fish biomass (FB) or average weight (AFW), average fish length (AFL), average fish daily weight gain (DWG), survival ratio, and feed conversion ratio (FCR) were calculated using equations 3.16 to 3.21.



Figure 3.8 Measurement of tilapia's weight (Left) and tilapia total length (Right)

$$FB(\text{kg} / \text{m}^3) = \frac{TFW(\text{g})}{V(\text{L})} \times \frac{1\text{kg}}{1,000\text{g}} \times \frac{1,000\text{L}}{1\text{m}^3} \quad (3.16)$$

$$AFW(\text{g}) = \frac{TFW(\text{g})}{n} \quad (3.17)$$

$$AFL(\text{cm}) = \frac{TFL(\text{cm})}{n} \quad (3.18)$$

$$DWG(\text{g} / \text{day}) = \frac{FinalAFW(\text{g}) - InitialAFW(\text{g})}{D} \quad (3.19)$$

$$\%SR = \frac{n_F \times 100}{n_I} \quad (3.20)$$

$$FCR = \frac{TI_F(\text{g})}{FinalTFW(\text{g}) - InitialTFW(\text{g})} \quad (3.21)$$

Where FB is Fish biomass (kg/m^3), TFW is Total fish lived weight (g), V is Total working volume in fish tank (L), n is Number of fish (g), AFW is Average fish lived weight (g), AFL is Average fish total length (cm), TFL is Total fish length (cm), DWG is Average daily weight gained (g/day), D is Cultivating time (days), $\%SR$ is

Fish survival rate (%), n_i is Initial number of fish, n_f is Final number of fish, FCR is Feed conversion ratio and TI_f is Total feed input (g)

Cell number density, determined by counting cells with haemocytometer under microscope, was used to calculate the specific growth rate of microalgae according to the equation $\mu = (\ln N_1 - \ln N_2)/(t_2 - t_1)$ where μ is the specific growth rate (d^{-1}) and N_1 and N_2 are cell number density at time t_1 and t_2 , respectively. Cell growth was also followed by using cell dried weight (i.e. biomass concentration), which was analyzed following the procedure for total suspended solids analysis [59]. Cell dried weight concentration were used to calculate biomass productivity for the batch cultivation according to $P = (B_2 - B_1) / (t_2 - t_1)$, where P is the biomass productivity for batch cultivation ($mg/L \cdot d$); and B_1 and B_2 are biomass concentration (mg/L) at time t_1 and t_2 , respectively.

Nitrate concentration, measured by a spectrophotometer, based on the UV screening method, was carried out by passing cultured samples through a Whatmann GF/C filter and measuring light absorption at 220 and 275 nm [59]. Ammonia and nitrite were quantified using spectrophotometer by measuring the light absorbance at 660 nm for ammonia based on Salicylate–Hypochlorite method and at 543 nm for nitrite based on colorimetric method [59, 60]. Analysis of phosphate concentration measured the light absorbance at 885 nm according to the ascorbic acid method [59]. Chlorophyll and carotenoids were measured according to Strickland and Parson by separating cell biomass from liquid by centrifugation, and followed by acetone extraction [61]. The clear liquid obtained was subjected to light absorption at 480 nm for carotenoids and from 630 to 665 nm for chlorophyll. Concentrations of chlorophyll and carotenoids were calculated according to Equation 3.22 and 3.23 as follows:

$$A = (11.6E_{665} - 1.31E_{645} - 0.014E_{630}) \times (V_a/V_b) \quad (3.22)$$

$$B = 4 \times (E_{480}) \times (V_a/V_b) \quad (3.23)$$

where A is chlorophyll concentration in mg/L , B is carotenoids concentration in mg/L , E_{480} is light absorbance at 480 nm, E_{630} is light absorbance at 630 nm, E_{645} is light

absorbance at 645 nm, and E_{665} is light absorbance at 665 nm, V_a is volume of solvent that was used for extraction, V_b is volume of sample that was extracted. Carotenoid composition of biomass were identified by HPLC equipped with photo-diode array detector based on comparing their retention time and UV spectrums from standards. Extracted carotenoid samples (20 μ L) were injected into C18 column, which was operated under the following condition: mixture of acetonitrile (79.9% v/v), dichloromethane (10% v/v), ethanol (10% v/v) and water (0.1% v/v) as mobile phase and flow rate of mobile phase at 1.0 mL min⁻¹. Light intensity was measured by Digicon-LX lux meter. Carbon dioxide concentration (ppm) were measured using Carbon dioxide meter (Extech CO-210). Total alkalinity was analyzed base on titration method (APHA, 2005). Dissolved carbon dioxide and dissolved inorganic carbon were obtained from total alkalinity and pH with carbon dioxide and forms of alkalinity by calculation (APHA, 2005). Experimental data were statistically tested by using one-way ANOVA and performing Tukey HSD post-hoc test with the significance level of 0.05 [62].

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Feasibility of using high nitrate water from nitrifying biofilter of commercial aquaculture farm to cultivate microalgae

4.1.1 Effluent qualities

Nitrifying biofilter was used to treat water from tilapia cultivating tank, producing the effluent containing nitrate and other compounds as shown in Table 4.1. Nitrate (25.6 mg N/L) was the major inorganic nitrogen compound in the effluent at time of collection as compared to nitrite and ammonia. Small amounts of phosphate and suspended solids were also detected. Previous works reported that nitrate less than 15 mg N/L was able to support growth of several freshwater green microalgae including *Scenedesmus* [63, 64], and thus the effluent from nitrifying biofilter in this experiment should be able to support growth of *S. armatus*.

Table 4.1. Effluent qualities from nitrifying biofilter of commercial tilapia farm.

Parameter	Concentration
Total ammonia nitrogen	0.01 mg N/L
Nitrite	0.004 mg N/L
Nitrate	25.6 mg N/L
Phosphate	0.519 mg P/L
Total suspended solids	11 mg SS/L
Alkalinity	220 mg CaCO ₃ /L

4.1.2 Growth of *Scenedesmus armatus* and nutrient utilization

Results of batch cultivation of *S. armatus* using nitrifying biofilter effluent and BG-11 growth media are demonstrated in Fig 4.1A. The maximum specific growth rate of microalgae from the cultivation using nitrifying biofilter effluent was

1.55 day⁻¹, which was approximately 1.75 folds higher than that using BG-11 media. The maximum cell number density subjected to nitrifying biofilter effluent and BG-11 growth media were 6.49×10^6 cells/mL on day 7 and 6.04×10^6 cells/mL on day 9, respectively, which were statistically insignificant difference ($p > 0.05$) because as both cultures already attained the stationary phase. Nitrogen to phosphorous ratio of nitrifying biofilter effluent ranged from 22.9 to 463.7 during the growth phase and were greater than the stoichiometric ratio of nitrogen to phosphorus for microalgal biomass that was determined at 16 [29, 65]. This finding explained why nitrifying biofilter effluent could support the growth of *S. armatus* as compared to the standard BG-11 growth media. Moreover, nitrogen to phosphorus ratio of nitrifying biofilter effluent decreased below the stoichiometric ratio since day 7. This suggested phosphorus limitation in the cultivating system and might explain an early establishment of stationary phase in this case.

The final biomass for the cultivation using BG-11 growth media were 675 mg/L (Fig. 4.1B). This corresponded to the average productivity over 9-days period of 71.7 mg/L·day. The biomass productivity obtained was slightly higher than that subjected to nitrifying biofilter effluent that reported the final biomass concentration and productivity over the same period at 664 mg/L and 73.0 mg/L·day, respectively. Based on the results presented, the cultivation of *S. armatus* using the effluent from nitrifying biofilter yielded similar growth performance, measured in terms of cell number density and biomass production, as compared to the cultivation using more expensive BG-11 growth media, and therefore could be substituted as low cost media to reduce operating expense during the future cultivation.

Batch cultivation of *S. armatus* using nitrifying biofilter effluent indicated that microalgal growth was accompanied by the reduction of nitrate concentration from 25.59 to 3.01 mg N/L (i.e., 88% reduction) (Fig. 4.2A). In contrast, batch cultivation using BG-11 growth media utilized more nitrate that led to 230 mg N/L of nitrate remaining at the end of cultivation (Fig. 4.2A). Significant amount of nitrate remaining in BG-11 growth media implied that the initial amount of nitrate was excessive for growth, and therefore needed to be optimized or recycled to avoid unnecessary wasting through effluent discharge. By comparing the results obtained with those from previous works, it was apparent that the level of nitrate affected the

extent of nitrate utilization by microalgae, for examples, Ansari et al. (2017) [8] reported the cultivation of *S. obliquus* using aquaculture effluent containing 41 mg N/L of nitrate that yielded the final biomass productivity of 89.61 mg /L·day and reduced approximately 78% of nitrate at the end of 14-day cultivation. Subsequent work by Habibi et al. (2018) [66] reported that the cultivation of *Scenedesmus* sp. using the effluent containing 100 mg N/L of nitrate yielded the average biomass productivity of 161.11 mg /L·day and was able to decrease nitrate concentration in the effluent as high as 78%. The cultivation using nitrifying biofilter effluent decreased phosphate from 0.52 to 0.29 mg P/L (i.e., 44% reduction) while the cultivation using BG-11 growth media was able to utilize more phosphate available in the media, hence decreasing phosphate concentration from 4.96 to 2.24 mg P/L (i.e., 55% reduction) (Fig. 4.2B). Although phosphate is considered as an important macronutrient for microalgae, results from previous works appeared to overlook its importance as compared to nitrate [67]. In conclusion, by considering the growth data and nitrate consumption, it can be concluded that nitrifying biofilter effluent could be employed as low-cost growth media for the cultivation of *S. armatus*.

4.1.3 Pigment content of *Scenedesmus armatus*

Microalgal cultivation using BG-11 growth media yielded higher pigment contents than the cultivation using nitrifying biofilter effluent although the statistical test revealed their magnitudes were insignificantly different ($p > 0.05$) between growth media used (Table 4.2). Results of pigment analysis concurred with earlier observation that nitrifying biofilter effluent could substitute BG-11 media for the cultivation of *S. armatus*. In this experiment, lutein was a major carotenoid in biomass, accounting for 36.25% to 40.15% of total carotenoids, yet significant fraction ranged from 41.75% to 48.65% remained unidentified while another unidentified peak accounted for 11.2% to 22% of total carotenoids available. It should be pointed out that lutein contents obtained in this experiment were lower than those reported in literature, which already optimized the production strategies, for examples by screening suitable strains or adjusting environmental parameters such as light intensity, photo-period, nitrogen availability and salinity [46, 68].

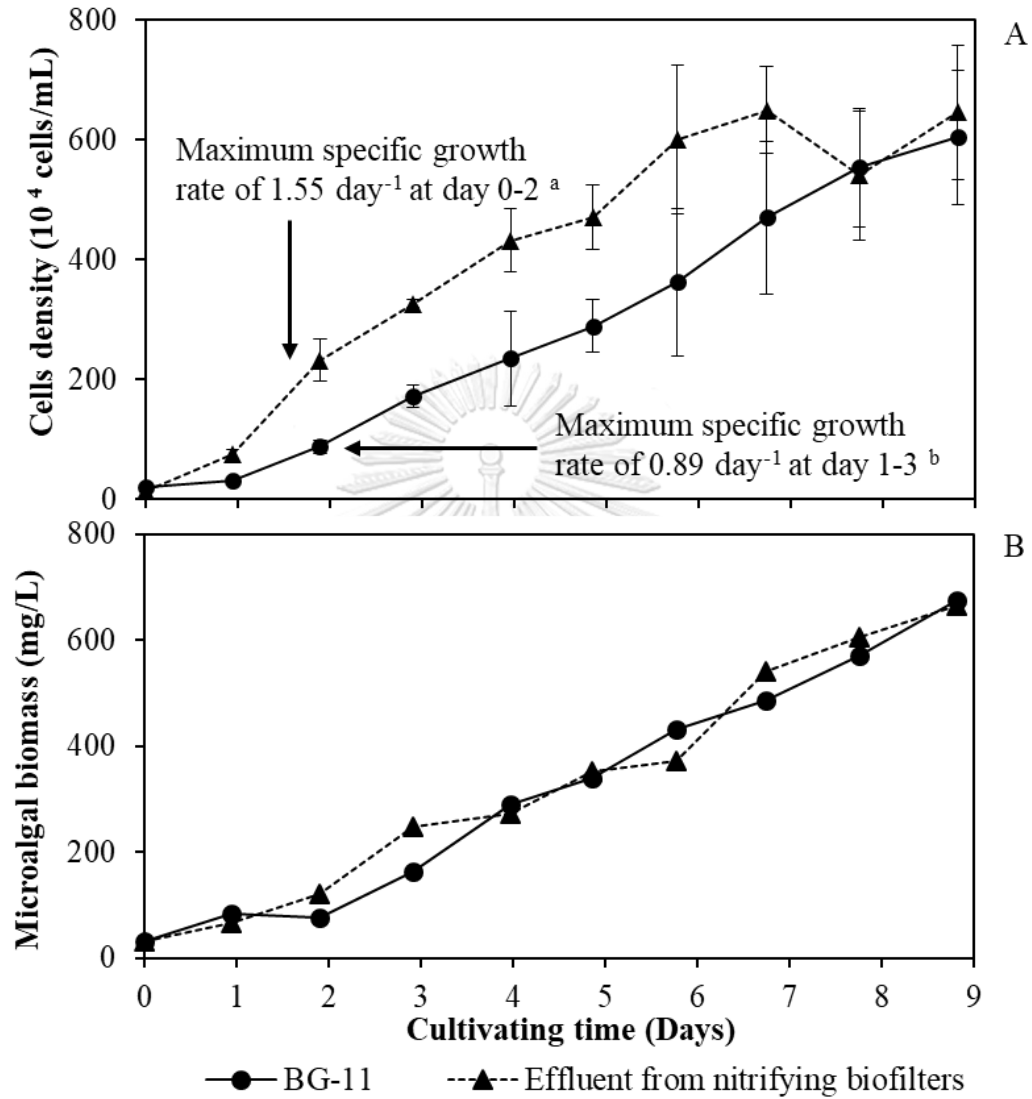


Figure 4.1. (A) Cell number density and (B) Microalgal biomass concentration of *Scenedesmus armatus* during the batch cultivation using BG-11 growth media and effluent from nitrifying biofilter.

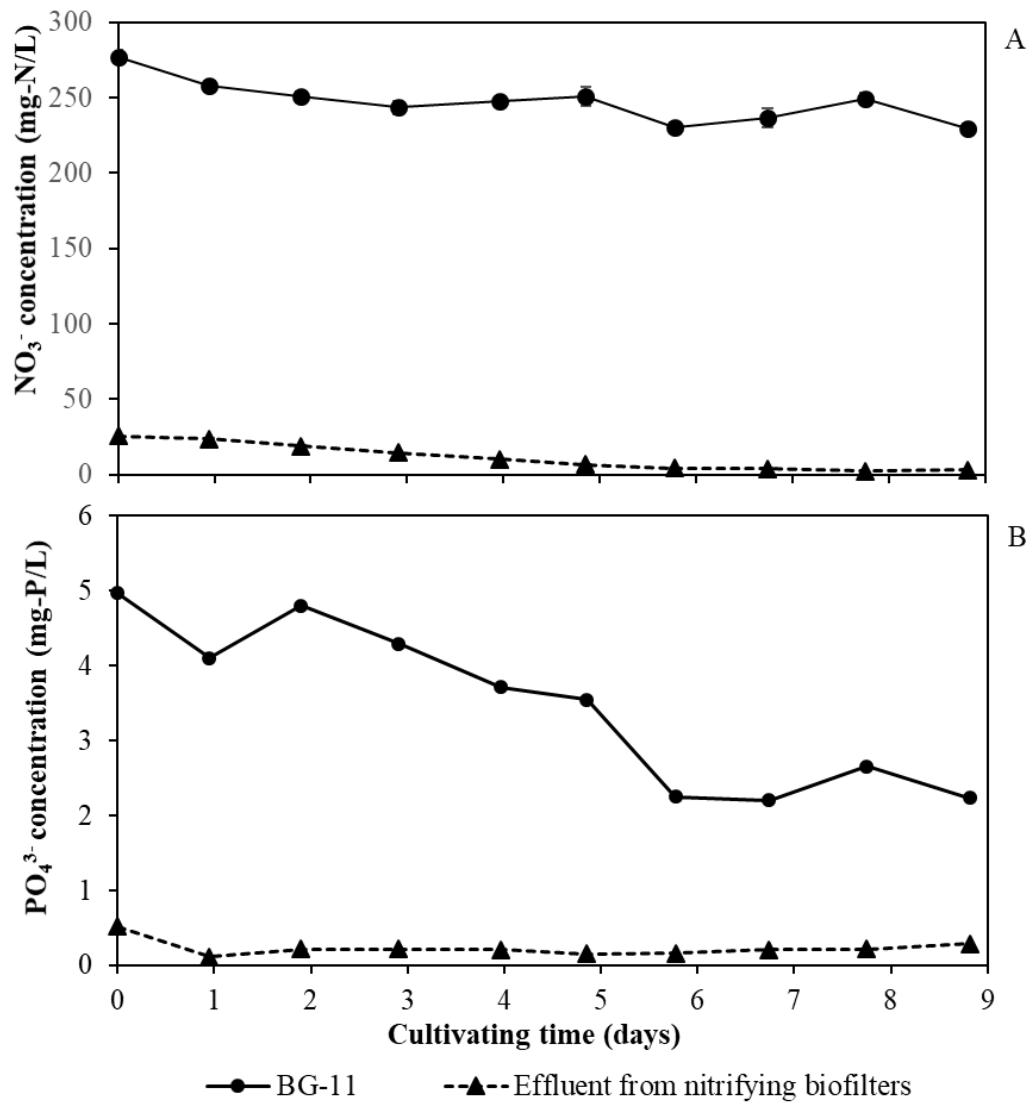


Figure 4.2. (A) Nitrate and (B) phosphate concentration during the batch cultivation of *Scenedesmus armatus* using BG-11 media and effluent from nitrifying biofilter

Table 4.2. Pigment contents in biomass of *Scenedesmus armatus* at the end of batch cultivation using BG-11 and nitrifying biofilter effluent as growth media.

Pigments	Growth media	
	BG-11	Nitrifying biofilter effluent
Chlorophyll a (mg/g)	16.72 ± 7.40 ^a	10.52 ± 6.06 ^a
Total carotenoids (mg/g)	3.96 ± 1.00 ^a	3.09 ± 2.00 ^a
Lutein (mg/g)	1.59 ± 0.62 ^a (40.15%)	1.12 ± 0.71 ^a (36.25%)
Beta-carotene	ND	ND
Astaxanthin	ND	ND
Unidentified carotenoid 1	48.65%	41.75%
Unidentified carotenoid 2	11.20%	22%

Different letters within the same row indicate statistically significant difference ($p < 0.05$)

ND, non-detectable

4.2 Feasibility of using carbon dioxide from recirculating aquaculture system for microalgal cultivation

4.2.1 Carbon dioxide production from aquaculture tank

Tilapia was released into rectangular fish tank (54 L working volume) to obtain the initial biomass about 3.09 kg/m³. During tilapia cultivation, the average carbon dioxide concentration from tank inlet (i.e., ambient air) were 566 ± 13.9 ppm (1,019 ± 25.1 mg/m³) and increased to 935 ± 93.4 ppm (1,683 ± 168.1 mg/m³) in tank headspace and outlet air (Fig. 4.3). The obtained carbon dioxide concentration in fish tank headspace (i.e., 935 ± 93.4 ppm) was used to calculate the maximum carbon dioxide solubility based on Henry's law [69]. However, by using pH and alkalinity data, carbon dioxide concentration in water was oversaturated at 3.6 × 10⁻⁵ mol/L, thus leading to carbon dioxide diffusion across the interface into headspace region. Increasing carbon dioxide in headspace and outlet was related to fish respiration and, to lesser extent, from microorganism in tank [70]. Detail examination of carbon dioxide profile from fish tank outlet revealed that carbon dioxide concentration

fluctuated with time and seemed to be influenced by fish feeding (Fig. 4.4). Carbon dioxide concentration peaked after tilapia was fed and decreased gradually from the peak back to the starting level within 24-hour period. Thus, the amount of carbon dioxide gained after feeding can be estimated from under peak area (i.e. black color area) while the baseline production by fish respiration was the difference between values before feeding and ambient carbon dioxide concentration (i.e., dark gray area). As illustrated in Fig. 4.4, the excessed carbon dioxide from feeding process during the 8-day period were 3.33 and 4.92 g for the first and second trials, respectively, while carbon dioxide production by basic respiration were 15.24 g for the first cultivation and 12.03 g for the second cultivation. Therefore, on average, the rate of excessed carbon dioxide production as a result of feeding and that from basic fish respiration were calculated at 106.65 and 351.89 g CO₂/day·kg-feed, respectively, leading to the total carbon dioxide production rate of 458.54 g CO₂/day·kg-feed.

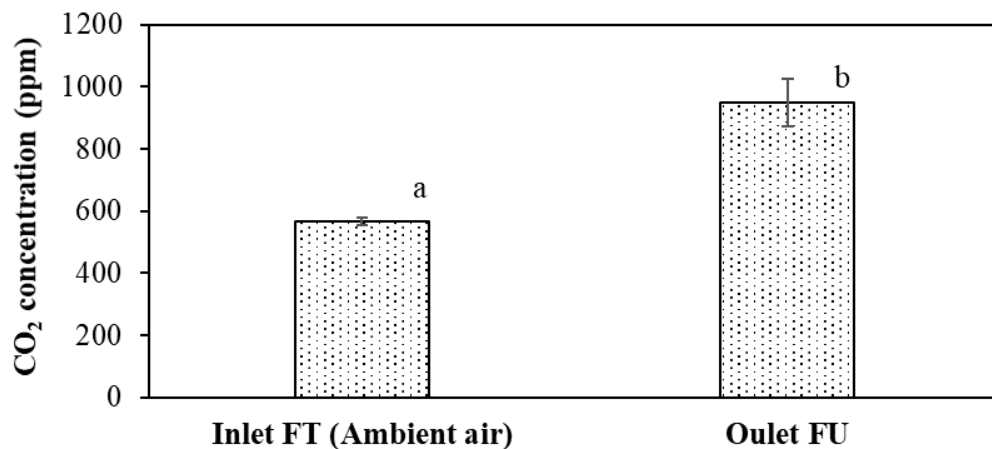


Figure 4.3. Average carbon dioxide concentration of inlet air (i.e., ambient air) and outlet air of fish tank (FT) when fish biomass about 3 kg/m³

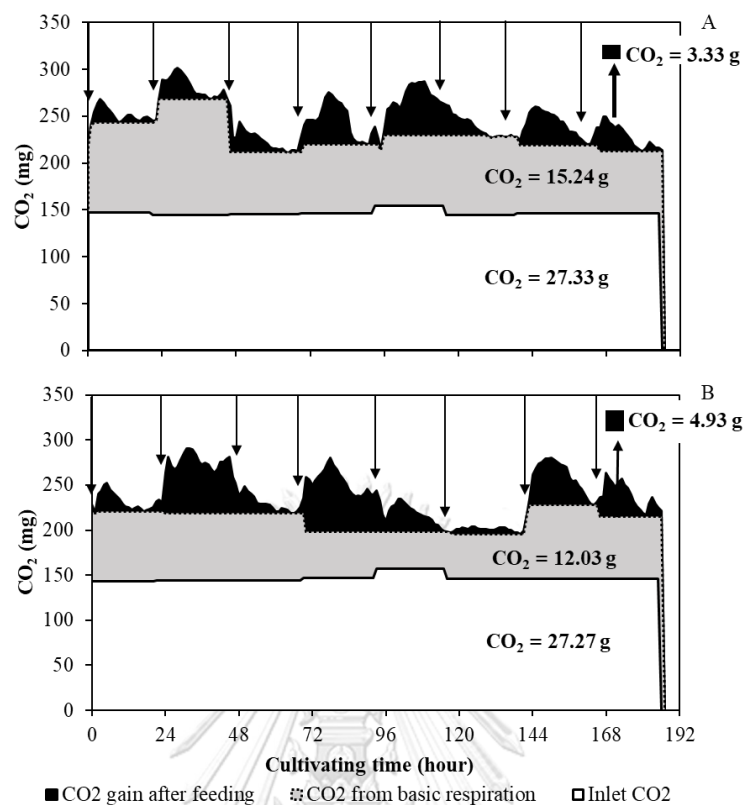


Figure 4.4. Profile of carbon dioxide concentration at the outlet of fish tank during the tilapia cultivation (arrows indicate fish feeding; A and B represent cultivation trial 1 and 2)

4.2.2 Utilization of carbon dioxide from fish tank to cultivate *Scenedesmus armatus*

Batch cultivation of *S. armatus* was conducted in 1-L bubble column photobioreactors, which were illuminated with white LEDs at 10,000 lux throughout the cultivation. Higher light intensity was employed in this experiment instead of 5,500 lux in the previous section in order to improve photosynthesis under higher carbon dioxide environment. The first set (C) was the cultivation using ambient air and BG-11 media while the second set (T1) was the cultivation using outlet air from fish tank and BG-11 media. The third set (T2) used ambient air and BG-11 media without Na₂CO₃ and finally the fourth set (T3) used air from fish tank and BG-11 media without Na₂CO₃. The final cell number densities were 7.83×10^6 , 12.0×10^6 , 4.14×10^6 and 10.4×10^6 cells/mL for the cultivation in C, T1, T2 and T3,

respectively (Fig. 4.5(A)). The maximum specific growth rates up to the end of growth period (i.e., day 5) were determined at 0.64 d^{-1} for C, 0.69 d^{-1} for T1, 0.52 d^{-1} for T2 and 0.64 d^{-1} for T3. T1 yielded the highest cell number density because it was supplied with the highest amount of carbon in the forms of carbon dioxide from fish tank and carbonate in BG-11 media. Statistical test indicated that cell number density from T1 was statistically significant different ($p < 0.05$) from cell number density from T2, which acquired carbon from ambient air only. The maximum cell dried weight ($916 \pm 81.7 \text{ mg/L}$) also occurred in T1 (Fig. 4.5(B)) and this was equivalent to biomass productivity over 8-days period of $110 \pm 11.4 \text{ mg/L}\cdot\text{day}$. Unlike the results of cell number density, the final cell dried weight from each treatment were insignificantly different ($p > 0.05$). Based on the results obtained, outlet air from fish tank containing higher carbon dioxide concentration was able to enhance growth of *S. armatus* relative to those using ambient air regardless to the availability of Na_2CO_3 in BG-11 growth media. Since microalgae including *Scenedesmus* have biological pathways that used either carbon dioxide or bicarbonate as carbon source, the exclusion of Na_2CO_3 from BG-11 growth media was therefore intended to prevent microalgae from using dissolved carbonate but utilizing only carbon dioxide [71].

Nitrate consumption during the cultivation of *S. armatus* in bubble column photobioreactors indicated the decreasing trends and comparable concentration at the end of cultivation on day 8 (Fig. 4.6(A)). Like the results from previous section (i.e. 4.1.2), high amount of nitrate was still in water, and that required additional operation to recycle rather than discharging as wastewater. Phosphate consumption was apparent during the growth period, with T2 and T3 (i.e., no Na_2CO_3 addition) consuming more phosphate as compared to those using the standard BG-11 media (Fig. 4.6(B)). It was also observed that pH of T1 and T3 increased from approximately 8.0 on the first day to the values ranged from 10 to 11 at the end of growth period although these treatments were supplied with elevated carbon dioxide concentration ($948 \pm 76.9 \text{ ppm}$) that should instead lower pH (Fig. 4.7(A)). Similar observation (i.e., increasing pH) was noted for C and T1, which were supplied with ambient air. The increase of pH could be linked to biochemical activities of microalgae that released hydroxyl ions into water due to carbonate utilization [71, 72].

This could influence the pH in low buffer solution such as BG-11 media, which contained initial alkalinity at 52.50 ± 9.57 mg CaCO_3 (Fig. 4.7(B)).

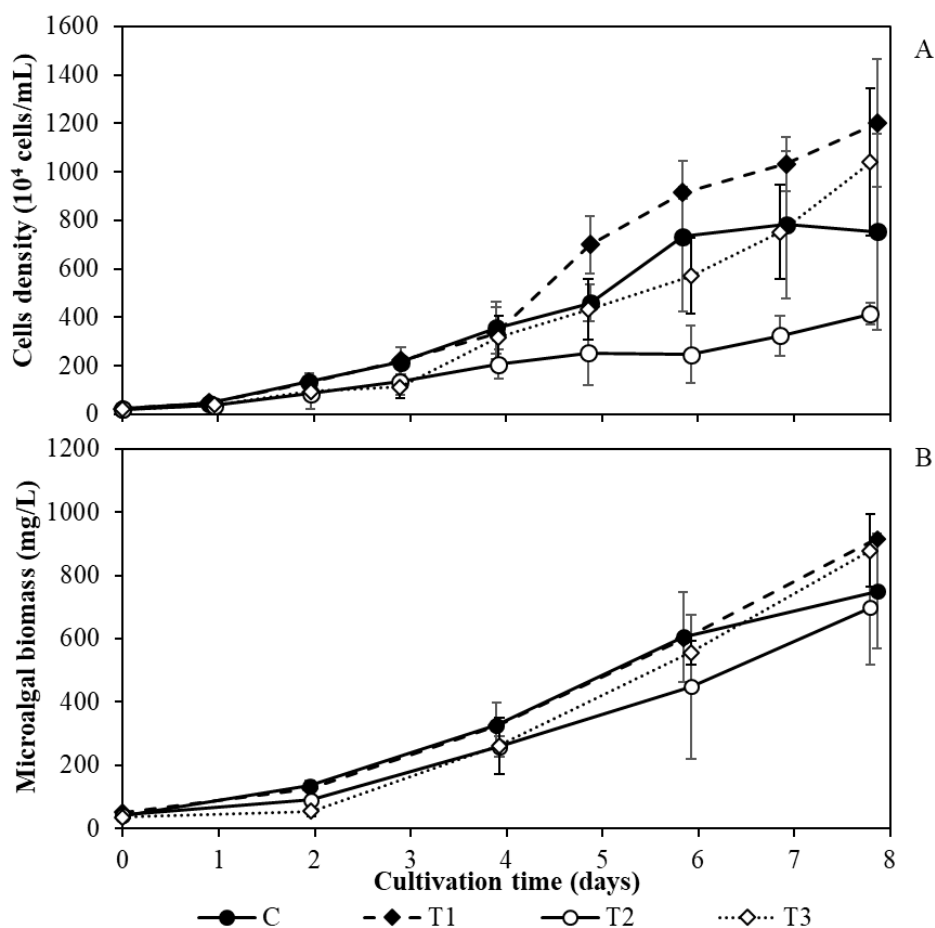


Figure 4.5. (A) Cell number density and (B) Microalgal biomass concentration of *Scenedesmus armatus* during the cultivation in 1-L bubble column photobioreactors: C using ambient air and BG-11 media, T1 using outlet air from fish tank and BG-11 media, T2 using ambient air and BG-11 media without NaHCO_3 and T3 using outlet air from fish tank and BG-11 media without Na_2CO_3 .

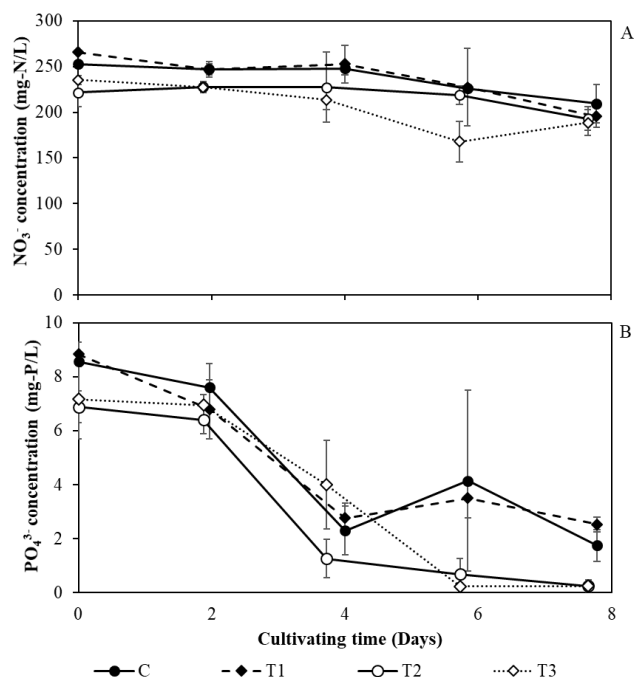


Figure 4.6. Profiles of (A) nitrate and (B) phosphate during the cultivation of *Scenedesmus armatus* in 1-L bubble columns: C using ambient air and BG-11 media, T1 using air from fish tank and BG-11 media, T2 using ambient air and BG-11 media without Na₂CO₃ and T3 using air from fish tank and BG-11 media without Na₂CO₃.

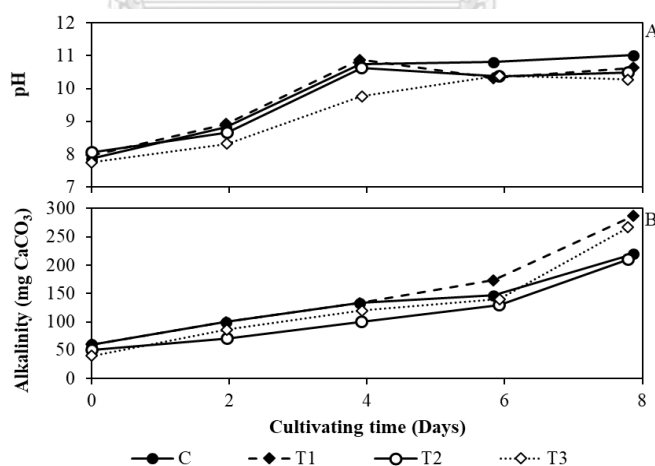


Figure 4.7. Profiles of (A) pH and (B) alkalinity during the cultivation of *Scenedesmus armatus* in 1-L bubble column: C using ambient air and BG-11 media, T1 using air from fish tank and BG-11 media, T2 using ambient air and BG-11 media without Na₂CO₃ and T3 using air from fish tank and BG-11 media without Na₂CO₃.

4.2.3 Pigments content of *Scenedesmus armatus*

Biomass of *S. armatus* were analyzed for chlorophyll and carotenoids contents after completing the cultivation (Table 4.3). T3, which was supplied with air from fish tank and BG-11 media without NaHCO₃, produced the highest contents of chlorophyll, total carotenoids and lutein although their magnitudes were not significant difference ($p > 0.05$) as compared to those from other treatments. Like the results of previous section, beta-carotene and astaxanthin was non-detectable, and lutein was still the major carotenoid (42% to 51%), while one unidentified peak (i.e., unidentified carotenoid 1 in Table 4.2) accounted for significant fraction of total carotenoids available.

Table 4.3. Pigment contents in *Scenedesmus armatus* at the end of cultivation on day 8.

Pigment contents	Treatment			
	C	T1	T2	T3
Chlorophyll (mg/g)	4.25 ± 1.80 ^a	3.57 ± 0.02 ^a	6.05 ± 4.07 ^a	7.23 ± 1.66 ^a
Total carotenoids (mg/g)	1.58 ± 0.54 ^a	1.57 ± 0.44 ^a	1.41 ± 0.31 ^a	2.17 ± 0.41 ^a
Lutein (mg/g)	0.81 ± 0.21 ^a (51.27%)	0.67 ± 0.06 ^a (42.68%)	0.67 ± 0.03 ^a (47.52%)	0.94 ± 0.14 ^a (43.32%)
Beta-carotene	ND	ND	ND	ND
Astaxanthin	ND	ND	ND	ND
Unidentified carotenoid	(48.73%)	(57.32%)	(52.48%)	(56.68%)

Different letters within the same row indicate statistically significant difference ($p < 0.05$)

ND, is non-detect.

4.2.4 Water quality in fish cultivation tank

Fish tank was connected to the solid separating unit, which was employed to remove suspended solids and carry out nitrification. Tilapia with initial fish biomass of 3.09 kg/m³ were cultivated in fish tank for 8 days to attain the final fish biomass of 3.43 kg/m³. Flow rate of water between fish tank and solid separating unit was maintained at approximately 4,500 L/day so that it was reasonable to assume that the well-mixed condition was established in the recirculating aquaculture system. The average DO concentration, pH, temperature and alkalinity were comparable ($p > 0.05$) between fish tank and solid separating unit (Table 4.4), hence confirming good mixing assumption, and were within acceptable ranges for tilapia cultivation [10]. Dissolved carbon dioxide concentration in fish tank was greater than that of the solid separating unit ($p < 0.05$), perhaps due to more aeration and mixing that stripped carbon dioxide out of water. It should be pointed out that water used during this cultivation was from fish storage tank, where nitrification was already established. Nitrate concentration of water in fish storage tank at the time of using was roughly 50 mg N/L. By the end of experiment, nitrate concentration in fish tank and solid separating unit increased slightly from 47.9 to 54.9 mg/L and from 50.3 to 55.2 mg N/L, respectively, while ammonium and nitrite were well below the acceptable level of 1.0 mg N/L (Fig. 4.8). The rise of nitrate with negligible ammonium and nitrite signaled the occurrence of nitrification in the fish cultivating system.

Table 4.4. DO concentration, pH, temperature and alkalinity of water in fish tank and solid separating unit during the 8-day cultivation of Nile tilapia.

Parameters	Fish tank	Solid separating unit	Recommended range (Timmons et al., 2002)
Dissolved oxygen (mg/L)	6.5 ± 0.8 ^a	6.7 ± 0.8 ^a	> 5.0 mg/L
pH	8.2 ± 0.2 ^a	8.3 ± 0.2 ^a	6.5 – 8.5
Temperature (°C)	30.6 ± 0.9 ^a	30.8 ± 1.0 ^a	28 – 32 °C
Alkalinity (mg CaCO ₃ /L)	111 ± 12 ^a	119 ± 7 ^a	50 – 300 mg CaCO ₃ /L
Dissolved carbon dioxide (mg/L)	1.6 ± 0.9 ^a	1.3 ± 0.5 ^b	< 60 mg/L

Different letters within the same row indicate statistically significant difference ($p < 0.05$)

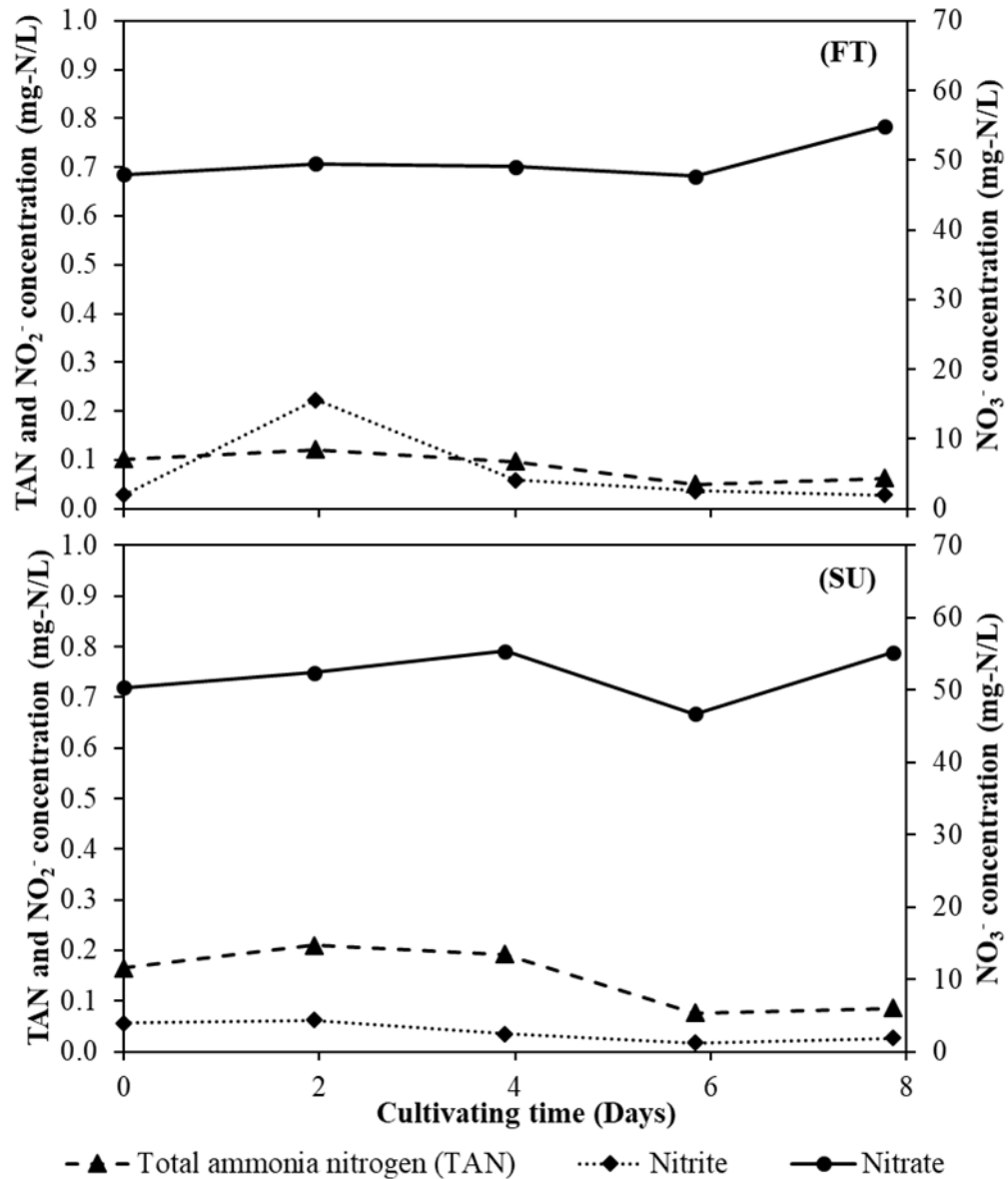


Figure 4.8. Ammonium, nitrite and nitrate concentration during the cultivation of Nile tilapia in recirculating aquaculture system consisting of fish tank (FT) and solid separating unit (SU).

4.3 Effect of fish biomass on carbon dioxide production and utilization of carbon dioxide and nitrate from recirculating aquaculture system for microalgal cultivation

4.3.1 Performance of recirculating aquaculture system

Recirculating aquaculture system consisted of fish tank to grow tilapia and solid separating unit to control suspended solids and inorganic nitrogen concentration within acceptable range. The average DO concentration, pH, temperature, alkalinity and dissolved carbon dioxide were within acceptable ranges for tilapia cultivation (Table 4.5). The average DO concentration in solid separating unit were greater than those in fish tank in each fish biomass tested. The possible explanation might be linked to the presence of fish and suspended solids in fish tank at the same time, whereas only suspended solids were present in the solid separating unit and aeration rate to solid separating unit was 4-times greater than that in fish tank.

Table 4.5. Dissolved oxygen concentration, pH, temperature and alkalinity water in fish tank (FT) and solid separating unit (SU) of the recirculating aquaculture system at different fish densities.

Physical property	3 kg/m ³		5 kg/m ³		10 kg/m ³	
	FT	SU	FT	SU	FT	SU
DO (mg/L)	6.6 ± 0.4	7.1 ± 0.3	6.6 ± 0.4*	7.2 ± 0.4*	6.3 ± 0.5	7.7 ± 0.6
pH	7.8 ± 0.2	7.9 ± 0.2	8.1 ± 0.2*	8.2 ± 0.1*	8.1 ± 0.1	8.1 ± 0.2
Temperature (°C)	30.5 ± 0.5	30.7 ± 0.5	29.6 ± 0.5*	29.3 ± 1.0*	30.3 ± 0.4	30.4 ± 0.5
Alkalinity (mg CaCO ₃ /L)	152 ± 11	158 ± 8	188 ± 11*	184 ± 20*	176 ± 17	172 ± 23
Dissolved carbon dioxide (mg/L)	5.5 ± 3.0	3.5 ± 1.3	2.9 ± 1.2*	2.5 ± 0.9*	3.1 ± 0.3	2.8 ± 1.2

*The results were obtained from 5 kg/m³ tilapia cultivation may be underestimated due to 1 fish was died on the 5th day of cultivation.

The solid separating unit was divided into 2 compartments. The first compartment was used primarily to retain suspended solids and to carry out nitrification while the second compartment was employed for additional filtration. However, significant solid clogging occurred in the second compartment shortly after starting the operation that eventually led to the failure of water pumping equipment. As a result, filters (pored size 150 μm) in the second compartment was removed to allow water to flow more easily from the second compartment back to fish tank. The average suspended solid concentration in fish tank after the system modification were 64 ± 20 , 69 ± 22 and 79 ± 24 mg SS/L for fish stocking at 3, 5 and 10 kg/m^3 , respectively. The average suspended solid concentration was within the acceptable level for aquacultures although there were fluctuations occurred occasionally that reached as high as 106 mg SS/L (Fig 4.9). This was caused by clogging on stainless steel screen separating the first and second compartments, causing liquid overflow from the first to second compartments. At the end of tilapia cultivation, the total amount of solids in the solid separating unit was measured to determine the percentage of solids retention, and it was clear that the majority of solids (84.54% for 3 kg/m^3 , 85.88% for 5 kg/m^3 and 77.51% kg/m^3) in the recirculating aquaculture system was kept inside the solid separating unit (Fig. 4.10). The performance of solid separating unit in this work was comparable with previous work employing other types of solid-liquid separator such as inclined plate (100 μm pored size), cone-shape column equipped with stainless steel screen (100 μm pored size) or upflow clarifier [30, 35, 52].

Fig. 4.11 illustrates the profiles ammonium, nitrite and nitrate in fish tank and solid separating unit for each tilapia biomass. The solid separating unit as nitrifying unit for the recirculating aquaculture system was able to maintain ammonium and nitrite in fish tanks below 0.8 mg N/L for each fish biomass considered. Ammonium and nitrite concentration were less than the acceptable range recommended for tilapia cultivation [10]. Nitrate was the major inorganic nitrogen compound detected in fish tank and solid separating unit, measured at 37.7 ± 1.74 and 40.1 ± 2.28 mg N/L for 3 kg/m^3 , 33.2 ± 2.32 and 35.7 ± 3.95 mg N/L for 5 kg/m^3 and 42.1 ± 1.60 and 45.3 ± 2.61 mg N/L for 10 kg/m^3 , respectively. Relatively constant nitrate concentration in fish tank was an unexpected as nitrate was the product of

nitrification. Thus, it was possible that denitrification was responsible for nitrate reduction in the system. Although DO concentration in the recirculating aquaculture system indicated aerobic condition, establishment of anaerobic environment was possible, especially within biofilm layer on tank wall and in the thick solid sediment on tank floor (Fig. 4.12). Measurement of oxygen concentration in sediment layer indicated dissolved oxygen concentration less than 1.0 mg/L. Previous works also reported the occurrence of denitrification when excessive organic solids were present in nitrifying biofilter unit [73, 74].

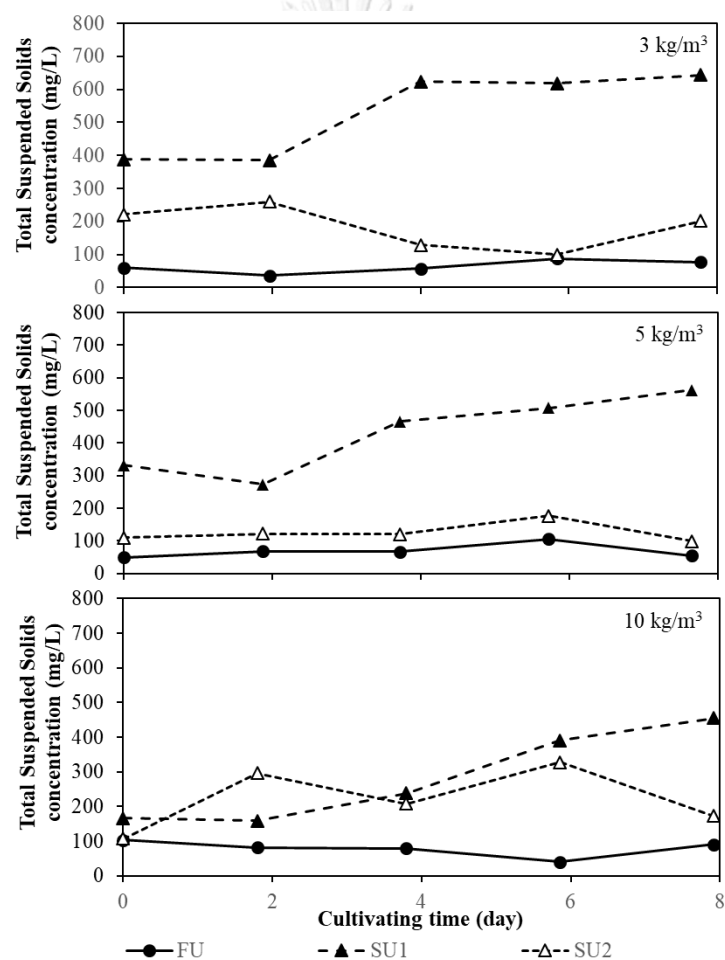


Figure 4.9. Suspended solid concentration in recirculating aquaculture system at different fish stocking densities: FT is fish tank; SU1 is the first chamber of solid separating and SU2 is the second chamber of solid separating unit. Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

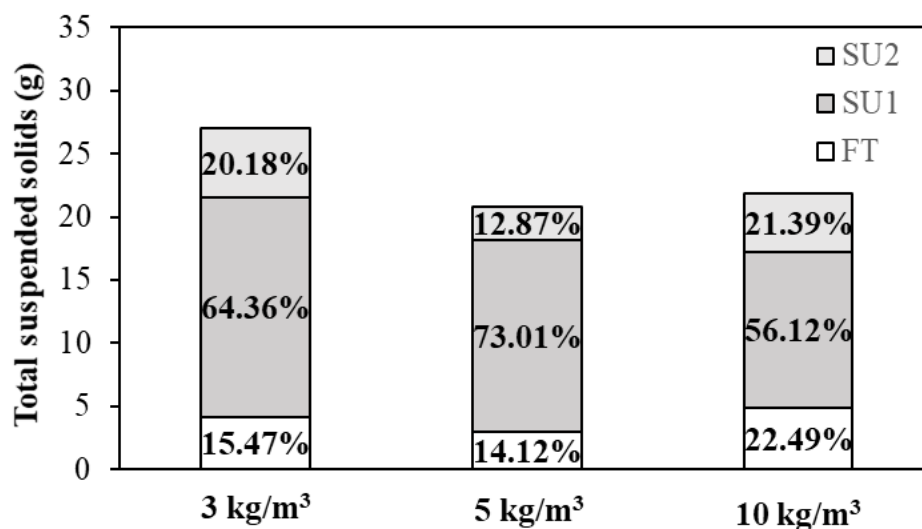


Figure 4.10. Distribution of solids in recirculating aquaculture system: FT is fish tank, SU1 is the first chamber of solid separating unit and SU2 is the second chamber of solid separating unit. Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

Table 4.6. Growth data for tilapia cultivation in recirculating aquaculture system at different fish stocking densities.

Parameter	3 kg/m ³		5 kg/m ³		10 kg/m ³	
	Day 0	Day 8	Day 0	Day 8	Day 0	Day 8
Fish biomass (kg/m ³)	3.2	3.6	5.1*	5.4	10.5	11.8
Average weight (g)	34.4 ± 6.8	38.6 ± 6.8	39.2 ± 6.8*	41.6 ± 6.8*	47.4 ± 6.8	53.0 ± 6.8
Average length (cm)	12.7 ± 6.8	12.8 ± 6.8	13.1 ± 6.8*	13.2 ± 6.8*	13.8 ± 6.8	13.9 ± 6.8
Feeding rate (g/day)	5.15		8.24*		17.05	
Survival rate (%)	100		85.71*		100	
Average daily weight gain (g/day)	0.53		0.29*		0.70	
Feed conversion ratio (FCR)	1.95		4.02*		2.02	

*Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

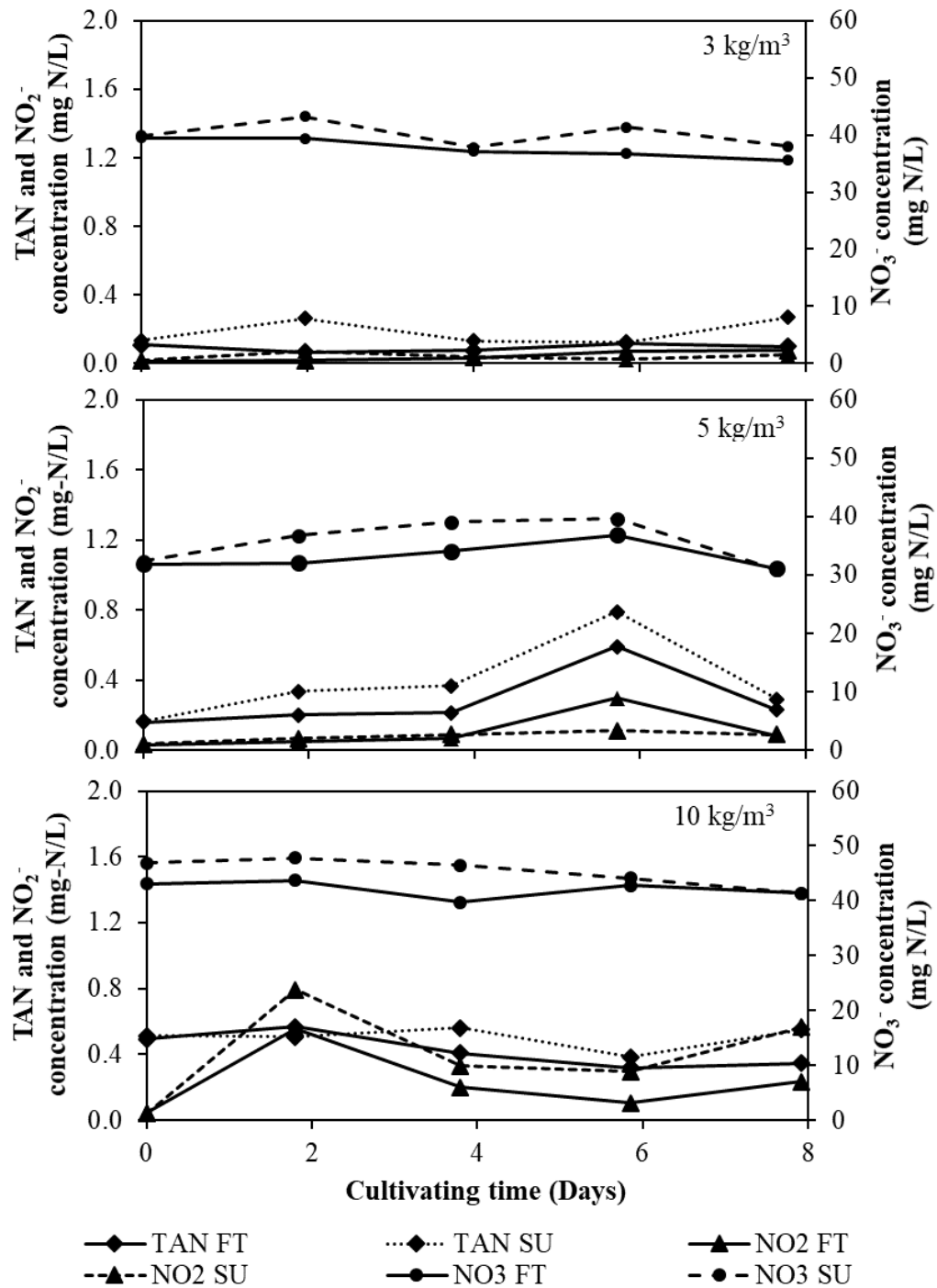


Figure 4.11. Concentration of ammonium, nitrite and nitrate in fish tank (FT) and solid separating unit (SU) during the cultivation of Nile tilapia at fish biomass about 3, 5 and 10 kg/m³. Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.



Figure 4.12. Solid sediments on tank floor of solid separating unit and biofilm formation

Total survival (i.e., 100%) was achieved for fish biomass at 3 and 10 kg/m³. The survival rate was 85% when fish biomass was 5 kg/m³ (Table 4.6). Lower survival rate in this treatment was perhaps due to the presence of weak or sick tilapia among the population that showed signs of lower immune system such as no eating and eventually died couple days later. Nonetheless, it was possible to assume that the length of cultivation (i.e., 8 days) did not significantly affect the rate of carbon dioxide and inorganic nitrogen waste production by fish as the average weight, length and fish biomass only increased slightly. The average daily weight gain in this work (0.29 to 0.70 g/day) was lower than the results from other recirculating systems using nitrifying biofilter that reported daily weight gain ranged from 0.82 to 3.35 g/day [73, 75-77]. However, it should be pointed out that the cultivating period in this work was only 8 days and the total wastes accumulated in the system should be significantly less than other works, which performed the cultivation as long as 1 to 4 months [73, 75-77].

4.3.2 Carbon dioxide production from recirculating aquaculture system

Fig. 4.13 summarizes the average carbon dioxide concentration at inlets of recirculating aquaculture system and outlets of fish tank and solid separating unit. Inlet carbon dioxide concentration were comparable ($p > 0.05$) for all fish biomass, ranged from 537 to 591 ppm, which were similar to the values of carbon dioxide in ambient air. Increasing carbon dioxide concentration in the outlet of fish tank was observed after increasing fish biomass from 3 to 10 kg/m³. This resulted in the highest carbon dioxide concentration (1,618 ± 398 ppm) in the outlet of fish tank. Similar observation was noted for the outlet of solid separating unit, with the highest carbon dioxide concentration at 915 ± 88 ppm when fish biomass was maintained at 10 kg/m³. Similar to the results in section 4.2.1, carbon dioxide concentration in water exceeded its soluble limitation, implying that excess carbon dioxide should be transported across the air-water interface into the headspace (Table 4.7)

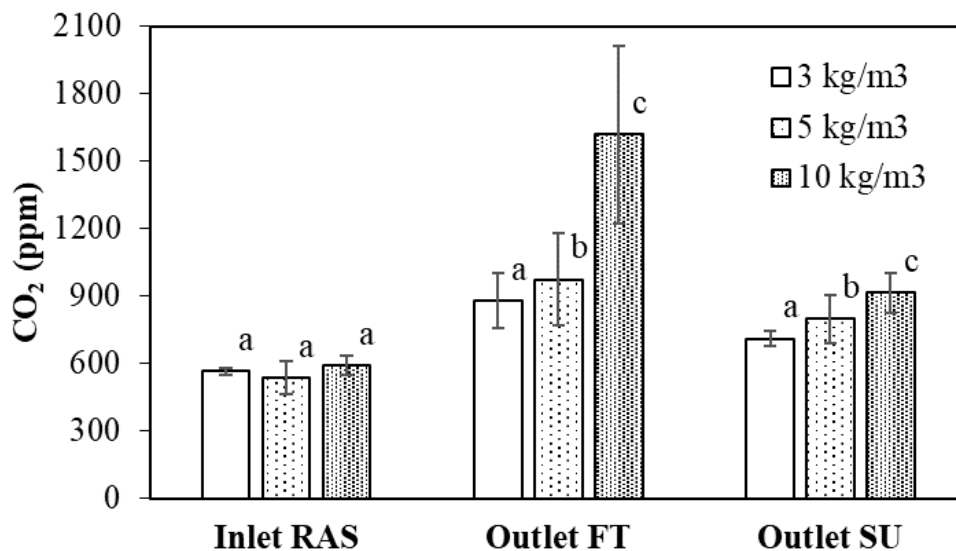


Figure 4.13. Average carbon dioxide concentration at the inlets of recirculating aquaculture system and at the outlets of fish tank (FT) and solid separating unit (SU) at different fish biomass. Different letters within each group of the x-axis indicates statistically significant difference ($p < 0.05$). Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

Table 4.7. Dissolved carbon dioxide concentration and solubility of carbon dioxide in water in the recirculating aquaculture system.

Water in each compartment of recirculating aquaculture system	Solubility of CO ₂ (mol/L) [Calculated from Henry's law]	Dissolved CO ₂ (mol/L) [Calculated total alkalinity and pH]
Fish tank at 3 kg/m ³	3.0×10^{-5}	12.5×10^{-5}
Solid separating unit at 3 kg/m ³	2.4×10^{-5}	8.0×10^{-5}
Fish tank at 5 kg/m ³	3.3×10^{-5}	6.6×10^{-5}
Solid separating unit at 5 kg/m ³	2.7×10^{-5}	5.8×10^{-5}
Fish tank at 10 kg/m ³	5.5×10^{-5}	7.0×10^{-5}
Solid separating unit at 10 kg/m ³	3.1×10^{-5}	6.3×10^{-5}

Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

Figure 4.14(A) illustrate the profile of carbon dioxide concentration at the inlets and outlets of recirculating aquaculture system for 3 kg/m³ fish biomass. Relatively constant carbon dioxide concentration at the inlets were measured at 565 ± 14.7 ppm. Carbon dioxide concentration at the outlets of fish tank fluctuated substantially depending on fish feeding process despite maintaining constant outlet flow rate. Carbon dioxide concentration increases rapidly after feeding, reaching the maximum and then gradually retreating back to the initial concentration. The volumetric flow rate of inlet air was maintained at 2.4 L/min throughout the cultivation, leading to 27.43 g of carbon dioxide into the system during the cultivation (i.e. white color in Fig 4.14(B)). As a result of feeding, the total amount of carbon dioxide gained was the sum of area under the outlet concentration from the point of feeding to the next one (i.e., black area), which was determined at 3.83 g, whereas the total carbon dioxide production from the basic respiration (11.47 g) was the gray area, which is the difference between carbon dioxide gained from feeding and based line from inlets. For the solid separating unit, the total carbon dioxide production from microorganism activities was 28.95 g as compared to 114.28 g of carbon dioxide entering the system during the cultivation (Fig 4.14(C)). Profiles of carbon dioxide concentration associated with the remaining fish stocking densities of 5 and 10 kg/m³

are displayed in Fig. 4.15 and 4.16, and the total carbon dioxide production was summarized into Table 4.8. It should be pointed out that the total carbon dioxide gained from feeding when fish biomass was maintained approximately 10 kg/m^3 was significantly higher than the amount from other fish stocking densities, and this is directly linked to significantly higher carbon dioxide concentration in the outlet. The times required for carbon dioxide concentration in the outlet of fish tank to reach the maximum were comparable in all fish biomass, with the average of 5.5 ± 3.25 hours after feeding began. The plastic lid of fish tank must be opened daily to the atmosphere for about 15 to 45 minutes to accommodate fish feeding and liquid sampling and measurement. As a result, carbon dioxide concentration at the outlet of fish tank decreased dramatically during this period, and this decrease became clearer as the fish biomass increased. In addition, the total amounts of carbon dioxide gained from microbial activities in solid separating unit were 28.95, 52.73 and 64.63 g for fish biomass of 3, 5 and 10 kg/m^3 , respectively (Table 4.8). This excess carbon dioxide was approximately 2 to 3 folds greater than the amounts gained from feeding and basic fish respiration. Higher carbon dioxide production associated with solid separating unit was due to maintaining higher volumetric flow rates.



Table 4.8. Total mass of carbon dioxide during tilapia cultivation in recirculating aquaculture system at different stocking densities

Parameter	Tilapia biomass		
	3 kg/m ³	5 kg/m ³	10 kg/m ³
CO ₂ from inlet of fish tank (g)	27.43	26.02*	28.34
CO ₂ gained from basic respiration (g)	11.47	15.88*	36.73
CO ₂ gained from fish feeding (g)	3.83	5.30*	9.50
Rate of CO ₂ production from fish tank (g CO ₂ /day·kg feed)	378.37 ± 119 ^a	335.94 ± 113 ^{a*}	377.31 ± 110 ^a
CO ₂ from inlet of solid separating unit (g)	114.28	108.42*	118.10
CO ₂ gained from microbial activities in solid separating unit (g)	28.95	52.73*	64.63
Rate of CO ₂ production from solid separating unit (g CO ₂ /day·kg feed)	729.9 ± 205 ^a	834.3 ± 107 ^{a*}	1004.3 ± 286 ^a
Cultivating time (hr)	187	184	185
Rate of total CO ₂ production from RAS (g CO ₂ /day·kg feed)	1,108.3	1,170.2*	1,381.6

Different letters within the same row indicate statistically significant difference ($p < 0.05$)

Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

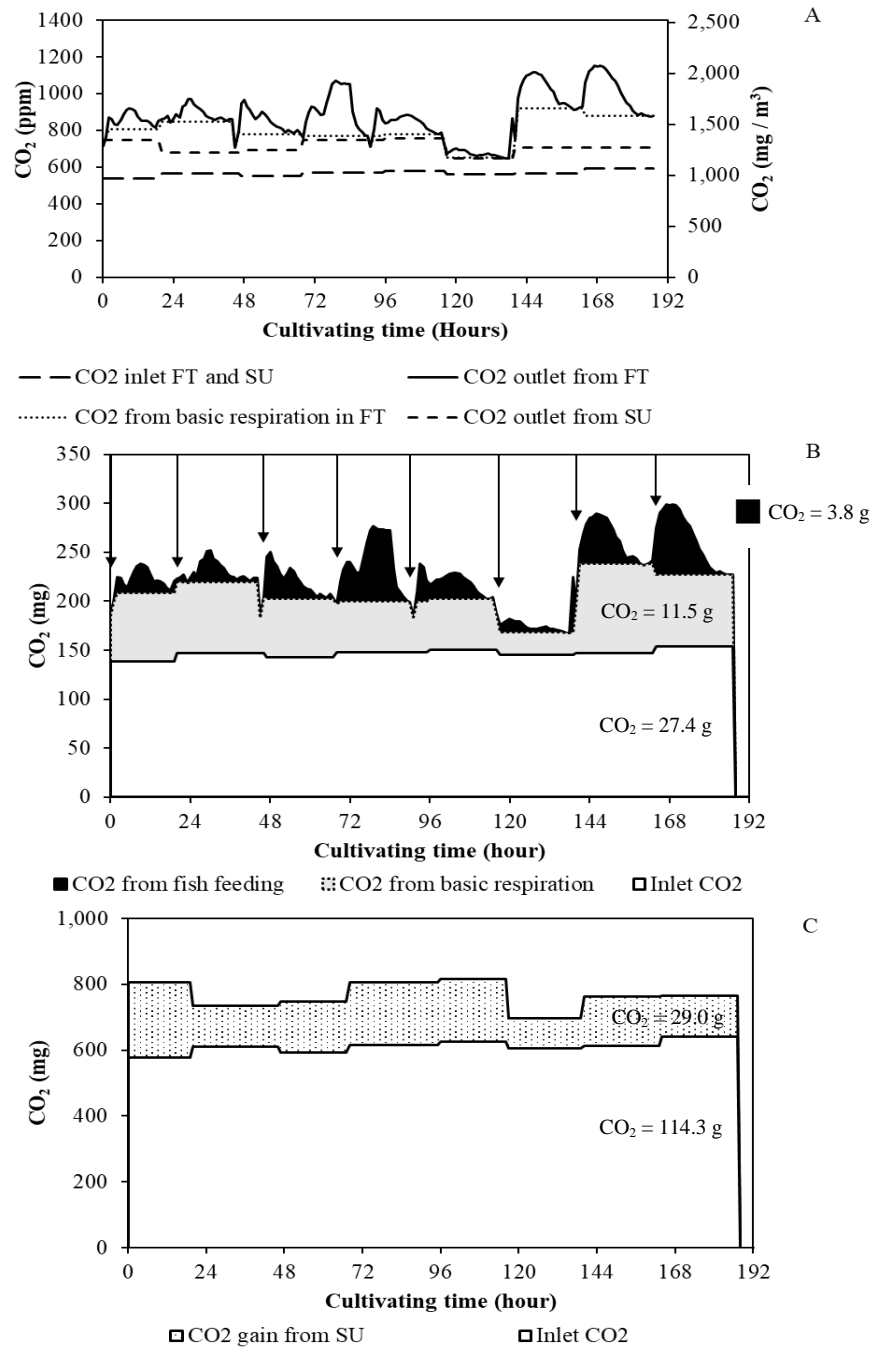


Figure 4.14. (A) carbon dioxide concentration from the inlets of recirculating system and the outlets from fish tank (FT) and solid separating unit (SU); (B) total mass of carbon dioxide from inlet air of FT, carbon dioxide gained from basic respiration and carbon dioxide gained from feeding; and (C) total mass of carbon dioxide from inlet air of SU and carbon dioxide gained from microbial activities in SU during fish cultivation at 3 kg/m³ (Arrows indicate fish feeding).

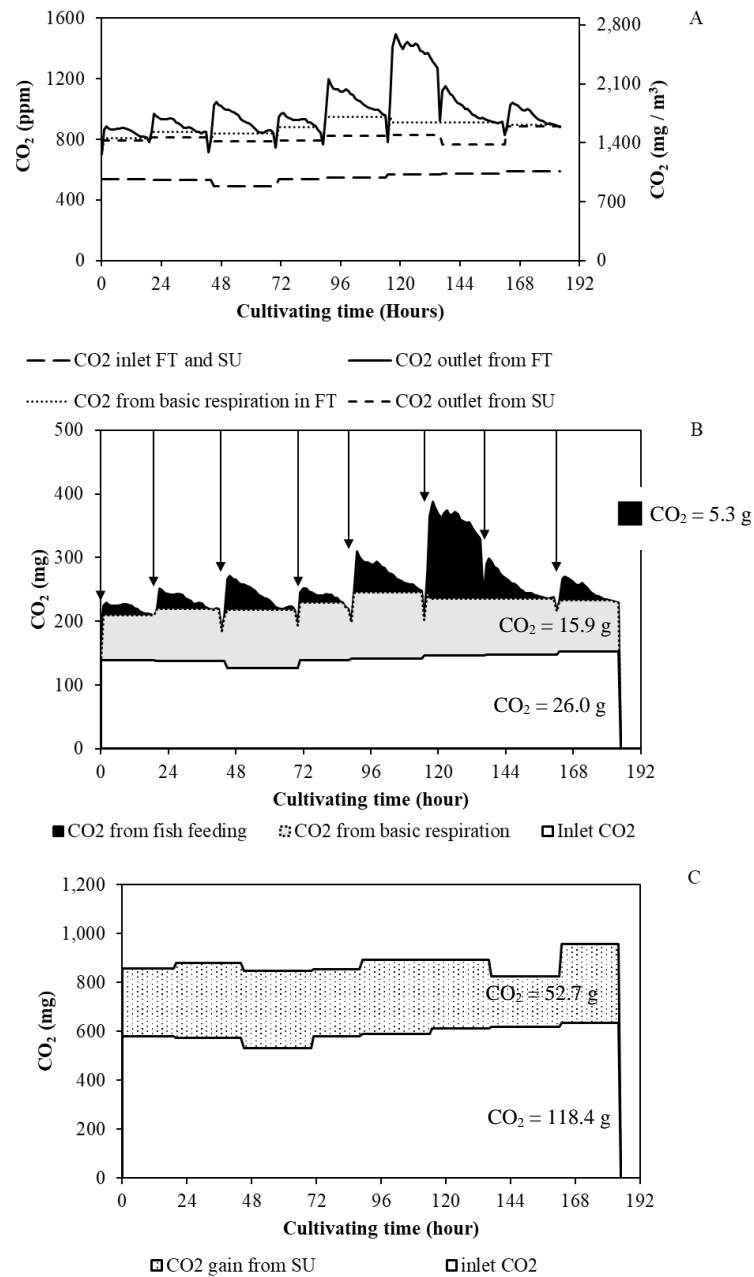


Figure 4.15. (A) carbon dioxide concentration from the inlets of recirculating system and the outlets from fish tank (FT) and solid separating unit (SU); (B) total mass of carbon dioxide from inlet air of FT, carbon dioxide gained from basic respiration and carbon dioxide gained from feeding; and (C) total mass of carbon dioxide from inlet air of SU and carbon dioxide gained from microbial activities in SU during fish cultivation at 5 kg/m³ (Arrows indicate fish feeding)

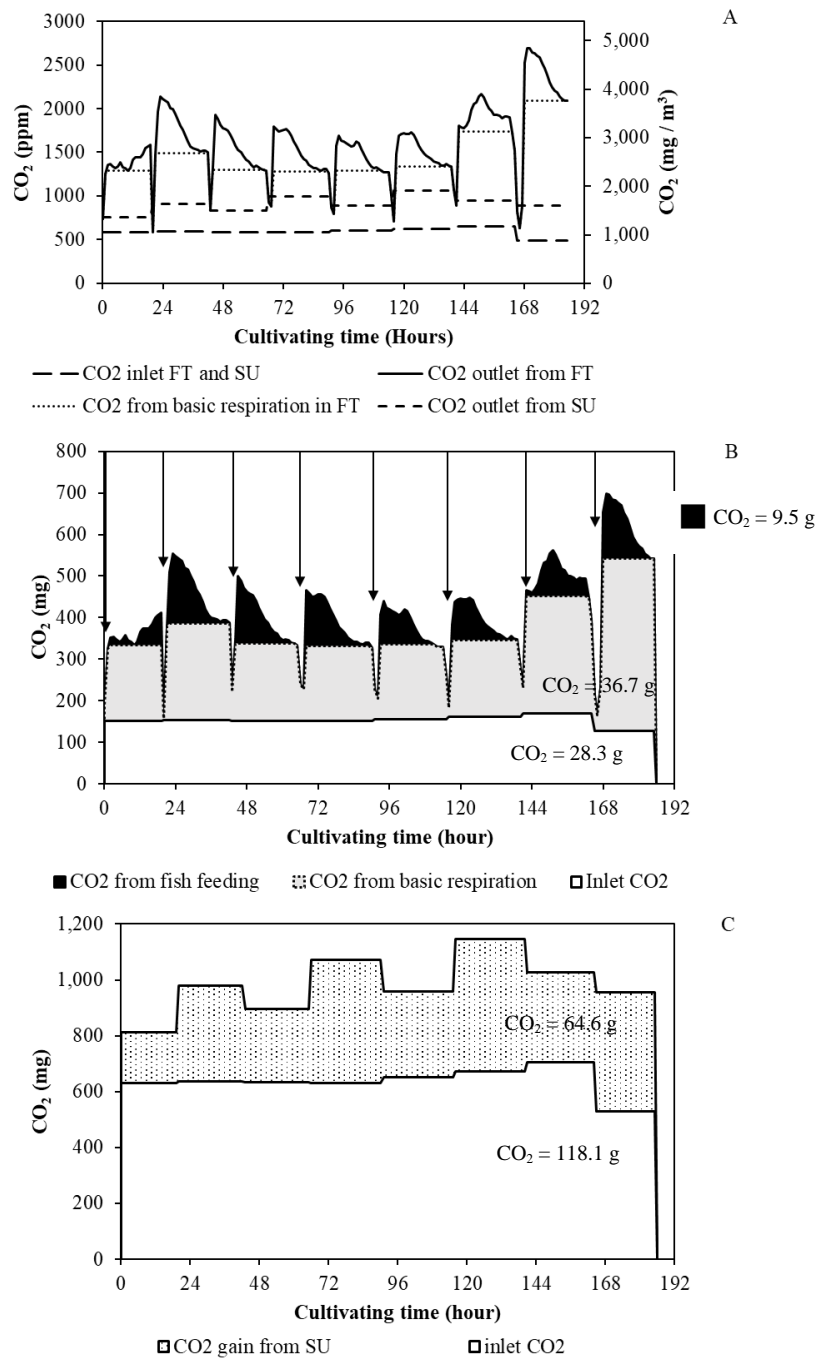


Figure 4.16. (A) carbon dioxide concentration from the inlets of recirculating system and the outlets from fish tank (FT) and solid separating unit (SU); (B) total mass of carbon dioxide from inlet air of FT, carbon dioxide gained from basic respiration and carbon dioxide gained from feeding; and (C) total mass of carbon dioxide from inlet air of SU and carbon dioxide gained from microbial activities in SU during fish cultivation at 10 kg/m³ (Arrows indicate fish feeding)

4.4 Utilization of carbon dioxide and nitrate from recirculating aquaculture system for microalgal cultivation

4.4.1 Growth of *Scenedesmus armatus*

Fig 4.17 displays cell number density from the cultivation of *S. armatus* in 1-L bubble column photobioreactors using nitrate-rich effluent (41.6 ± 0.0 mg N/L) from the solid separating unit and outlet air from fish tank containing elevated carbon dioxide concentration (881 ± 123.7 ppm) when fish biomass was maintained approximately 3 kg/m^3 . This condition yielded the maximum cell number density of 2.53×10^6 cells/mL and maximum specific growth rate of 0.89 d^{-1} , which were insignificant difference ($p > 0.05$) to the results from microalgal cultivation using the same effluent and ambient air. Tilapia cultivation at 5 kg/m^3 fish biomass yielded the outlet carbon dioxide concentration from fish tank at 973 ± 204.2 ppm. This air was used to cultivate *S. armatus* along with effluent containing nitrate (35.0 ± 2.1 mg N/L) from the solid separating unit, and consequently yielded the maximum specific growth rate (1.33 d^{-1}) and maximum cell number density (5.07×10^6 cells/mL) that were comparable ($p > 0.05$) to those using the same effluent and ambient air.

Unlike earlier results associated with lower fish biomass, tilapia cultivation at 10 kg/m^3 produced the outlet air containing elevated carbon dioxide concentration ($1,652 \pm 269.9$ ppm) and nitrate-rich effluent (46.1 ± 1.2 mg N/L) from the solid separating unit. Cultivation of *S. armatus* using the described carbon dioxide and nitrate-rich effluent resulted in the maximum cell number density of 5.53×10^6 cells/mL, which was approximately 2-folds higher the results (2.72×10^6 cells/mL) using ambient air. In as compared to the cultivation using ambient air although the maximum specific growth rates from both treatments were similar (i.e., 1.83 day^{-1} for the cultivation with outlet air from fish tank and 1.66 day^{-1} for the cultivation using ambient air). Clearly, tilapia biomass in fish tank must be maintained at least 10 kg/m^3 in order to produce sufficient carbon dioxide to enhance cell growth. However, the stationary phase was established after 2 or 3 days into the cultivation, whereas it required longer period (i.e., 8 days) for the cultivation using BG-11 growth media or nitrifying biofilter effluent from commercial farm. It was speculated that the effluent

from solid separating unit might lack other important nutrients or trace elements essential for growth as tilapia cultivating period lasted only 8 days. Calculation of nitrogen to phosphorus ratio indicated that it was lower than the required ratio for microalgal biomass.

Similar results were obtained for biomass concentration (Fig. 4.18). Final biomass concentrations were 238 ± 13.4 , 354 ± 83.7 and 410 ± 22.3 mg/L for the cultivation using nitrate from solid separating unit and carbon dioxide from outlet air from fish tank at 3, 5 and 10 kg/m³, respectively. Microalgal cultivation subjected to the same effluent and ambient air produced biomass concentrations at 219 ± 27.6 , 349 ± 41.7 and 234 ± 54.6 mg/L when maintaining fish biomass closed to 3, 5, and 10 kg/m³, respectively. Clearly, the cultivation of *S. armatus* using outlet air from fish tank at 10 kg/m³ was preferred since the described condition produced the highest carbon dioxide concentration and yielded the highest biomass concentration and cell number density.

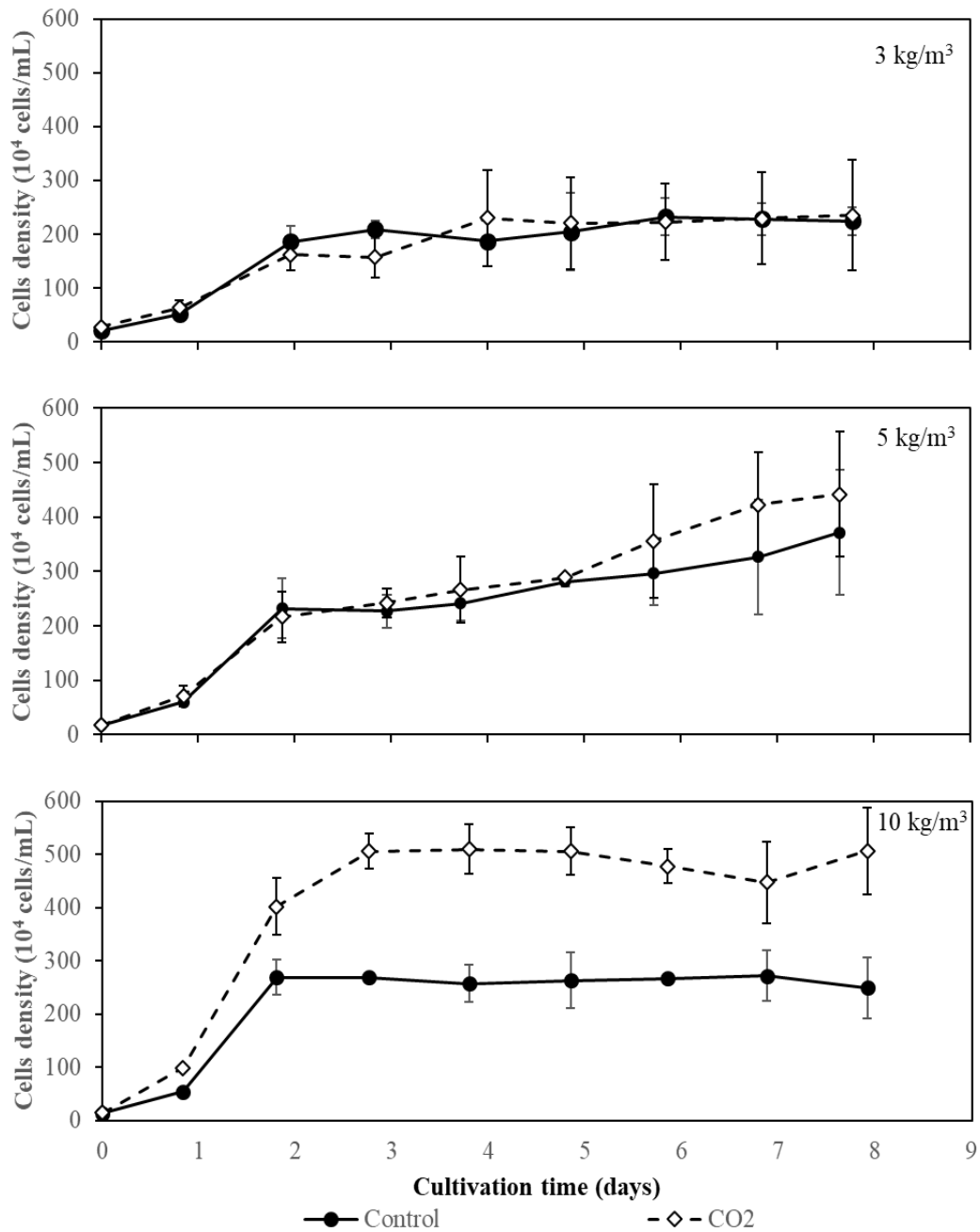


Figure 4.17. Cell number density during the cultivation of *Scenedesmus armatus* using the effluent from solid separating unit and outlet air from fish tank at different tilapia biomass. Control represents the cultivation using ambient air while CO₂ represents the cultivation using outlet air from fish tank. Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

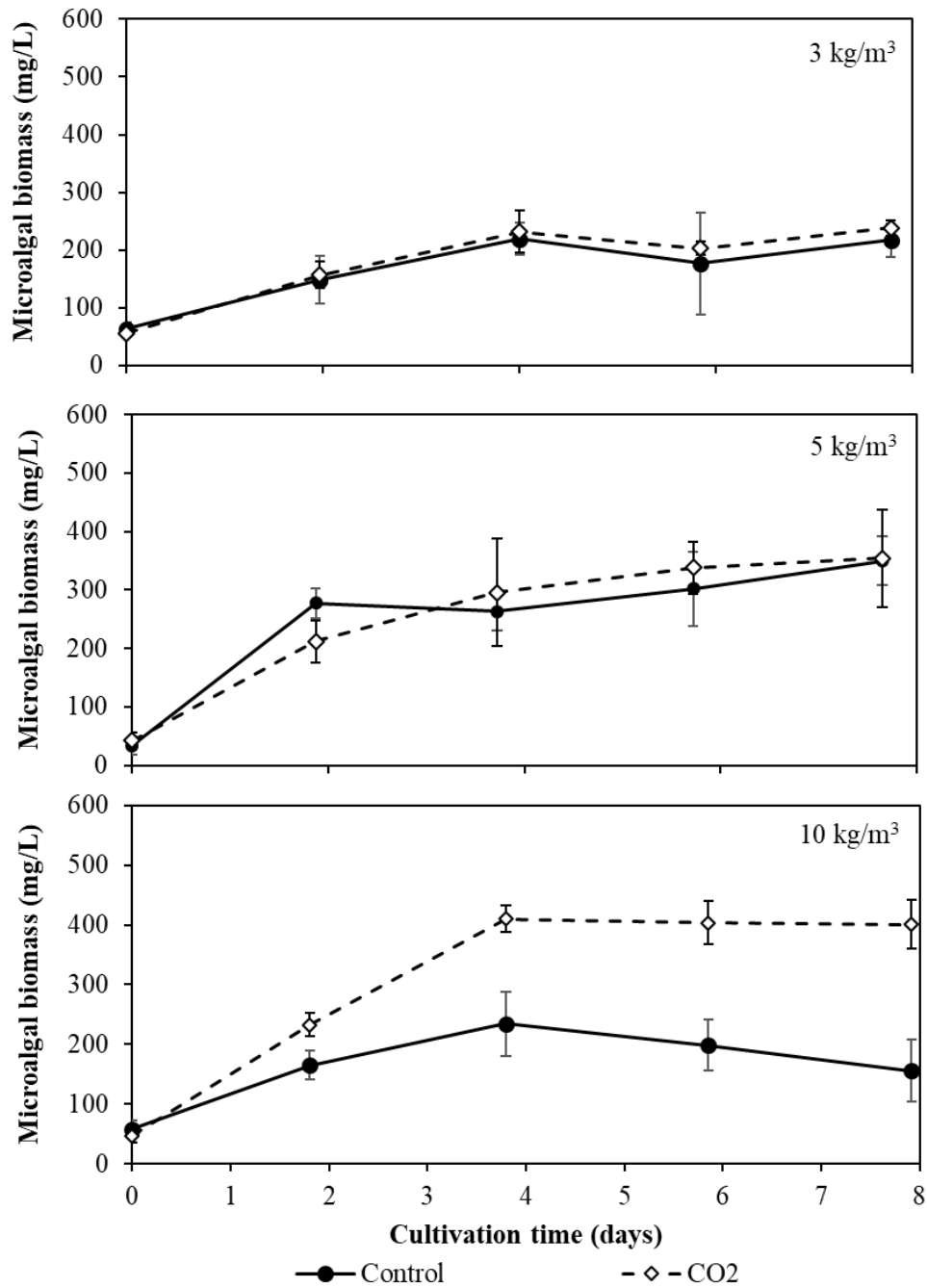


Figure 4.18. Biomass concentration during the cultivation of *Scenedesmus armatus* using the effluent from solid separating unit and outlet air from fish tank at different tilapia biomass. Control represents algal cultivation using ambient air while CO₂ represents algal cultivation using outlet air from fish tank. Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

4.4.2 Nutrients utilization during algal cultivation

Profiles of nitrate in photobioreactors are displayed in Fig. 4.19 for different fish biomass. Microalgal cultivation using the effluent from 3 kg/m³ fish tank reduced nitrate from 41.6 mg N/L to 28.2 mg N/L and to 24.8 mg N/L for the cultivation using ambient air and outlet air from fish tank, respectively. The amounts of nitrate consumed by microalgae were not significantly different ($p > 0.05$) between both treatments. For fish biomass of 5 kg/m³, microalgal cultivation lowered nitrate concentration from 36.4 mg N/L to 6.3 mg N/L and to 5.4 mg N/L for the cultivation using ambient air and outlet air from fish tank, respectively. Statistical test gave similar outcome that nitrate consumption was insignificantly different ($p > 0.05$) between both treatments. In contrast, the cultivation using outlet air from 10 kg/m³ fish tank decreased nitrate concentration in the effluent from 47.3 to 11.14 mg N/L, more than the cultivation using ambient air that reported the final nitrate concentration at 30 mg N/L. Clearly, the most effective nitrate utilization was associated with fish cultivation that yielded the highest carbon dioxide and highest microalgal growth. Similar observation was noted by Habibi et al. (2019) that high nitrate consumption in *Scenedesmus* sp. tended to associate with the cultures with highest growth that could be accelerated by supplying elevated carbon dioxide concentration. Other inorganic nitrogen (i.e., TAN and nitrite) were relatively constant less than 1.0 mg N/L throughout the cultivation (Table 4.9).

Profiles of phosphate concentration in photobioreactors are illustrated in Fig. 4.20. Decreasing phosphate concentration was observed in all fish biomass. For fish biomass at 3 kg/m³, phosphate concentration in photobioreactors were reduced from the initial to final values measured at 3.14 and 4.31 mg P/L for the treatments using ambient air and outlet air, respectively. For fish biomass of 5 kg/m³, similar phosphate profiles were observed between both treatments, with the final phosphate concentration measured at 3.26 and 3.24 mg P/L for the treatment using ambient air and outlet air from fish tank, respectively. Final phosphate concentration associated with 10 kg/m³ fish biomass were 2.59 mg P/L for the cultivation using ambient air and 2.85 mg P/L for the cultivation supplying with air from fish tank. For this particular fish biomass, phosphate consumption by microalgae supplied with air from fish tank was clearly greater than the cultivation with ambient air. This observation

differed from the lower fish biomass that saw similar phosphate consumption between both treatments.

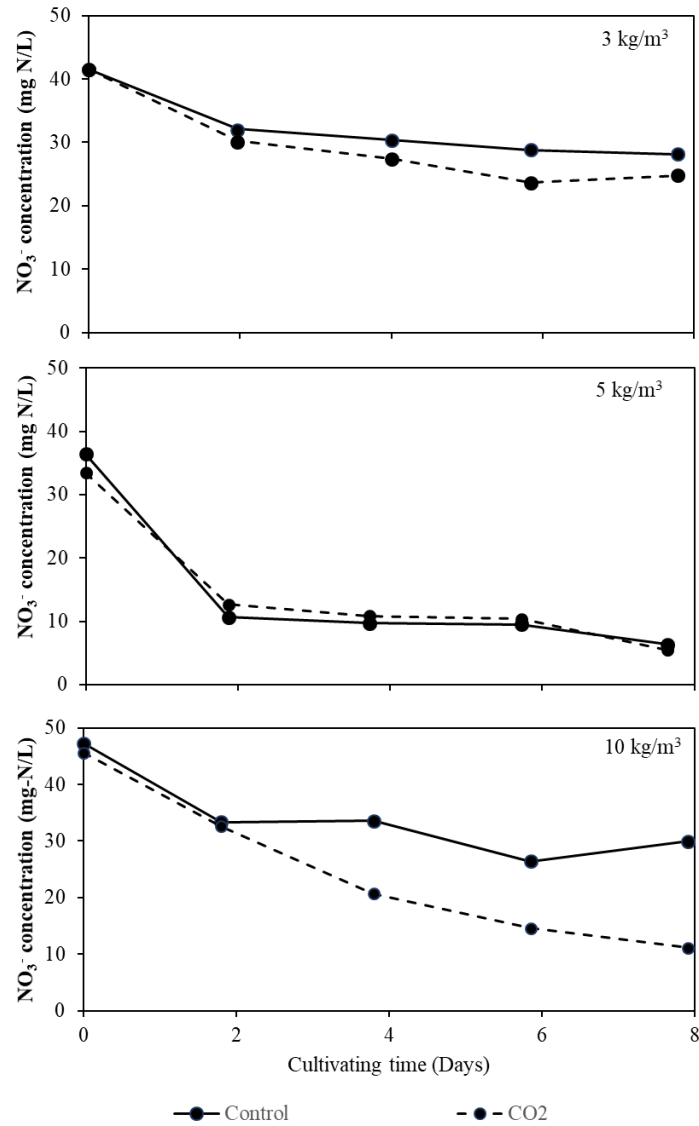


Figure 4.19. Nitrate concentration during the cultivation of *Scenedesmus armatus* using nitrate from the effluent of solid separating unit and carbon dioxide from the ambient air or outlet air from fish tank at different tilapia biomass. Control represents microalgal cultivation using ambient air while CO₂ represents microalgal cultivation using outlet air from fish tank. Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

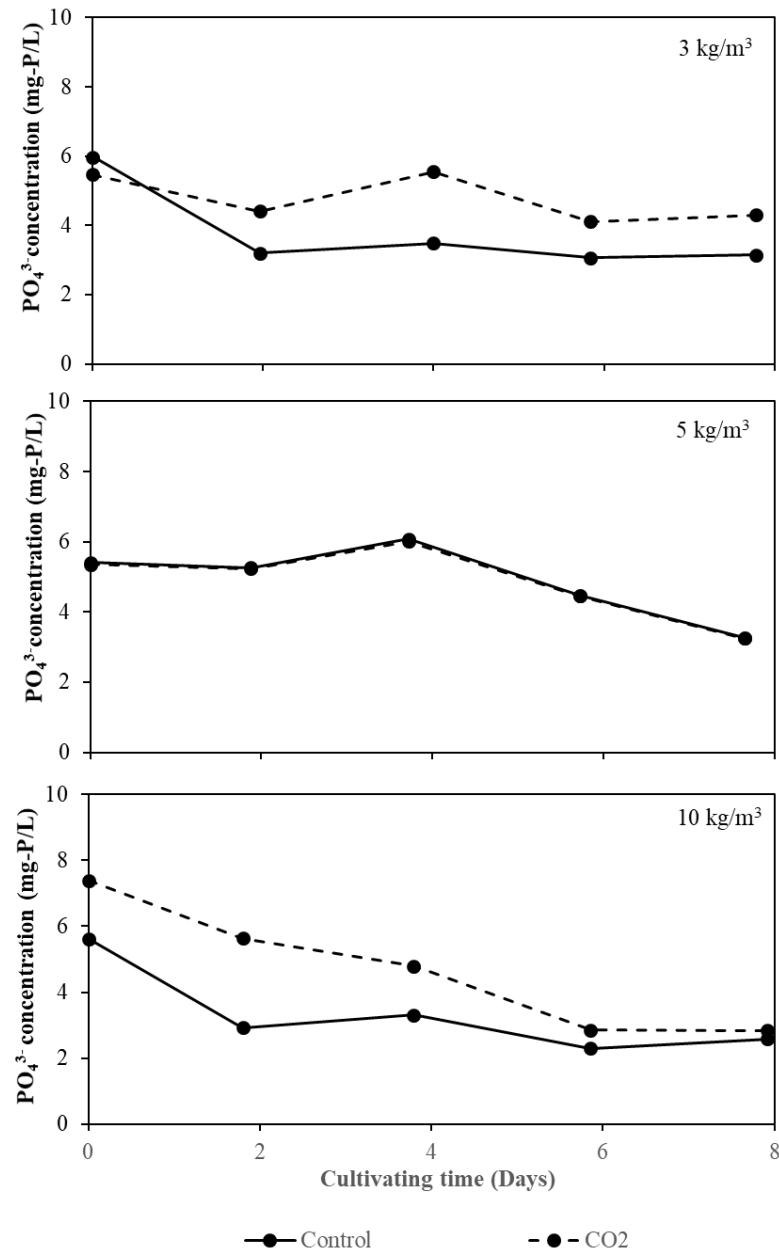


Figure 4.20. Phosphate concentration during the cultivation of *Scenedesmus armatus* using nitrate from the effluent of solid separating unit and carbon dioxide from ambient air or outlet air from fish tank at different tilapia biomass. Control represents microalgal cultivation using ambient air while CO₂ represents microalgal cultivation using outlet air from fish tank. Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

Table 4.9. Total ammonia nitrogen and nitrite concentration during the cultivation of *Scenedesmus armatus* using the effluent of solid separating unit and carbon dioxide from ambient air or outlet air from fish tank at different tilapia biomass.

Treatment	Concentration (mg N/L)	
	TAN	Nitrite
Control at fish 3 kg/m ³	0.04 ± 0.03	0.44 ± 0.07
CO ₂ at fish 3 kg/m ³	0.07 ± 0.06	0.24 ± 0.06
Control at fish 5 kg/m ³	0.41 ± 0.29*	0.09 ± 0.01*
CO ₂ at fish 5 kg/m ³	0.49 ± 0.32*	0.05 ± 0.03*
Control at fish 10 kg/m ³	0.12 ± 0.04	0.26 ± 0.13
CO ₂ at fish 10 kg/m ³	0.24 ± 0.13	0.14 ± 0.07

*Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

4.4.3 Carbon dioxide consumption during algal cultivation

Average carbon dioxide concentration in inlet air to photobioreactors depended on fish biomass, measured at 881 ± 123.1 , 974 ± 203.8 and $1,619 \pm 397.7$ ppm for fish biomass of 3, 5 and 10 kg/m³, respectively (Fig. 4.21). Significant carbon dioxide consumption was observed in the treatment associated with 10 kg/m³ fish biomass. Carbon dioxide concentration in outlet air from photobioreactors, ranged from 596 ± 25.4 to 615 ± 37.4 ppm, were similar to ambient air values. Carbon dioxide concentration in inlet air (i.e., from fish tank) varied with time, with the sharp increase observed after each fish feeding before starting to decrease (Fig. 4.22). Relatively constant carbon dioxide concentration about that of ambient air was observed in outlet air of photobioreactors for all fish biomass considered. The total mass of carbon dioxide that was consumed during microalgal cultivation is illustrated in dark gray, which is the difference between the total carbon dioxide supplied into photobioreactors and that remaining after the cultivation was completed (Fig. 4.23). It was estimated that 13.79 g of carbon dioxide were consumed from the total of 42.73 g during microalgal cultivation when fish biomass was closed to 3 kg/m³. For 5 kg/m³ fish biomass, higher carbon dioxide consumption at 19.13 g was accomplished during

microalgal cultivation as compared to the total carbon dioxide supplied into photobioreactors at 47.21 g. Approximately 3-folds higher carbon dioxide consumption at 48.10 g out of 77.58 g were reported when fish biomass was maintained closed to 10 kg/m³.

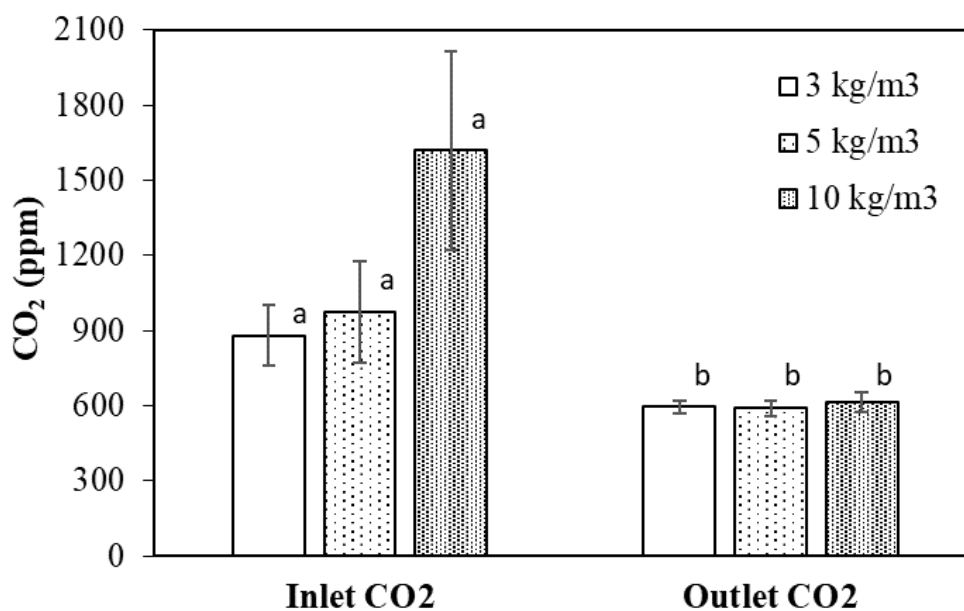


Figure 4.21. Average carbon dioxide concentrations in inlet air (i.e., outlet of fish tank) and outlet air from photobioreactors at different fish stocking densities (i.e., 3, 5 and 10 kg/m³). Different letters with the same color pattern indicate statistically significant difference in CO₂ concentration ($p < 0.05$). Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

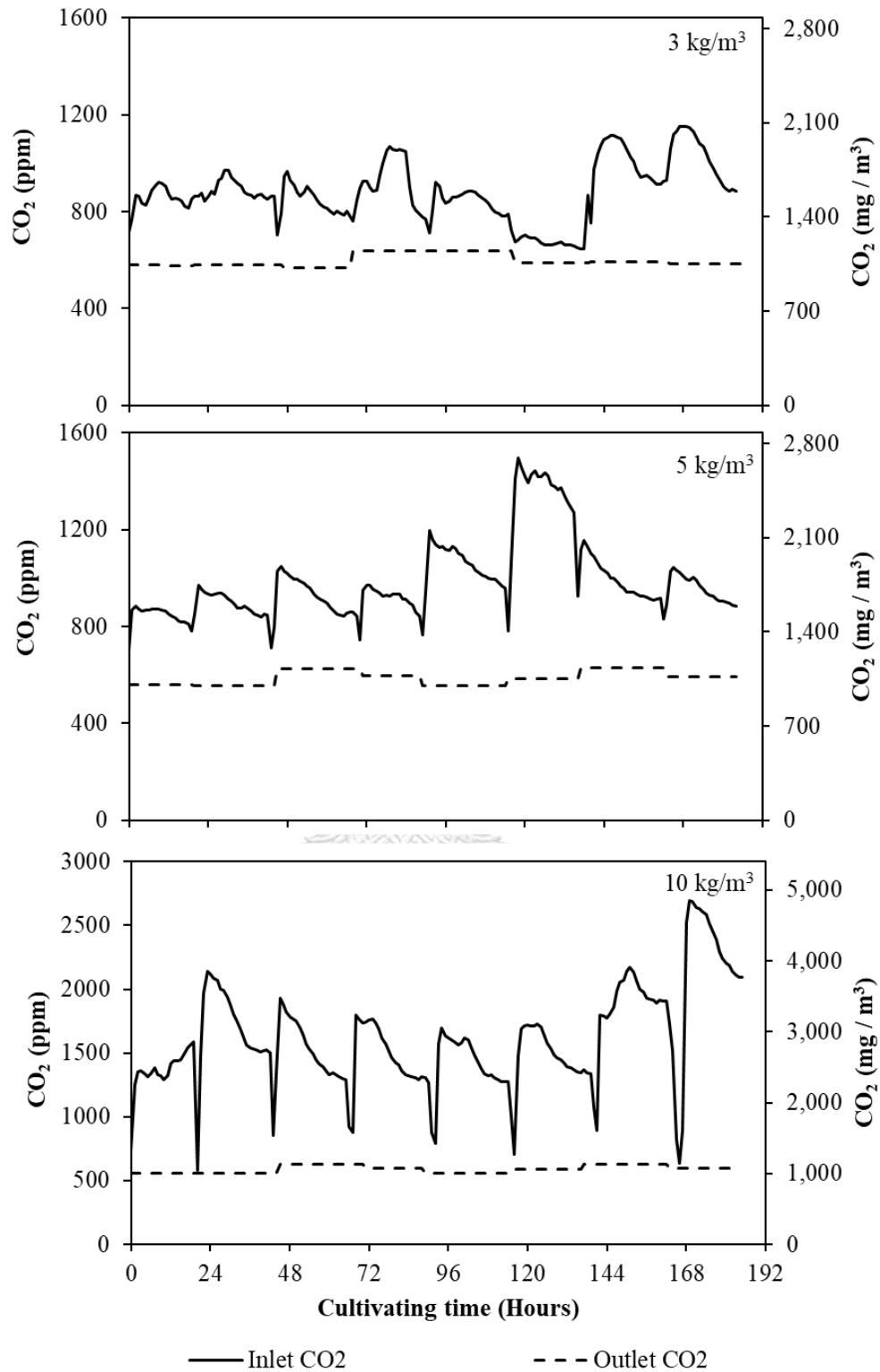


Figure 4.22. Carbon dioxide concentration in inlet air (i.e., outlet of fish tank) and outlet air of photobioreactors for microalgal cultivation at different fish biomass

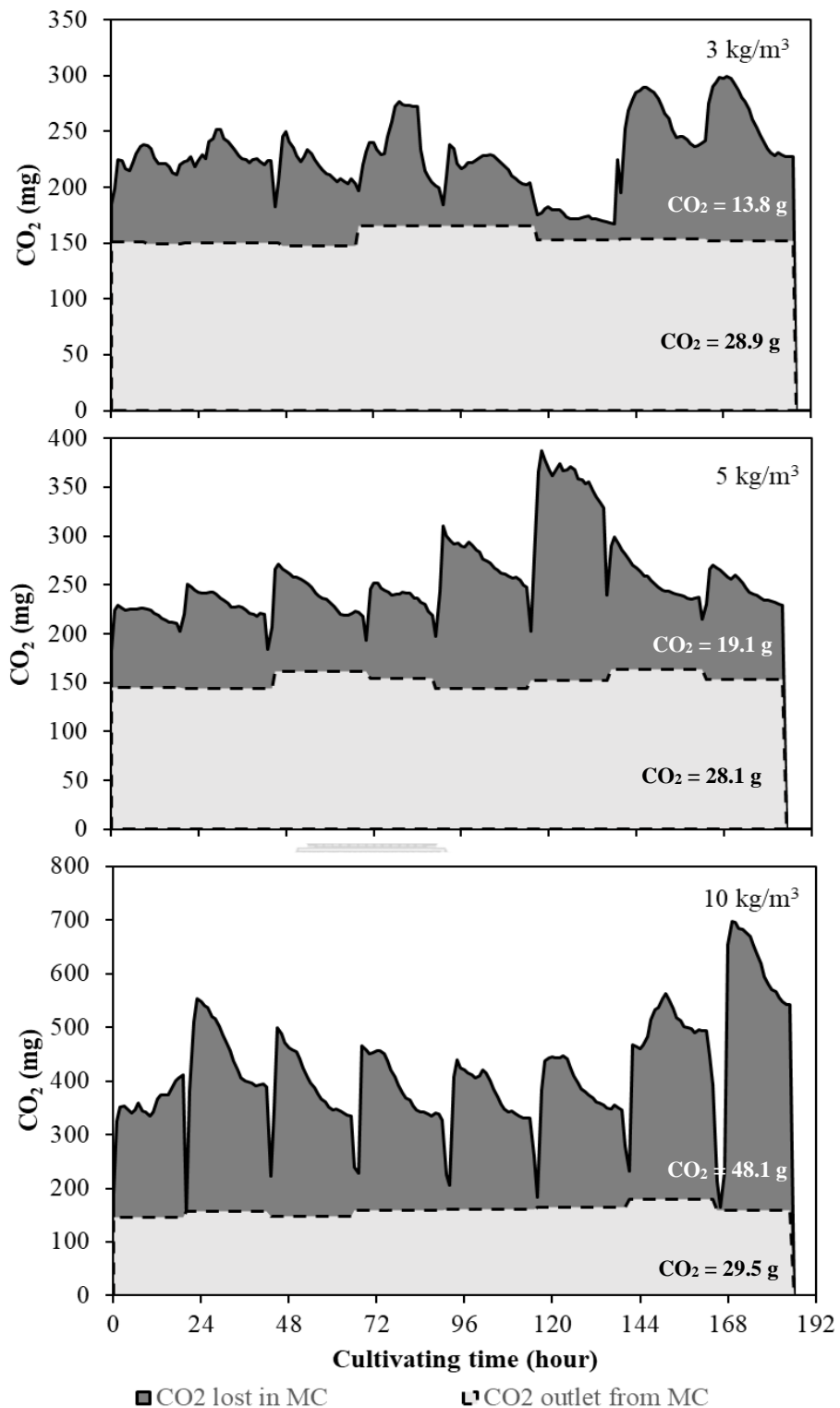


Figure 4.23. Mass of carbon dioxide into (i.e., from outlet of fish tank) and out of photobioreactors at different fish biomass. Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

4.5 Measurement of carbon dioxide concentration by gas chromatography

Samples (i.e., approximately 0.5 to 1.0 L) of inlet air into fish tank and outlet air from fish tank and solid separating unit were collected on the last day of microalgal cultivation for fish biomass of 10 kg/m³, and subsequently sent for analysis by gas chromatography. As demonstrated in Table 4.10, carbon dioxide concentration from carbon dioxide meter and gas chromatography were different so that calibrating graph were constructed as shown in Fig. 4.24. It should be mentioned that all carbon dioxide concentration reported in this chapter are post-calibrated concentration using this figure.

Table 4.10. Comparison between carbon dioxide concentration measured by Carbon dioxide meter and gas chromatography for fish biomass of 10 kg/m³.

Air sample	CO ₂ meter (ppm)	Gas chromatography (ppm)
Inlet of fish tank	339	545
Outlet of fish tank	1,165	1,341
Outlet of solid separating unit	728	871
Outlet of photobioreactors supplied with ambient air	379	509
Outlet of photobioreactors supplied with air from fish tank	456	580

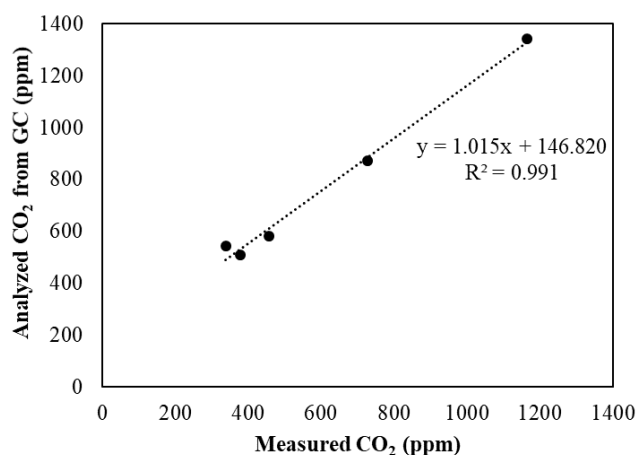


Figure 4.24. Calibrated graph for carbon dioxide concentration

4.6 Carbon and nitrogen mass balance in recirculating aquaculture system

Carbon and nitrogen mass balance was conducted to their distribution in input and output under different forms. Table 4.11 displays the results of carbon and nitrogen contents of feed, tilapia and suspended solids. Carbon input into the recirculating aquaculture system came from various parts, namely tilapia biomass, feed given to tilapia, and suspended solids in fish tank and solid separating unit. As shown in Table 4.12 and Fig. 4.25, large fraction of carbon input was in fish feed, biomass of tilapia and carbon dioxide into the system. Similar distribution of carbon input was observed for all fish biomass except that tilapia biomass accounted for large portion at 42.08% when fish biomass was at 10 kg/m³. For carbon output, major fractions were in the forms of fish biomass and carbon dioxide. Fractions of carbon emitted from solid separating unit was quite large relative to that from fish tank, and the likely explanation was linked to higher volumetric flow rate of air into the solid separating unit compared to that into fish tank (i.e., about 4 times higher).

Fish feed was the only carbon input that was converted to other forms, namely fish weight gained, formed suspended solids and carbon dioxide. According to Table 4.13, the fraction of carbon in tilapia weight gained accounted for 7% to 16% of total carbon in feed, but the largest fraction of feed carbon conversion was in the form carbon dioxide gained from respiration, accounting for 54% to 74% of the total carbon in feed. The results obtained were different from the carbon mass balance

calculation reported by Yogev et al. (2017) [12] that indicated the carbon fraction for fish biomass, suspended solids and carbon dioxide at 27%, 37% and 36% of total carbon in feed, respectively. The likely reason for the difference might be related to the activities of microorganisms (i.e., suspended solids as source of microorganisms) accumulated in solid separating that produced carbon dioxide into the system via respiration as well as other biological pathways, whereas carbon dioxide production from microorganisms in the work of Yogev et al. (2017) [12] was less likely because solids continuously removed from the aquaculture system.

Table 4.11. Carbon and nitrogen content in feed, fish biomass and suspended solids and *Scenedesmus armatus* biomass.

Parameter	% carbon	% nitrogen	Reference
Nile tilapia	49.9	6.76	Appendix G
Suspended solids	29.73	3.14	Appendix G
Feed	41.68	2.97	Appendix G
<i>S. armatus</i>	47.87	8.11	Appendix G

Table 4.12. Carbon mass distribution during the cultivation of tilapia in recirculating aquaculture system at fish biomass of 3, 5 and 10 kg/m³.

Carbon source	3 kg/m ³		5 kg/m ³		10 kg/m ³	
	g Carbon	%	g Carbon	%	g Carbon	%
Input						
Fish feed	17.2	19.3%	27.5*	24.9%*	56.8	31.5%
Nile tilapia	23.0	25.8%	36.7*	33.3%*	76.0	42.1%
Suspended solids	5.9	6.6%	4.4*	3.9%*	3.9	2.1%
CO ₂ fed to FT	7.5	8.4%	7.1*	6.4%*	7.7	4.3%
CO ₂ fed to SU	31.2	35.1%	29.6*	26.9%*	32.2	17.8%

Carbon source	3 kg/m ³		5 kg/m ³		10 kg/m ³	
	g Carbon	%	g Carbon	%	g Carbon	%
Dissolved inorganic carbon	4.2	4.8%	4.9*	4.5%*	3.9	2.2%
Sum	88.9	100.0%	110.1*	100.0%*	180.6	100.0%
Output						
Nile tilapia	25.8	29.0%	38.9*	35.3%*	85.0	47.1%
Suspended solids	8.0	9.0%	6.2*	5.6%*	6.5	3.6%
CO ₂ emitted from FT	11.7	13.1%	12.9*	11.7%*	21.2	11.7%
CO ₂ emitted from SU	39.1	44.0%	44.0*	39.9%*	49.9	27.6%
Dissolved inorganic carbon	3.8	4.3%	4.4*	4.0%*	5.2	2.9%
<i>Lost</i>	0.5	0.6%	3.8*	3.5%*	12.8	7.1%
Sum	88.9	100.0%	110.1*	100.0%*	180.6	100.0%

*Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

Table 4.13. Feed carbon conversion into different forms during tilapia cultivation in recirculating aquaculture system at fish biomass of 3, 5 and 10 kg/m³

Carbon source	3 kg/m ³		5 kg/m ³		10 kg/m ³	
	g Carbon	%	g Carbon	%	g Carbon	%
Input						
Feed input	17.19	100%	27.46*	100%*	56.84	100%
Output						
Fish biomass gained	2.82	16.42%	2.19*	7.99%*	9.01	15.86%
Suspended solids gained	2.17	12.64%	1.84*	6.69%*	2.65	4.67%
CO ₂ gained	12.08	70.27%	20.17*	73.46%*	31.08	54.67%
Lost	0.12	0.67%	3.26*	11.86%*	14.10	24.81%
Sum	17.19	100%	27.46*	100%*	56.84	100%

*Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

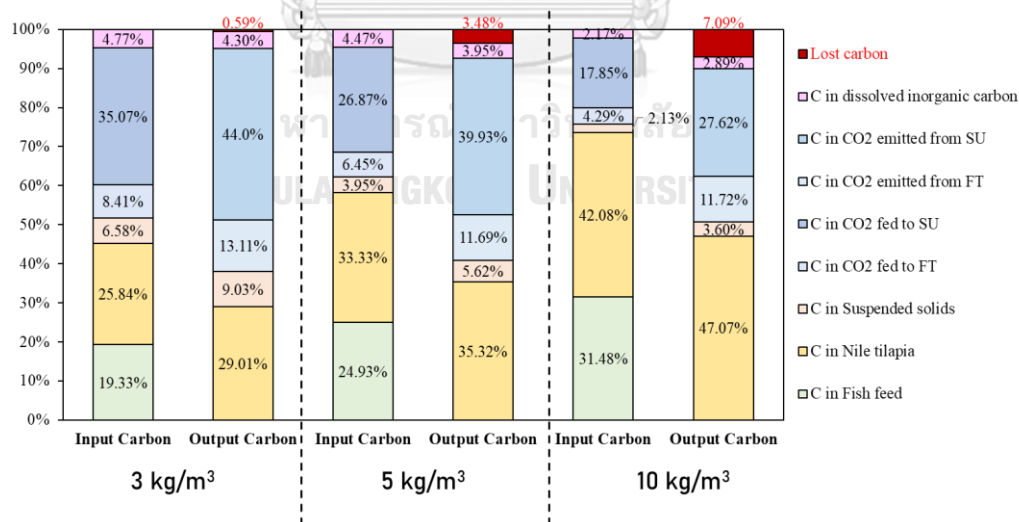


Figure 4.25. Carbon mass distribution during the cultivation of tilapia in recirculating aquaculture system at fish biomass of 3, 5 and 10 kg/m³. Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

Nitrogen contents in feed, tilapia and suspended solids are summarized in Table 4.14 and Fig. 4.26. Results of nitrogen mass balance indicated that fish feed was the only nitrogen input that was distributed to other forms, specifically nitrogen in fish biomass, nitrogen in suspended solids in fish tank and solid separating unit, inorganic nitrogen dissolved in water and unaccounted fraction assumed as nitrogen gas. Large fractions of nitrogen output were dissolved inorganic nitrogen compounds (i.e., ammonia, nitrite and nitrate), accounting for 32% to 55% of nitrogen input. Fraction of dissolved inorganic nitrogen were significantly larger than the range reported in previous works employing bioflocs as nitrifying sludge that indicated inorganic nitrogen fraction from 15% to 25% [51, 78]. Another large fraction of nitrogen output was the unaccounted portions assumed as nitrogen gas. The magnitude of unaccounted nitrogen in this work was smaller than the previous works, which reported unaccounted portion about 30% to 50% [78-80]. Another limitation of this work that led to different nitrogen distributions might be linked to short cultivation period only 8 days.

Table 4.14. Nitrogen mass balance displaying nitrogen distribution in recirculating aquaculture system for tilapia biomass of 3, 5 and 10 kg/m³.

Nitrogen source	3 kg / m ³		5 kg / m ³		10 kg / m ³	
	g Nitrogen	%	g Nitrogen	%	g Nitrogen	%
Input						
Fish feed	1.22	16.86%	1.96*	24.27%*	4.05	28.50%
Nile tilapia	1.55	21.38%	2.48*	30.79%*	5.14	36.15%
Suspended solids	0.18	2.53%	0.14*	1.70%*	0.12	0.85%
Dissolved inorganic nitrogen	4.30	59.22%	3.49*	43.25%*	4.90	34.50%

Nitrogen source	3 kg / m ³		5 kg / m ³		10 kg / m ³	
	Sum	7.26	100%	8.06	100%*	14.21
Output						
Nile tilapia	1.74	24.01%	2.63	32.63%*	5.75	40.43%
Suspended solids	0.25	3.47%	0.19	2.41%*	0.20	1.44%
Dissolved inorganic nitrogen	4.00	55.03%	3.38	41.95%*	4.60	32.36%
Unaccounted	1.27	17.48%	1.86	23.02%*	3.66	25.77%
Sum	7.26	100%	8.06	100%*	14.21	100%

* Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

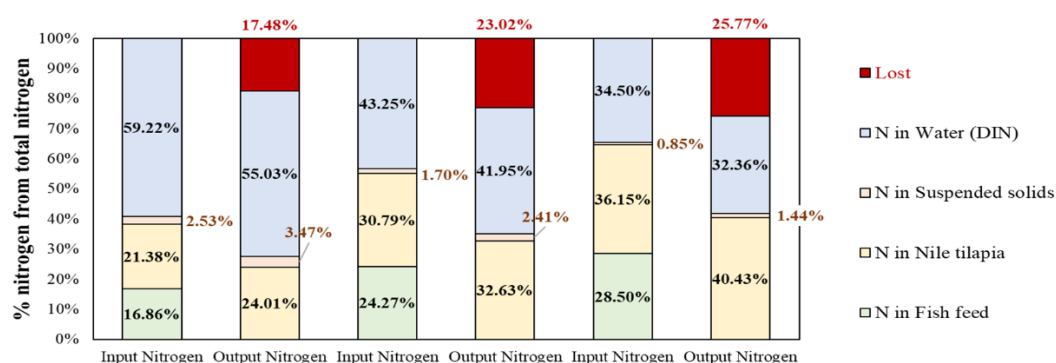


Figure 4.26. Nitrogen mass distribution during the cultivation of tilapia in recirculating aquaculture system at fish biomass of 3, 5 and 10 kg/m³. Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

4.7 Carbon and nitrogen mass balance during microalgal cultivation using nitrate and carbon dioxide from recirculating aquaculture system

CHN analysis revealed that biomass of *S. armatus* contained carbon and nitrogen at 47.87% and 8.11%, respectively (Table 4.9). Carbon dioxide input for microalgal cultivation was the carbon dioxide output from fish tank. Carbon dioxide was almost accounted entirely (98.16% to 99.07%) for carbon input while the

remaining (0.61 to 1.37%) was dissolved inorganic carbon (Table 4.15 and Fig 4.27). For carbon output, carbon dioxide was still the highest fraction accounted for 37.64% to 66.57% depending on fish biomass, whereas the fraction of carbon in microalgae was only 2.68% to 3.84% and the remaining fractions (29.05% to 58.60%) were unaccounted carbon, which may be dissolved organic carbon (DOC). These findings implied that microalgae cultivation was ineffective for carbon recovery and the process needed further optimization to improve efficiency of carbon recycle.

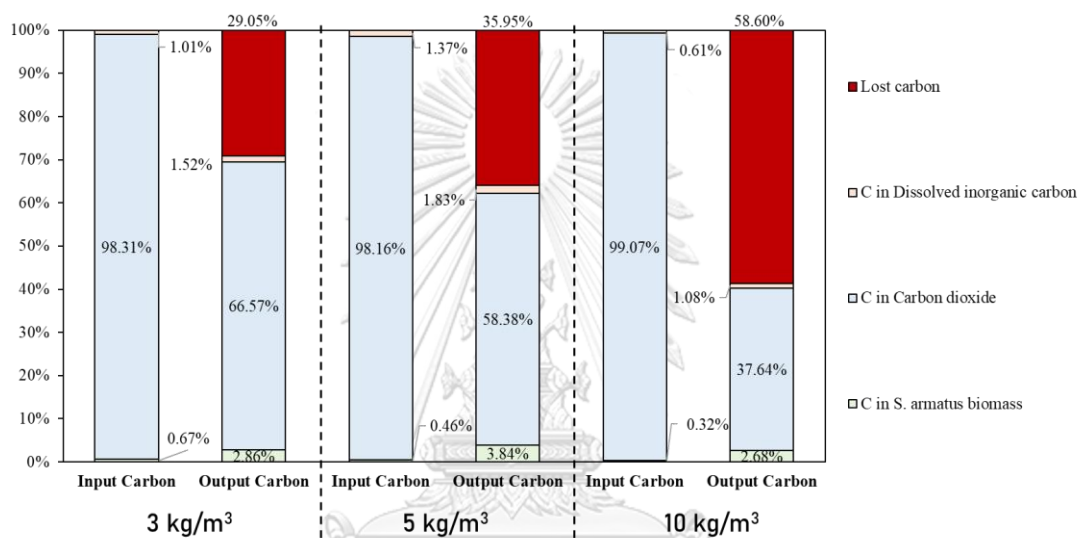


Figure 4.27. Carbon mass balance for the cultivation of *S. armatus* using outlet air from fish tank and nitrate from solid separating unit for 3, 5 and 10 kg/m³ tilapia biomass. Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

Table 4.15. Carbon mass balance for the cultivation of *S. armatus* using outlet air from fish tank and nitrate from solid separating unit for 3, 5 and 10 kg/m³ tilapia biomass.

Carbon source	CO2-3		CO2-5		CO2-10	
	g-C	%	g-C	%	g-C	%
Input						
<i>S. armatus</i> biomass	0.080	0.68%	0.061*	0.47%*	0.068	0.32%
CO ₂	11.660	98.86%	12.882*	98.89%*	21.172	99.41%
Dissolved inorganic carbon	0.055	0.47%	0.083*	0.64%*	0.058	0.27%
Sum	11.795	100.00%	13.027*	100.00%*	21.297	100.00%
Output						
<i>S. armatus</i> biomass	0.339	2.87%	0.504*	3.87%*	0.572	2.69%
CO ₂	7.895	66.94%	7.661*	58.81%*	8.044	37.77%
Dissolved inorganic carbon	0.089	0.75%	0.118*	0.91%*	0.110	0.52%
Unaccounted	3.472	29.44%	4.743*	36.41%*	12.570	59.02%
Sum	11.795	100.00%	13.027*	100.00%*	21.297	100.00%

*Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

Table 4.16 and Fig 4.28 demonstrate the results of nitrogen mass balance calculation showing nitrogen distribution during the microalgal cultivation. Clearly, nitrogen input was almost entirely nitrate (90.03 to 91.96%) while the remaining fraction was the initial biomass of microalgae. For nitrogen output, microalgal growth increased its nitrogen fraction to about 39.34% to 76.64% while the remaining nitrate after consumption was still a major portion ranged from 14.66% to 50.94%. The unaccounted fraction of nitrogen output was assumed as dissolved organic nitrogen. Production of nitrogenous gases was unlikely due to continuous aeration in

photobioreactors. Detailed examination revealed that the treatments associated with 5 and 10 kg/m³ fish biomass were more efficient in nitrogen recovery in the form microalgal biomass (39.34% to 76.64%) and consumed more nitrate as compared to the treatment associated with 3 kg/m³ biomass. In general, it can be said that nitrogen recovery fared better than carbon recovery for all fish stocking densities considered.

Table 4.16. Nitrogen mass balance for the cultivation of *S. armatus* using outlet air from fish tank and nitrate from solid separating unit for 3, 5 and 10 kg/m³ tilapia biomass.

Carbon source	CO2-3		CO2-5		CO2-10	
	mg Nitrogen	%	mg Nitrogen	%	mg Nitrogen	%
Input						
<i>S. armatus</i> biomass	13.52	9.26%	10.38*	9.33%*	11.46	7.71%
TAN	0.0089	0.01%	0.20*	0.17%*	0.17	0.11%
NO ₂ ⁻	1.02	0.70%	0.33*	0.30%*	0.33	0.22%
NO ₃ ⁻	131.38	90.03%	100.40*	90.20%*	136.76	91.96%
Sum	145.93	100%	111.31*	100%*	148.71	100%
Output						
<i>S. armatus</i> biomass	57.41	39.34%	85.31*	76.64%*	96.89	65.15%
TAN	0.29	0.20%	0.71*	0.64%*	1.13	0.76%
NO ₂ ⁻	0.54	0.37%	0.08*	0.08%*	0.28	0.19%
NO ₃ ⁻	74.33	50.94%	16.32*	14.66%*	33.43	22.48%
Unaccounted	13.37	9.16%	8.88*	7.98%*	16.99	11.43%
Sum	145.93	100%	111.31*	100%*	148.71	100%

* Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

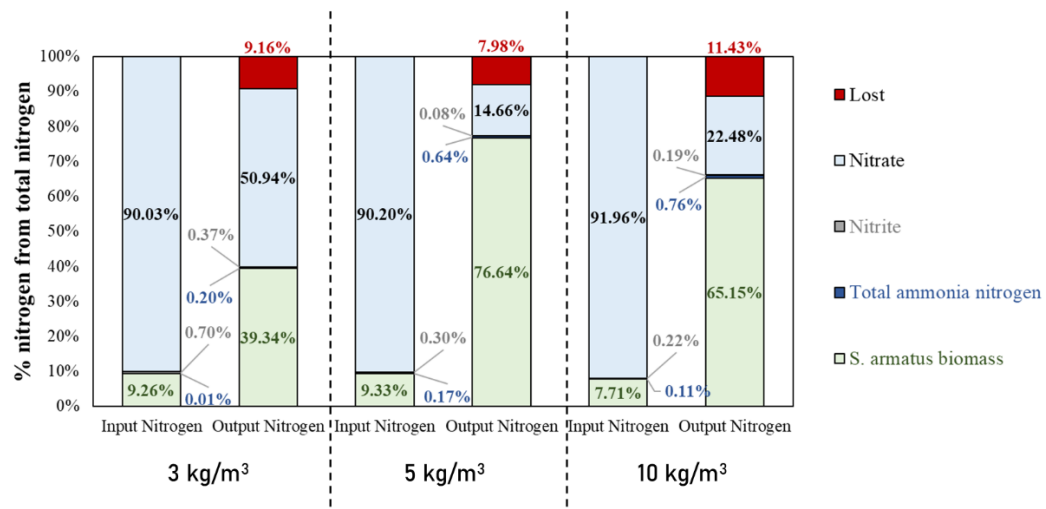


Figure 4.28. Nitrogen mass balance for the cultivation of *S. armatus* using outlet air from fish tank and nitrate from solid separating unit for 3, 5 and 10 kg/m³ tilapia biomass. Note that results for 5 kg/m³ tilapia cultivation may be underestimated due to fish death on day 5.

CHAPTER V

CONCLUSION AND SUGESTION FOR FUTURE WORK

5.1. Conclusion

This research proposed the concept of sustainable aquaculture by introducing photosynthetic process to utilize wastes generated from recirculating aquacultures system. Specifically, carbon dioxide from fish respiration and microbial activity from recirculating aquaculture system and nitrate-rich effluent from nitrifying unit were utilized by microalgae to obtain biomass and other valuable products. The recirculating aquaculture system used in this work consisted of fish tank as the main source for carbon dioxide and solid separating unit as nitrifying unit to produce nitrate. These wastes were subsequently directed to bubble column photobioreactors to cultivate green microalga *Scenedesmus armatus*. The first part of this research evaluated the feasibility of using effluent from nitrifying biofilter of commercial aquaculture system; the second part verified the concept of using carbon dioxide from aquaculture production as carbon source for microalgal cultivation; and the final part studied the effects of fish biomass on carbon dioxide and nitrate production and their utilization during microalgal cultivation in photobioreactors. The following conclusions can be drawn.

(1) The effluent from nitrifying biofilter of commercial tilapia farm, containing nitrate approximately 25.6 mg N/L, yielded similar cell dried weight (664 mg/L), cell number density (6.49×10^6 cells/mL), total carotenoids content (3.09 ± 2.00 mg/g) and lutein content (1.12 ± 0.71 mg/g) to BG-11 growth media during the batch cultivation of *S. armatus*, thereby implying that nitrifying biofilter effluent can be used as substitute for a more expensive BG-11 growth media in the future.

(2) It was possible to capture carbon dioxide from tilapia cultivation even at moderated fish stocking at 3 kg/m³. This resulted in the average carbon dioxide concentration at 935 ± 93.4 ppm ($1,683 \pm 168.1$ mg/m³) in outlet air of fish tank as compared to the average carbon dioxide concentration of 566 ± 13.9 ppm ($1,019 \pm 25.1$ mg/m³) in ambient air. Calculation showed that the amounts of carbon dioxide

gained from fish feeding and basic respiration were 3.33 to 4.92 g and 12.03 to 15.24 g over the 8-days fish cultivating period, respectively, for this particular fish biomass (i.e., 3 kg/m³). This corresponded to the total carbon dioxide production rate of 458.54 g/day·kg-feed. The cultivation of *S. armatus* using the captured carbon dioxide from fish tank resulted in higher cell number density than the cultivation using ambient air. Therefore, it can be concluded that elevated carbon dioxide concentration could be obtained from recirculating aquaculture system and was effective for microalgal cultivation relative to supplying ambient air.

(3) Recirculating aquaculture system consisting of fish tank and solid separating unit was effective in maintaining suspended solids, ammonia and nitrite within the acceptable range for tilapia cultivation. For fish biomass at 10 kg/m³, nitrate concentration in the effluent of solid separating unit were relatively constant, with the average value at 45.3 ± 2.61 mg N/L. This observation was linked to denitrification that occurred in anaerobic region of biofilm or within sediment layer on tank floor. Increasing fish biomass in tank from 3, 5 to 10 kg/m³ resulted in higher carbon dioxide concentration in the outlet of fish tank. Carbon dioxide concentration in fish tank outlet were measured at $1,618 \pm 398$ ppm for the biomass of 10 kg/m³. The obtained carbon dioxide was approximately 2-folds higher in concentration than those from the outlet of fish tank when maintaining lower fish biomass at 3 and 5 kg/m³. Nitrate and carbon dioxide from 10 kg/m³ fish biomass were used to cultivate *S. armatus* in bubble column photobioreactors and found that it yielded higher growth than the cultivation using ambient air. This was in contrast to the results when maintaining lower fish biomass at 3 and 5 kg/m³ that yielded comparable growth between treatments supplying carbon dioxide from fish tank and ambient air.

(4) Results of carbon and nitrogen mass balance calculation of tilapia cultivation in recirculating aquaculture system found that only 15.86% of carbon and 15.04% of nitrogen from feed were transferred to carbon and nitrogen in fish biomass, whereas the major fraction (54.7% to 73.5%) of carbon from feed was converted to carbon dioxide.

(5) Results of carbon mass balance calculation indicated that carbon dioxide was the major carbon input during microalgal cultivation, accounting for 98.86% to 99.41% of the total carbon input. Carbon dioxide was still the highest

fraction of carbon output for microalgal cultivation, accounting for 37.77% to 66.94%. However, the fraction of carbon in microalgae was only 2.69% to 3.87%, implying that for this work microalgal cultivation was ineffective for carbon recovery and the process needed further optimization to improve efficiency. Results of nitrogen mass balance calculation indicated that nitrogen input was almost entirely nitrate (90.03% to 91.96%) while the remaining fraction was the initial biomass of microalgae. For nitrogen output, microalgal growth increased nitrogen fraction to about 39.34% to 76.64% while the remaining nitrate after consumption was still a major portion ranged from 14.66% to 50.94%.

5.2. Suggestion for future works

(1) More efficient liquid mixing and oxygen transfer in solid separating unit should be explored to improve nitrate production and reduce the likelihood of denitrification occurring in the system. Suitable range of suspended solid should be identified for higher fish biomass to balance energy requirement and nitrification effectiveness. The excessed solids should be periodically removed from the solid separating unit and identified the utilization method, for example using as substrates for digestion to produce additional carbon dioxide to be used during microalgal cultivation or undergoing fermentation to produce intermediates for high-value products.

(2) Results of carbon mass balance indicated that solid separating unit generated significant amount of carbon dioxide, which can be combined with carbon dioxide from fish tank. As a result, optimal operating condition of solid separating unit must be determined to lessen the dilution effect from high volumetric flow rate, such as reducing the aeration just enough to maintain adequate oxygen for complete nitrification.

(3) Optimization of cultured condition for microalgae should be conducted to improve carbon dioxide utilization. Moreover, exploring other photosynthetic processes (e.g. aquaponic or greenhouse planting) to be used in parallel to microalgal cultivation should be considered as significant amount of carbon dioxide from recirculating aquaculture system remained unused.

(4) Continuous or semi-batch microalgal cultivation should be considered to increase waste utilization, and hence improving biomass productivity. Remaining water after harvesting microalgal biomass can be recirculated to fish tank or solids separating unit to ensure the zero-discharge concept.



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Appendix A

A1. BG-11 medium recipe (Stainier et al., 1971)

Stocks	Per 500 mL
1. NaNO_3	75.0 g
	Per 500 mL
2. K_2HPO_4	2.0 g
3. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	3.75 g
4. $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	1.80 g
5. Citric acid	0.30 g
6. Ammonium ferric citrate green	0.30 g
7. Na_2EDTA	0.05 g
8. Na_2CO_3	1.00 g
9. Trace metal solution	Per 1 L
9.1. H_3BO_3	2.86 g
9.2. $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	1.81 g
9.3. $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.22 g
9.4. $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.39 g
9.5. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.08 g
9.6. $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$	0.05 g
Medium	Per 1 L
- Stock solution 1 – 8	10.0 mL each
- Stock solution 9	1.0 mL

Make up to 1 litre with deionized water. Adjust pH to 7.1 with 1M NaOH or HCl. Autoclave at 15 psi for 15 minutes.

Appendix B

B1. Cells numbers density analysis with Haemocytometer [81]

Analysis procedure

The haemocytometer was used for counting the *Scenedesmus armatus* cells number through the view from light microscope using 10 – 40 x objective lens. There was gridded area of the hemocytometer that consists of nine 1 x 1 mm (1 mm²) squares as in Fig B1. The cells counting procedure was started when place the cover glass on 2 mounting support of haemocytometer. Then fill the microalgal cells suspension into haemocytometer at the edge of the cover glass. The cell suspension was sucked into the void by capillary action which completely fills the chamber with the sample. The number of cells in the chamber can be determined by direct counting the cells within 1 mm² squares at gridded area. The void between cover glass and haemocytometer was 0.1 mm thickness that made each 1 mm² squares at gridded area contain 10⁻⁴ mL suspension volume. The cells numbers density could analyze from the equation below.

$$\text{Cells number density } \left(\frac{\text{Cells}}{\text{mL}} \right) = \frac{(\text{NC}(\text{cells}) \times \text{NSq})}{10^{-4} \text{mL}}$$

Where NC = Number of counted cells (cells)
 NSq = Number of squares

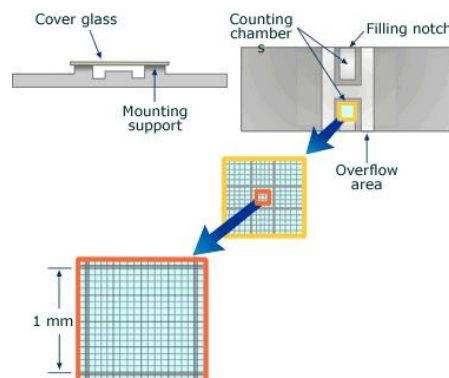


Figure B1 Feature of haemocytometer

Source : [http://www.odec.ca/projects/2013/beso13s/images/haemgrid_1_2_3\[1\].jpg](http://www.odec.ca/projects/2013/beso13s/images/haemgrid_1_2_3[1].jpg)

(07/11/2019)

B2. Analysis of total ammonia nitrogen concentration

The method that was used in this study was applied from salicylate-hypochlorite method [60] that had procedure as follow.

Reagent preparation

1. Salicylate-catalyst solution

Dissolved 440 g sodium salicylate and 0.28 g sodium nitroprusside dehydrate in ammonia-free water and diluted to 1 L with ammonia-free water. Stored this reagent in a light-resistant container at temperature below 5 °C.

2. Alkaline citrate solution

Dissolved 18.5 g sodium hydroxide and 100 g sodium citrate dehydrate in ammonia-free water and adjusted the volume of solution to 1 L with ammonia-free water. Stored this reagent in a light-resistant container at temperature below 5 °C.

3. Sodium hypochlorite solution

The commercial 1.5 N Sodium hypochlorite solution could be use as this reagent.

4. Alkaline-hypochlorite solution

Mixed the Alkaline citrate solution with Sodium hypochlorite solution at the ratio of 1:9. This reagent could be used within 1 hour after preparation.

Analysis procedure

The water samples with the volume at least 15 mL had to be filtrated with Whatman GF/C filter. After that, the samples should be instantly analyzed or store them at temperature below -20°C if the analysis can't be performed right now.

The analysis will begin when the 5 mL water sample was mixed with 0.6 mL Salicylate-catalyst solution and 1.0 mL Alkaline-hypochlorite solution in test tube,

respectively. This solution was completely mix with vortex mixer and was stored in the dark for 1-3 hours. The ammonia-free water was mixed with these reagents and was stored in the same condition for using as blank. The sample was measured the absorbance at 660 nm with spectrophotometer. The absorbance from sample was compared with absorbance from analysis of standard ammonia solutions which had total ammonia concentrations of 0.01, 0.05, 0.1, 0.5 and 1 mg N/L, respectively. These standard solutions could be prepared by diluting the 100 mg N/L stock ammonia solution with ammonia-free water. The relative graph between total ammonia nitrogen concentrations and absorbances was showed in Fig B2.

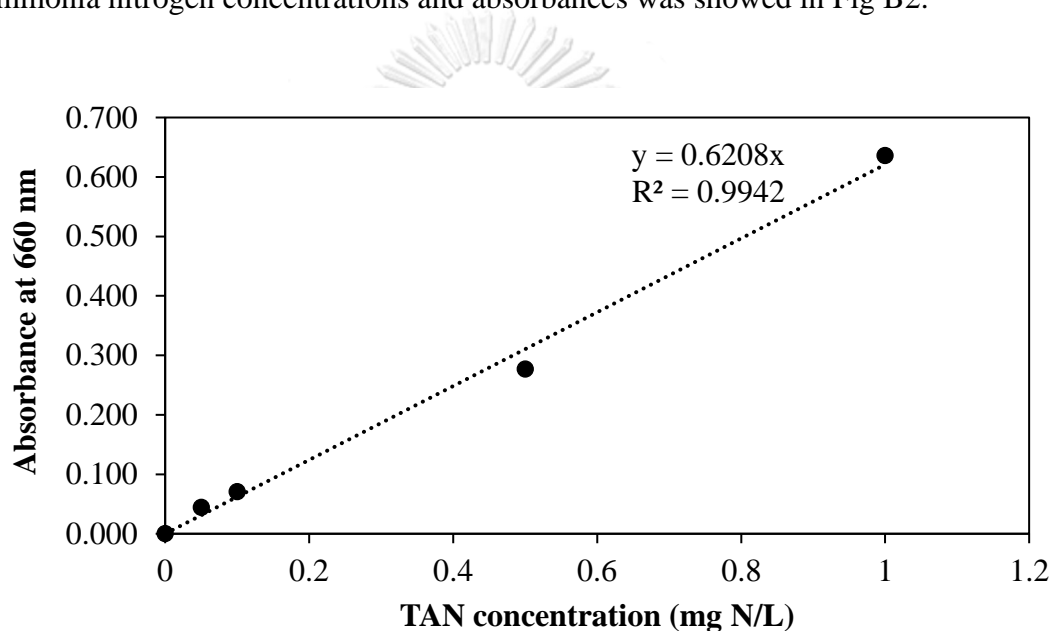


Fig B2 Relative graph between total ammonia nitrogen concentrations and absorbances at 660 nm

B3. Analysis of nitrite concentration

The method that was used in this study was applied from 4500-NO₂⁻ B. Colorimetric method [59] that had procedure as follow.

Reagent preparation

1. Sulfanilamide solution

Dissolved 5 g Sulfanilamide in 50 mL Concentrated hydrochloric acid and diluted to 1 L with deionized water. Stored this reagent in a light-resistant container at temperature below 5 °C.

2. Naphthylethylenediamine solution (NED)

Dissolved 0.5 g N-(1-Naphthyl)ethylenediamine in deionized water and adjusted the volume of solution to 500 mL with this water. Stored this reagent in a light-resistant container at temperature below 5 °C.

Analysis procedure

The water samples with the volume at least 15 mL had to be filtrated with Whatman GF/C filter. After that, the samples should be instantly analyzed or store them at temperature below -20°C if the analysis can't be performed right now.

The analysis will begin when the 5 mL water sample was mixed with 0.1 mL Sulfanilamide solution in test tube and was completely mixed with vortex mixer. The solution should be waited for 2-10 minutes after that mixer this solution with 0.1 mL Naphthylethylenediamine solution then completely mixed this solution and wait for 30-120 minutes. The deionized water was mixed with these reagents and was stored in the same condition for using as blank. The sample was measured the absorbance at 543 nm with spectrophotometer. The absorbance from sample was compared with absorbance from analysis of standard nitrite solutions which had total nitrite concentrations of 0.01, 0.05, 0.1, 0.5 and 1 mg N/L, respectively. These standard solutions could be prepared by diluting the 100 mg N/L stock nitrite solution with deionized water. The relative graph between nitrite concentrations and absorbances was showed in Fig B3.

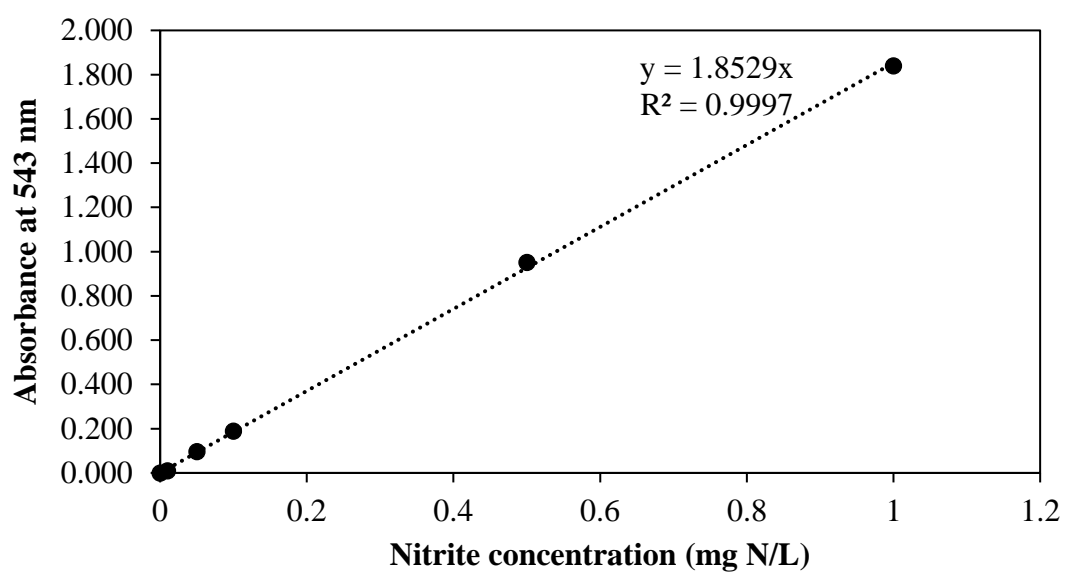


Fig B3 Relative graph between nitrite concentrations and absorbances at 543 nm



B4. Analysis of nitrate concentration

The method that was used in this study was applied from 4500-NO₃⁻ B. UV-screening method [59] that had procedure as follow.

Analysis procedure

The water samples with the volume at least 15 mL had to be filtrated with Whatman GF/C filter. After that, the samples should be instantly analyzed or store them at temperature below -20°C if the analysis can't be performed right now.

The analysis will begin when the water sample was measured the absorbance at 220 nm and 275 nm with spectrophotometer. The deionized water was used as blank in this analysis. The different value between absorbance at 220 nm and absorbance at 275 nm from sample was compared with The different value between absorbance at 220 nm and absorbance at 275 nm from analysis of standard nitrate solutions which had total nitrate concentrations of 1, 3, 5 and 7 mg N/L, respectively. These standard solutions could be prepared by diluting the 100 mg N/L stock nitrate solution with deionized water. The relative graph between nitrate concentrations and absorbances was showed in Fig B3.

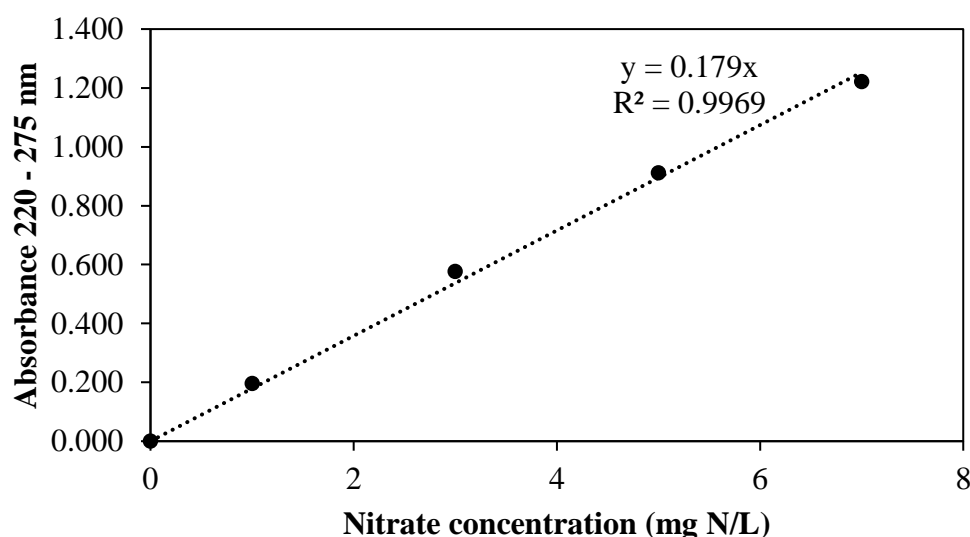


Fig B4 Relative graph between nitrate concentrations and different values between absorbance at 220 nm and absorbance at 275 nm

B5. Analysis of orthophosphate concentration

The method that was used in this study was applied from 4500-P E. ascorbic acid method [59] that had procedure as follow.

Reagent preparation

1. Ammonium molybdate solution

Dissolved 15 g sodium salicylate in deionized water and diluted to 500 mL with deionized water. Stored this reagent in a light-resistant container at temperature below 5 °C.

2. Sulfuric acid solution

Dilute 140 mL of Concentrated sulfuric acid with 900 mL deionized water. Stored this reagent in a glass container at temperature below 5 °C.

3. Ascorbic acid solution

Dissolved 27 g ascorbic acid in deionized water and diluted to 500 mL with deionized water. Stored this reagent in a light-resistant container at temperature below 5 °C.

4. Potassium antimony tartrate solution

Dissolved 0.34 g potassium antimony tartrate in deionized water and diluted to 250 mL with deionized water. Stored this reagent in a light-resistant container at temperature below 5 °C.

5. Mix reagent

Mix the Ammonium molybdate solution, Sulfuric acid solution, Ascorbic acid solution and potassium antimony tartrate solution together with the ratio of 2 mL :5 mL :2 mL:1 mL for preparing 10 mL Mix reagent. This reagent could be used within 2 hours after preparation.

Analysis procedure

The water samples with the volume at least 15 mL has to be filtrated with Whatman GF/C filter. After that, the samples should be instantly analyzed or store them at temperature below -20°C if the analysis can't be performed right now.

The analysis will begin when the 5 mL water sample was mixed with 0.5 mL Mix reagent. This solution was completely mix with vortex mixer and was stored in the dark for 30 minutes to 2 hours. The deionized water was mixed with this Mix reagent and was stored in the same condition for using as blank. The sample was measured the absorbance at 885 nm with spectrophotometer. The absorbance from sample was compared with absorbance from analysis of standard phosphate solutions which had phosphate concentration of 0.01, 0.05, 0.1, 0.5 and 1 mg P/L, respectively. These standard solutions could be prepared by diluting the 100 mg P/L stock ammonia solution with ammonia-free water. The relative graph between total ammonia nitrogen concentrations and absorbances was showed in Fig B5.

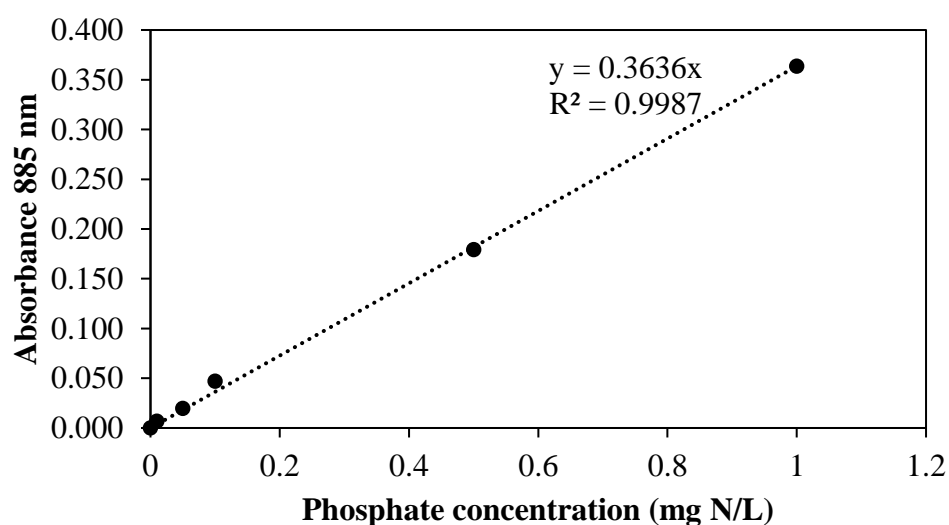


Fig B5 Relative graph between phosphate concentrations and absorbances at 885 nm

B6. Analysis of Total suspended solids concentration

Analysis procedure

The method that was used in this study was applied from 2540 B. Total suspended solids method [59] that had procedure as follow.

47 mm diameter GF/C filter was dried with hot air oven using the temperature of 103 - 105 °C at least 1 hours. After that keep this dried GF/C filter in desiccator before weighing it with 4-digit Analytical Balance when the temperature of this filter was equal to ambient temperature. Collect the weight of this GF/C filter (B). Then, this GF/C filter was used to filtrate the suspended solids inside the known volume water sample by using the vacuum flask and vacuum pump. After the filtration, GF/C paper with suspended solid on it was dried with hot air oven using the temperature of 103 - 105 °C at least 1 hours and keep this GF/C paper in desiccator after drying. Weigh this GF/C filter again with with 4-digit Analytical Balance and collect the weight of GF/C filter with suspended solids (A). Calculate the total suspended solids with the equation below.

$$TSS \left(\frac{mg}{L} \right) = \frac{[(A(mg) - B(mg))] \times 1,000 \left(\frac{mL}{L} \right)}{V(mL)}$$

Where	TSS	= Total suspended solids (mg/L)
	A	= Weight of GF/C filter with suspended solids (mg)
	B	= Weight of GF/C filter before filtration (mg)
	V	= Volume of sample (mL)

B7. Analysis of total amount of suspended solid and percentage of solids retention

The total amounts of suspended solid in each unit (i.e., fish tank and suspended solid separating unit) were calculated using their final total suspended solids concentration and their working volume as the following equations.

$$TAS_{FT} (mg) = finalTSS_{FT} \left(\frac{mg}{L} \right) \times 54 L$$

$$TAS_{SU1} (mg) = finalTSS_{SU1} \left(\frac{mg}{L} \right) \times 27 L$$

$$TAS_{SU2} (mg) = finalTSS_{SU2} \left(\frac{mg}{L} \right) \times 27 L$$

$$TAS_{RAS} (mg) = TAS_{FT}(mg) + TAS_{SU1}(mg) + TAS_{SU2} (mg)$$

Where

TAS_{FT} = Total amounts of suspended solid in fish tank (mg)

TAS_{SU1} = Total amounts of suspended solid in the 1st chamber of solid separating unit (mg)

TAS_{SU2} = Total amounts of suspended solid in the 2nd chamber of solid separating unit (mg)

TAS_{RAS} = Total amounts of suspended solid in recirculating aquaculture system (mg)

Final TSS_{FT} = Total suspended solids concentration in fish tank at day 8 (mg/L)

Final TSS_{SU1} = Total amounts of suspended solid in the 1st chamber of solid separating unit at day 8 (mg/L)

Final TSS_{SU2} = Total amounts of suspended solid in the 2nd chamber of solid separating unit at day 8 (mg/L)

The total amounts of suspended solid in solid separating unit was compared with total amounts of suspended solid in recirculating aquaculture system for finding the percentage of solids retention in solid separating unit as the following equation.

$$\text{percentage of solids retention} = \frac{TAS_{SU1} + TAS_{SU2}}{TAS_{RAS}}$$

B8. Analysis of total alkalinity

The method that was used in this study was applied from 2320 B. titration method [59] that had procedure as follow.

Reagent preparation

1. Sodium carbonate solution, approximately 0.05N

Dry 3 to 5 g primary standard Na_2CO_3 at 250°C for 4 h and cool in a desiccator. Weigh 2.5 ± 0.2 g (to the nearest mg), transfer to a 1-L volumetric flask, fill flask to the mark with distilled water, and dissolve and mix reagent. Do not keep longer than 1 week.

2. Standard sulfuric acid solution 0.1000 N

Prepare acid solution of approximate normality as indicated under Preparation of Desk Reagents. Standardize against 40.00 mL 0.05N Sodium carbonate solution, with about 60 mL water, in a beaker by titrating potentiometrically to pH of about 5. Lift out electrodes, rinse into the same beaker, and boil gently for 3 to 5 min under a watch glass cover. Cool to room temperature, rinse cover glass into beaker, and finish titrating to the pH inflection point. Calculate normality:

$$N = \frac{(M \times V)}{(53.00 \times C)}$$

where

M	=	Na_2CO_3 weighed into 1-L flask (g)
V	=	Na_2CO_3 solution taken for titration (mL)
C	=	Acid used (mL)

3. Standard sulfuric acid, 0.02N

Dilute 200.00 mL 0.1000 N standard acid to 1,000 mL with distilled or deionized water. Standardize by potentiometric titration of 15.00 mL 0.05N Na_2CO_3 according to the procedure of Standard sulfuric acid 0.1000 N preparation; 1 mL = 1.00 mg CaCO_3 .

4. Methyl orange indicator solution, pH 4.4 indicator

Dissolve 500 mg methyl orange, in distilled water and adjust the solution's volume to 100 mL with distilled water.

5. Sodium thiosulfate solution, 0.1M

Dissolve 25 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ and dilute to 1,000 mL with distilled water.

Analysis procedure

The water samples with the volume at least 15 mL has to be filtrated with Whatman GF/C filter. After that, the samples should be instantly analyzed or store them at temperature below -20°C if the analysis can't be performed right now.

Adjust sample to room temperature, if necessary, and with a pipet discharge sample into an erlenmeyer flask, while keeping pipet tip near flask bottom. If free residual chlorine is present add 0.05 mL (1 drop) 0.1M Sodium thiosulfate solution or destroy with ultraviolet radiation. Add 0.2 mL(5 drops) methyl orange indicator solution and titrate over to a persistent color change characteristic of the equivalence point.(pH = 4.4 Commercial indicator solutions or solids designated for the appropriate pH(4.4) may be used. Check color at end point by adding the same concentration of indicator used with sample to a buffer solution at the designated pH. The total alkalinity could calculate with the equation below.

$$\text{Total alkalinity} \left(\frac{\text{mg CaCO}_3}{\text{L}} \right) = \left(\frac{A \times N \times 50,000}{V} \right)$$

$$\text{Total alkalinity, mg CaCO}_3 / \text{L} = (A \times N \times 50,000) / \text{mL sample}$$

Where

A	=	Volume of standard acid used (mL)
N	=	Normality of standard acid (N)
V	=	Volume of sample (mL)

B9. Calculation of dissolved carbon dioxide and dissolved inorganic carbon

The dissolved carbon dioxide can compute from sample total alkalinity and pH base on 4500-CO₂ D. Carbon Dioxide and Forms of Alkalinity by Calculation [59]. Total alkalinity that is allowed to use is due almost entirely to hydroxides, carbonates, or bicarbonates, and the total dissolved solids is not greater than 500 mg/L. The alkalinity forms and free CO₂ can be calculated from the sample pH and total alkalinity with the following equation.

a. Bicarbonate alkalinity:

$$HCO_3^- \text{ as mg } CaCO_3/L = \frac{T - 5.0 \times 10^{(pH-10)}}{1 + 0.94 \times 10^{(pH-10)}}$$

where:

$$T = \text{total alkalinity, mg } CaCO_3/L$$

b. Carbonate alkalinity:

$$CO_3^{2-} \text{ as mg } CaCO_3/L = 0.94 \times B \times 10^{(pH-10)}$$

where:

$$B = \text{bicarbonate alkalinity, from a.}$$

c. Hydroxide alkalinity:

$$OH^- \text{ as mg } CaCO_3/L = 5.0 \times 10^{(pH-10)}$$

d. Free carbon dioxide:

$$\text{Dissolved } CO_2 \text{ as mg } CO_2/L = 2.0 \times B \times 10^{(6-pH)}$$

where:

$$B = \text{bicarbonate alkalinity, from a.}$$

e. Dissolved inorganic carbon (DIC):

$$DIC \text{ as mg } CO_2/L = A + 0.44(2B + C)$$

where:

$$A = \text{mg free } CO_2/L$$

$$B = \text{bicarbonate alkalinity from a}$$

$$C = \text{carbonate alkalinity from b.}$$

B10. Chlorophyll-a and total carotenoids content analysis

The method that was used in this study was applied from Spectrophotometric determination of Chlorophylls and Total Carotenoids [61] that had procedure as follow.

Pigments extracted procedure

5 mL of microalgal cells suspension was filled in 15 ml centrifuge tube and was separated the liquid out by centrifugation. Then, the microalgal cells was broken by homogenization in the dark and was extracted the pigments with 90% acetone. The extracted suspension was kept in refrigerator in completely darkness for 24 hours. After that the extracted suspension was centrifuged and separated the supernatant to analyze the pigments content. The remained microalgal cells was extracted again if there were some pigments left in microalgal cells and the supernatant from extraction was pooled together with the supernatant from the first extraction before analyzing the pigments content.

Analysis procedure

The supernatant from extraction was measured the absorbance at 665, 645, 630 and 480 nm with spectrophotometer and using glass or quartz cuvette for measurement. Calculate the chlorophyll-a content and total carotenoid content by using the equation below.

$$\text{Chlorophyll} - a \left(\frac{mg}{g} \right) = \frac{(11.6E_{665} - 1.31E_{645} - 0.014E_{630}) \times \left(\frac{V_a}{V_b} \right)}{DW}$$

$$\text{Total carotenoid} \left(\frac{mg}{g} \right) = \frac{(4 \times E_{480}) \times \left(\frac{V_a}{V_b} \right)}{DW}$$

Where E_{665} , E_{645} , E_{630} and E_{480} = Absorbance at 665, 645, 630 and 480 nm

V_a = Volume of 90% acetone (mL)

V_b = Volume of microalgal cells suspension (mL)

DW = Cells dried weight of microalgal cells suspension (g/L)

B11. Carotenoids profile and lutein content analysis

The method that was used in this study was applied from Spectrophotometric determination of Total Carotenoids [61] and pigment determination from the literature of Mantoura and Wrigh (1997) [82] that had procedure as follow.

Pigments extracted procedure

5 mL of microalgal cells suspension was filled in 15 ml centrifuge tube and was separated the liquid out by centrifugation. Then, the microalgal cells was broken by homogenization in the dark and was extracted the pigments with 90% acetone. The extracted suspension was kept in refrigerator in completely darkness for 24 hours. After that the extracted suspension was centrifuged and separated the supernatant then this supernatant was filtrate through 0.45 μm pore size Nylon membrane filter before analyzing the pigments profile and lutein content with High Performance Liquid Chromatography. The remained microalgal cells was extracted again if there were some pigments left in microalgal cells and the filtrated supernatant from extraction was pooled together with the supernatant from the first extraction before analyzing the pigments profile and lutein content.

Mobile phase preparation

The mobile phase that was used in High-Performance Liquid Chromatography (Shimadzu Model SPD-M20A) was isocratic mobile phase that could prepare by filtrated the 1 mL deionized water, 100 mL HPLC grade methanol, 799 mL HPLC grade acetonitrile and 100 mL HPLC grade dichloromethane through 0.45 pore size membrane filter and mixed together in glass bottle.

Analysis procedure

20 μL supernatant sample from extraction was fed in C18 column of High-Performance Liquid Chromatography (Shimadzu Model SPD-M20A) with Diode Array Detector for detecting the chromatogram at 400 nm for 30 minutes. The type of carotenoids which were the peaks on chromatogram could determine by comparing the peak spectrum, e.g. lutein (Fig B.6) with data in literature of Mantoura and Wrigh (1997).

The area under lutein peak was compared with lutein standard curve (Fig B.7) for analyzing lutein concentration of supernatant and used this lutein concentration for calculating the lutein content with the equation below.

$$\text{Lutein } \left(\frac{\text{mg}}{\text{g}}\right) = \frac{\text{Lutein concentration} \left(\frac{\text{mg}}{\text{L}}\right)}{\text{DW}}$$

$$\text{Lutein (mg/g)} = \text{Lutein concentration (mg/L)} / \text{DW}$$

Where DW = Cells dried weight of microalgal cells suspension (g/L)

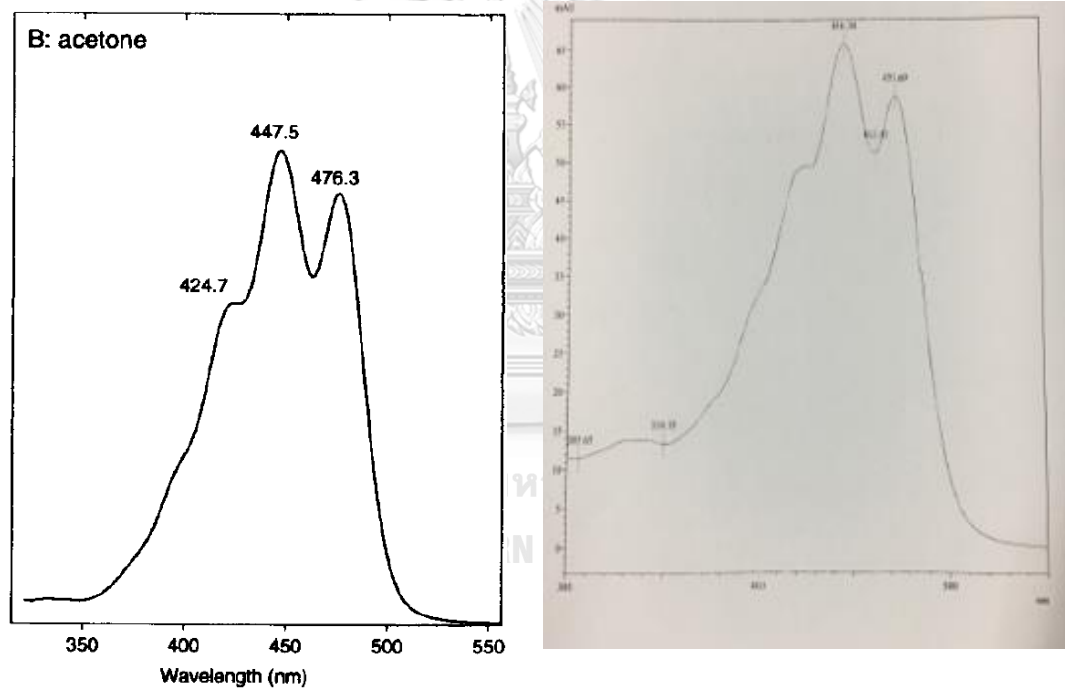


Figure B6 (Left) Lutein spectrum from Mantoura and Wrigh (1997) and (Right) Lutein spectrum from High-Performance Liquid Chromatography (Shimadzu Model SPD-M20A)

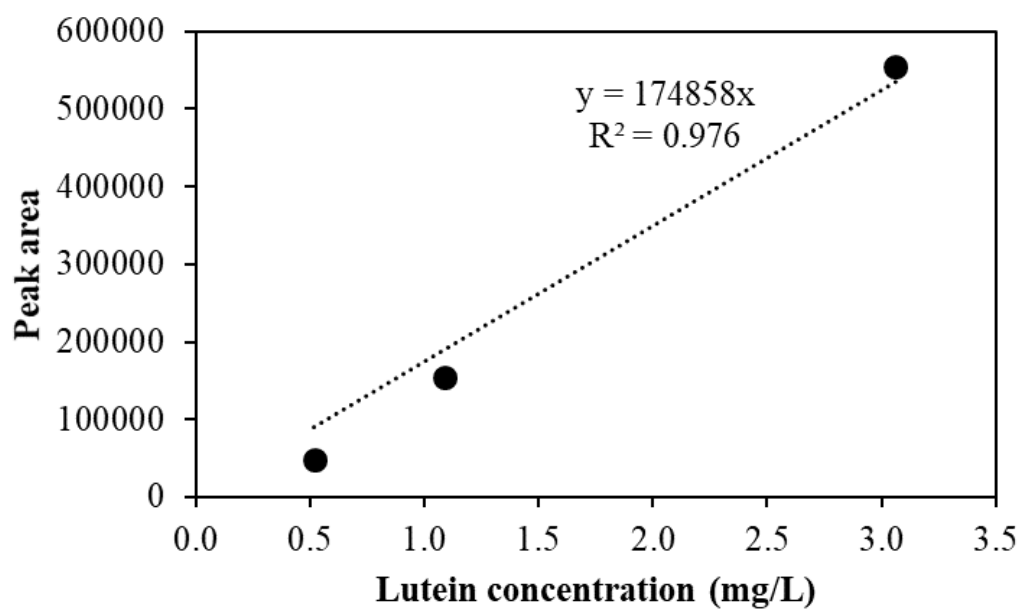


Figure B7 Lutein standard curve from High-Performance Liquid Chromatography (Shimadzu Model SPD-M20A)



Appendix C

C1. Carbon dioxide unit conversion

To calculate the mass of carbon dioxide, every hour carbon dioxide concentrations had to change their units from ppm to mg/m³ as following equation.

$$CCO_2\left(\frac{mg}{m^3}\right) = \frac{CCO_2(ppm) \times 44}{24.45}$$

Where CCO₂ (mg/m³) = Carbon dioxide concentration in mg/m³ unit

CCO₂ (ppm) = Carbon dioxide concentration in ppm unit

44 = molecular weight of carbon dioxide (g/mol)

24.45 = Volume of 1 mol air at 25°C 1 atm

Which 1 ppm carbon dioxide was equal to 1.80 mg/m³.

C2. Hourly carbon dioxide mass calculation

Each carbon dioxide concentrations in mg/m³ unit were calculation with volume of 1 hour flowed air to find the mass of carbon dioxide every hour by these following equations.

$$VA_x(m^3) = F_x\left(\frac{L}{min}\right) \times \frac{60(min)}{1,000\left(\frac{L}{m^3}\right)}$$

Where VA_x = Volume of 1 hour flowed air from x unit (m³)

F_x = Flow rate of aeration from x unit (L/min)

X unit = Carbon dioxide source; IF is Inlet Fish tank, IS is Inlet Solids-liquid separating unit, OF is Outlet Fish tank, OS is Outlet Solids-liquid separating unit, BR is Basic respiration and OM is Outlet microalgal cultivation

$$HCO2_{xn}(mg) = CCO2_{xn}\left(\frac{mg}{m^3}\right) \times VA_x(m^3)$$

- Where $HCO2_{xn}$ = Hourly carbon dioxide mass from x unit at t_n (mg)
 $CCO2_{xn}$ = Carbon dioxide concentration from x unit at t_n
 (mg/m³)
 VA_x = Volume of 1 hour flowed air from x unit (m³)
 t_n = Cultivating time (hour)

C3. Total carbon dioxide mass calculation

All values of hourly carbon dioxide mass from each unit were used for calculating total carbon dioxide mass of that unit as the following equations.

C3.1 Total of Fish tank inlet carbon dioxide

$$TCO2_{IF} = \left[\sum_{n=1}^{tc} HCO2_{IFn} \right] \div 1,000 \left(\frac{g}{mg} \right)$$

- Where $TCO2_{IF}$ = Total of Fish tank inlet carbon dioxide (g)
 tc = Total cultivating time (hour)

C3.2 Total of Solids-liquid separating unit inlet carbon dioxide

$$TCO2_{IS} = \left[\sum_{n=1}^{tc} HCO2_{ISn} \right] \div 1,000 \left(\frac{g}{mg} \right)$$

- Where $TCO2_{IS}$ = Total of solid separating unit inlet carbon dioxide (g)

C3.3 Total of Fish tank outlet carbon dioxide

$$TCO2_{OF} = \left[\sum_{n=1}^{tc} HCO2_{OFn} \right] \div 1,000 \left(\frac{g}{mg} \right)$$

- Where $TCO2_{OF}$ = Total of fish tank Outlet carbon dioxide (g)

C3.4 Total of Solid separating unit outlet carbon dioxide

$$TCO2_{OS} = \left[\sum_{n=1}^{tc} HCO2_{OSn} \right] \div 1,000 \left(\frac{g}{mg} \right)$$

- Where $TCO2_{OS}$ = Total of solid separating unit outlet carbon dioxide (g)

C3.5 Total of carbon dioxide production from basic respiration

$$TCO2_{BR} = \left\{ \left[\sum_{n=1}^{tc} HCO2_{BRn} \right] \div 1,000 \right\} - TCO2_{IS}$$

Where $TCO2_{BR}$ = Total of carbon dioxide production from basic respiration (g)

C3.6 Total of carbon dioxide production during feeding process

$$TCO2_{FEED} = TCO2_{OF} - TCO2_{BR}$$

Where $TCO2_{FEED}$ = Total of carbon dioxide production from basic respiration (g)

C3.7 Total of gained carbon dioxide from Fish Tank

$$TCO2_{GF} = TCO2_{FEED} + TCO2_{BR}$$

Where $TCO2_{GF}$ = Total of gained carbon dioxide from Fish tank (g)

C3.8 Total of gained carbon dioxide from Solid separating unit

$$TCO2_{GS} = TCO2_{OS} - TCO2_{IS}$$

Where $TCO2_{GS}$ = Total of gained carbon dioxide from Solid separating unit (g)

C3.9 Total of Microalgal cultivation inlet air using Fish tank outlet air

$$TCO2_{IM} = TCO2_{OF}$$

Where $TCO2_{IM}$ = Total of microalgal cultivation inlet air (g)

C3.10 Total of Microalgal cultivation outlet air

$$TCO2_{OM} = \left[\sum_{n=1}^{tc} HCO2_{OMn} \right] \div 1,000 \left(\frac{g}{mg} \right)$$

Where $TCO2_{OM}$ = Total of microalgal cultivation outlet air (g)

C3.11 Total sequestrated carbon dioxide during microalgal cultivation

$$TCO2_{SQ} = TCO2_{IM} - TCO2_{OM}$$

Where $TCO2_{SQ}$ = Total of sequestrated carbon dioxide during microalgal cultivation (g)

C4. Rate of carbon dioxide production calculation

The rate of carbon dioxide production could calculate from Total of carbon dioxide production from basic respiration, Total of carbon dioxide production during feeding process and Total feed input which were happen in Fish Tank by using the equation as follow.

C4.1 Rate of carbon dioxide production from basic respiration

$$RCO2_{BR} = \frac{TCO2_{BR}(g) \times 1,000\left(\frac{g}{kg}\right) \times 24\left(\frac{hour}{day}\right)}{DFI(g) \times tc(hour)}$$

Where $RCO2_{BR}$ = Rate of carbon dioxide production from basic respiration (g CO₂/kg feed · day)

DFI = Daily feed input to fish tank during Nile tilapia cultivation (kg)

C4.2 Rate of carbon dioxide production during feeding process

$$RCO2_{FEED} = \frac{TCO2_{FEED}(g) \times 1,000\left(\frac{g}{kg}\right) \times 24\left(\frac{hour}{day}\right)}{DFI(g) \times tc(hour)}$$

Where $RCO2_{FEED}$ = Rate of carbon dioxide production during feeding process (g CO₂/kg feed · day)

C4.3 Rate of carbon dioxide production from fish tank

$$RCO2_{FT} = RCO2_{BR} + RCO2_{FEED}$$

Where $RCO2_{FT}$ = Rate of carbon dioxide production from fish tank (g CO₂/kg feed · day)

Appendix D

Table D1 Cell number density of *Scenedesmus armatus* during the cultivation in 1-L photobioreactor using BG-11 medium and using effluent from nitrifying biofilter.

Cultivating time (days)	Cell number density of BG-11 (10^4 cells / mL)				
	1	2	3	Average	S.D.
0	19.93	19.03	19.23	19.40	0.47
1	31.90	26.90	31.47	30.09	2.77
2	96.83	75.00	88.67	86.83	11.03
3	149.67	180.83	185.00	171.83	19.31
4	195.83	182.50	325.00	234.44	78.71
5	240.83	298.33	326.67	288.61	43.73
6	239.17	483.33	362.50	361.67	122.09
7	325.83	516.67	566.67	469.72	127.09
8	446.67	568.33	644.17	553.06	99.63
9	493.33	600.83	716.67	603.61	111.69
Cultivating time (days)	Cell number density of Effluent from nitrifying biofilter (10^4 cells / mL)				
	1	2	3	Average	S.D.
0	13.57	10.70	12.40	12.22	1.44
1	74.00	68.67	83.17	75.28	7.33
2	245.00	256.67	190.83	230.83	35.13
3	318.33	329.17	330.00	325.83	6.51
4	386.67	417.50	490.83	431.67	53.51
5	421.67	463.33	528.33	471.11	53.76
6	464.17	626.67	708.33	599.72	124.29
7	730.83	625.00	590.83	648.89	72.99
8	416.67	591.67	612.50	540.28	107.56
9	524.17	745.83	666.67	645.56	112.33

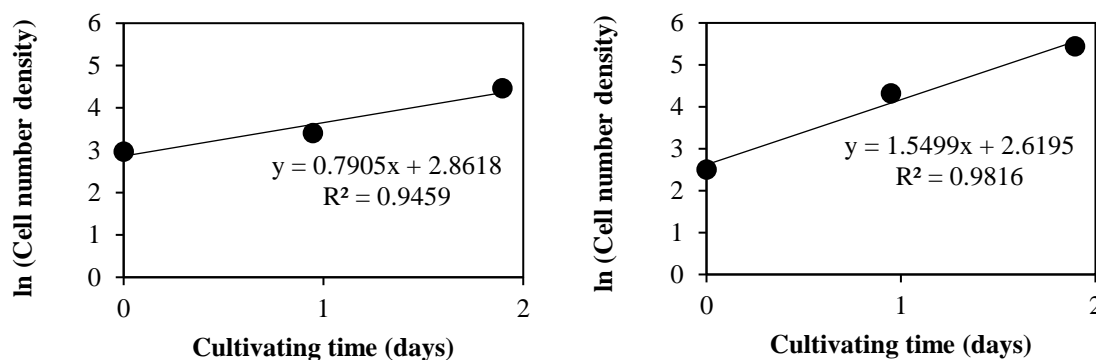


Figure D1 Maximum specific growth rate of *Scenedesmus armatus* during the cultivation in 1-L photobioreactor using BG-11 medium (left) and using effluent from nitrifying biofilter (Right).

Table D2 Biomass of *Scenedesmus armatus* during the cultivation in 1-L photobioreactor using BG-11 medium and using effluent from nitrifying biofilter.

Cultivating time (days)	Biomass (mg/L)	
	BG-11 (Control)	Effluent from nitrifying biofilter
0	32.67	32.67
1	84.44	66.67
2	75.56	122.22
3	162.22	246.67
4	291.11	273.33
5	340.00	353.33
6	431.11	371.11
7	486.67	540.00
8	571.11	604.44
9	675.56	664.44

Table D3 Nitrate concentration of *Scenedesmus armatus* during the cultivation in 1-L photobioreactor using BG-11 medium and using effluent from nitrifying biofilter.

Cultivating time (days)	Nitrate concentration (mg/L)	
	BG-11 (Control)	Effluent from nitrifying biofilter
0	277.20	25.59
1	257.70	23.51
2	250.59	18.96
3	243.37	14.64
4	247.68	10.19
5	251.13	6.59
6	230.66	4.17
7	236.37	3.92
8	249.30	2.35
9	229.58	3.01

Table D4 Phosphate concentration of *Scenedesmus armatus* during the cultivation in 1-L photobioreactor using BG-11 medium and using effluent from nitrifying biofilter.

Cultivating time (days)	Phosphate concentration (mg/L)	
	BG-11 (Control)	Effluent from nitrifying biofilter
0	4.97	0.52
1	4.10	0.11
2	4.80	0.22
3	4.29	0.22
4	3.71	0.21
5	3.55	0.16
6	2.25	0.16
7	2.21	0.21
8	2.65	0.22
9	2.28	0.290

Table D5 Pigments content in *Scenedesmus armatus* at the end of cultivation on day 9.

Pigment	Pigment content (mg/g)									
	BG-11 (Control)					Effluent from nitrifying biofilter				
	1	2	3	Average	S.D.	1	2	3	Average	S.D.
Chlorophyll a	22.48	19.43	8.34	16.72	7.40	14.37	13.64	3.54	10.52	6.06
Total carotenoids	5.10	3.48	3.29	3.96	1.00	4.78	3.61	0.89	3.09	2.00
Lutein	2.30	1.23	1.22	1.59	0.62	1.64	1.40	0.31	1.12	0.71



Appendix E

Table E1 the concentrations and mass of carbon dioxide in air that flow in/out Fish tank during Nile tilapia cultivation including basic respiration (replication 1).

Cultivating time (Hour)	Fish tank inlet carbon dioxide			Fish tank outlet carbon dioxide			Basic respiration		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)	CCO _{2BR} (ppm)	CCO _{2BR} (mg/m ³)	HCO _{2BR} (mg)
1	568.05	1,022.25	147.20	926.85	1,667.95	240.18	926.85	1,667.95	240.18
2	568.05	1,022.25	147.20	982.08	1,767.34	254.50	937.59	1,687.27	242.97
3	568.05	1,022.25	147.20	1,016.59	1,829.45	263.44	937.59	1,687.27	242.97
4	568.05	1,022.25	147.20	1,036.98	1,866.13	268.72	937.59	1,687.27	242.97
5	568.05	1,022.25	147.20	1,021.83	1,838.88	264.80	937.59	1,687.27	242.97
6	568.05	1,022.25	147.20	1,000.01	1,799.61	259.14	937.59	1,687.27	242.97
7	568.05	1,022.25	147.20	990.62	1,782.72	256.71	937.59	1,687.27	242.97
8	568.05	1,022.25	147.20	973.20	1,751.36	252.20	937.59	1,687.27	242.97
9	568.05	1,022.25	147.20	951.97	1,713.15	246.69	937.59	1,687.27	242.97
10	568.05	1,022.25	147.20	937.59	1,687.28	242.97	937.59	1,687.27	242.97
11	568.05	1,022.25	147.20	946.89	1,704.02	245.38	937.59	1,687.27	242.97
12	568.05	1,022.25	147.20	959.07	1,725.94	248.54	937.59	1,687.27	242.97
13	568.05	1,022.25	147.20	968.38	1,742.68	250.95	937.59	1,687.27	242.97
14	568.05	1,022.25	147.20	969.05	1,743.90	251.12	937.59	1,687.27	242.97
15	568.05	1,022.25	147.20	955.01	1,718.63	247.48	937.59	1,687.27	242.97
16	568.05	1,022.25	147.20	948.33	1,706.61	245.75	937.59	1,687.27	242.97
17	568.05	1,022.25	147.20	946.39	1,703.11	245.25	937.59	1,687.27	242.97
18	568.05	1,022.25	147.20	959.16	1,726.09	248.56	937.59	1,687.27	242.97
19	568.05	1,022.25	147.20	963.64	1,734.16	249.72	937.59	1,687.27	242.97
20	568.05	1,022.25	147.20	957.13	1,722.44	248.03	937.59	1,687.27	242.97
21	568.05	1,022.25	147.20	945.54	1,701.59	245.03	937.59	1,687.27	242.97
22	558.15	1,004.44	144.64	949.26	1,708.28	245.99	949.26	1,708.28	245.99
23	558.15	1,004.44	144.64	1,036.30	1,864.91	268.55	1,036.30	1,864.91	268.55
24	558.15	1,004.44	144.64	1,116.31	2,008.91	289.28	1,036.72	1,865.67	268.66
25	558.15	1,004.44	144.64	1,113.02	2,002.97	288.43	1,036.72	1,865.67	268.66
26	558.15	1,004.44	144.64	1,113.78	2,004.34	288.63	1,036.72	1,865.67	268.66
27	558.15	1,004.44	144.64	1,131.20	2,035.70	293.14	1,036.72	1,865.67	268.66
28	558.15	1,004.44	144.64	1,157.68	2,083.34	300.00	1,036.72	1,865.67	268.66
29	558.15	1,004.44	144.64	1,162.84	2,092.63	301.34	1,036.72	1,865.67	268.66
30	558.15	1,004.44	144.64	1,152.60	2,074.21	298.69	1,036.72	1,865.67	268.66
31	558.15	1,004.44	144.64	1,137.38	2,046.81	294.74	1,036.72	1,865.67	268.66
32	558.15	1,004.44	144.64	1,122.24	2,019.56	290.82	1,036.72	1,865.67	268.66
33	558.15	1,004.44	144.64	1,097.20	1,974.51	284.33	1,036.72	1,865.67	268.66
34	558.15	1,004.44	144.64	1,069.29	1,924.28	277.10	1,036.72	1,865.67	268.66
35	558.15	1,004.44	144.64	1,056.68	1,901.60	273.83	1,036.72	1,865.67	268.66
36	558.15	1,004.44	144.64	1,055.25	1,899.01	273.46	1,036.72	1,865.67	268.66
37	558.15	1,004.44	144.64	1,053.98	1,896.73	273.13	1,036.72	1,865.67	268.66
38	558.15	1,004.44	144.64	1,052.45	1,893.99	272.73	1,036.72	1,865.67	268.66
39	558.15	1,004.44	144.64	1,040.02	1,871.61	269.51	1,036.72	1,865.67	268.66
40	558.15	1,004.44	144.64	1,036.72	1,865.67	268.66	1,036.72	1,865.67	268.66
41	558.15	1,004.44	144.64	1,047.29	1,884.70	271.40	1,036.72	1,865.67	268.66
42	558.15	1,004.44	144.64	1,040.78	1,872.98	269.71	1,036.72	1,865.67	268.66
43	558.15	1,004.44	144.64	1,057.70	1,903.42	274.09	1,036.72	1,865.67	268.66
44	558.15	1,004.44	144.64	1,074.02	1,932.80	278.32	1,036.72	1,865.67	268.66
45	558.15	1,004.44	144.64	1,038.75	1,869.33	269.18	1,036.72	1,865.67	268.66
46	558.15	1,004.44	144.64	1,008.30	1,814.53	261.29	818.83	1,473.57	212.19
47	561.96	1,011.29	145.63	877.75	1,579.59	227.46	818.83	1,473.57	212.19
48	561.96	1,011.29	145.63	875.34	1,575.25	226.84	818.83	1,473.57	212.19
49	561.96	1,011.29	145.63	941.40	1,694.13	243.95	818.83	1,473.57	212.19
50	561.96	1,011.29	145.63	924.14	1,663.08	239.48	818.83	1,473.57	212.19

Cultivating time (Hour)	Fish tank inlet carbon dioxide			Fish tank outlet carbon dioxide			Basic respiration		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)	CCO _{2BR} (ppm)	CCO _{2BR} (mg/m ³)	HCO _{2BR} (mg)
51	561.96	1,011.29	145.63	903.16	1,625.33	234.05	818.83	1,473.57	212.19
52	561.96	1,011.29	145.63	892.25	1,605.69	231.22	818.83	1,473.57	212.19
53	561.96	1,011.29	145.63	889.38	1,600.51	230.47	818.83	1,473.57	212.19
54	561.96	1,011.29	145.63	894.03	1,608.89	231.68	818.83	1,473.57	212.19
55	561.96	1,011.29	145.63	883.46	1,589.86	228.94	818.83	1,473.57	212.19
56	561.96	1,011.29	145.63	873.22	1,571.44	226.29	818.83	1,473.57	212.19
57	561.96	1,011.29	145.63	863.41	1,553.78	223.75	818.83	1,473.57	212.19
58	561.96	1,011.29	145.63	852.58	1,534.30	220.94	818.83	1,473.57	212.19
59	561.96	1,011.29	145.63	837.87	1,507.82	217.13	818.83	1,473.57	212.19
60	561.96	1,011.29	145.63	831.27	1,495.94	215.42	818.83	1,473.57	212.19
61	561.96	1,011.29	145.63	831.52	1,496.40	215.48	818.83	1,473.57	212.19
62	561.96	1,011.29	145.63	826.19	1,486.81	214.10	818.83	1,473.57	212.19
63	561.96	1,011.29	145.63	830.85	1,495.18	215.31	818.83	1,473.57	212.19
64	561.96	1,011.29	145.63	818.83	1,473.57	212.19	818.83	1,473.57	212.19
65	561.96	1,011.29	145.63	814.27	1,465.35	211.01	814.27	1,465.35	211.01
66	561.96	1,011.29	145.63	825.35	1,485.29	213.88	818.83	1,473.57	212.19
67	561.96	1,011.29	145.63	822.81	1,480.72	213.22	818.83	1,473.57	212.19
68	561.96	1,011.29	145.63	820.10	1,475.85	212.52	818.83	1,473.57	212.19
69	561.96	1,011.29	145.63	876.69	1,577.68	227.19	818.83	1,473.57	212.19
70	564.49	1,015.86	146.28	927.69	1,669.47	240.40	847.25	1,524.71	219.56
71	564.49	1,015.86	146.28	948.59	1,707.07	245.82	847.25	1,524.71	219.56
72	564.49	1,015.86	146.28	948.08	1,706.15	245.69	847.25	1,524.71	219.56
73	564.49	1,015.86	146.28	948.42	1,706.76	245.77	847.25	1,524.71	219.56
74	564.49	1,015.86	146.28	946.81	1,703.87	245.36	847.25	1,524.71	219.56
75	564.49	1,015.86	146.28	958.90	1,725.64	248.49	847.25	1,524.71	219.56
76	564.49	1,015.86	146.28	1,002.97	1,804.94	259.91	847.25	1,524.71	219.56
77	564.49	1,015.86	146.28	1,042.81	1,876.63	270.24	847.25	1,524.71	219.56
78	564.49	1,015.86	146.28	1,061.59	1,910.43	275.10	847.25	1,524.71	219.56
79	564.49	1,015.86	146.28	1,051.78	1,892.77	272.56	847.25	1,524.71	219.56
80	564.49	1,015.86	146.28	1,037.48	1,867.04	268.85	847.25	1,524.71	219.56
81	564.49	1,015.86	146.28	1,025.13	1,844.82	265.65	847.25	1,524.71	219.56
82	564.49	1,015.86	146.28	1,017.86	1,831.73	263.77	847.25	1,524.71	219.56
83	564.49	1,015.86	146.28	1,001.53	1,802.35	259.54	847.25	1,524.71	219.56
84	564.49	1,015.86	146.28	985.38	1,773.28	255.35	847.25	1,524.71	219.56
85	564.49	1,015.86	146.28	896.57	1,613.45	232.34	847.25	1,524.71	219.56
86	564.49	1,015.86	146.28	871.36	1,568.09	225.81	847.25	1,524.71	219.56
87	564.49	1,015.86	146.28	860.20	1,548.00	222.91	847.25	1,524.71	219.56
88	564.49	1,015.86	146.28	855.04	1,538.72	221.58	847.25	1,524.71	219.56
89	564.49	1,015.86	146.28	859.94	1,547.54	222.85	847.25	1,524.71	219.56
90	564.49	1,015.86	146.28	853.26	1,535.52	221.11	847.25	1,524.71	219.56
91	564.49	1,015.86	146.28	847.25	1,524.71	219.56	847.25	1,524.71	219.56
92	564.49	1,015.86	146.28	895.13	1,610.87	231.96	847.25	1,524.71	219.56
93	564.49	1,015.86	146.28	921.77	1,658.81	238.87	847.25	1,524.71	219.56
94	596.72	1,073.85	154.63	874.32	1,573.42	226.57	847.25	1,524.71	219.56
95	596.72	1,073.85	154.63	824.33	1,483.46	213.62	824.33	1,483.46	213.62
96	596.72	1,073.85	154.63	903.68	1,626.26	234.18	885.67	1,593.84	229.51
97	596.72	1,073.85	154.63	995.25	1,791.05	257.91	885.67	1,593.84	229.51
98	596.72	1,073.85	154.63	1,001.15	1,801.67	259.44	885.67	1,593.84	229.51
99	596.72	1,073.85	154.63	1,022.26	1,839.65	264.91	885.67	1,593.84	229.51
100	596.72	1,073.85	154.63	1,014.79	1,826.21	262.97	885.67	1,593.84	229.51
101	596.72	1,073.85	154.63	998.89	1,797.60	258.85	885.67	1,593.84	229.51
102	596.72	1,073.85	154.63	1,020.88	1,837.17	264.55	885.67	1,593.84	229.51
103	596.72	1,073.85	154.63	1,067.83	1,921.65	276.72	885.67	1,593.84	229.51
104	596.72	1,073.85	154.63	1,094.11	1,968.95	283.53	885.67	1,593.84	229.51
105	596.72	1,073.85	154.63	1,101.74	1,982.69	285.51	885.67	1,593.84	229.51
106	596.72	1,073.85	154.63	1,098.78	1,977.36	284.74	885.67	1,593.84	229.51
107	596.72	1,073.85	154.63	1,104.18	1,987.07	286.14	885.67	1,593.84	229.51
108	596.72	1,073.85	154.63	1,103.29	1,985.47	285.91	885.67	1,593.84	229.51
109	596.72	1,073.85	154.63	1,107.12	1,992.36	286.90	885.67	1,593.84	229.51
110	596.72	1,073.85	154.63	1,078.53	1,940.91	279.49	885.67	1,593.84	229.51

Cultivating time (Hour)	Fish tank inlet carbon dioxide			Fish tank outlet carbon dioxide			Basic respiration		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)	CCO _{2BR} (ppm)	CCO _{2BR} (mg/m ³)	HCO _{2BR} (mg)
111	596.72	1,073.85	154.63	1,051.86	1,892.92	272.58	885.67	1,593.84	229.51
112	596.72	1,073.85	154.63	1,047.19	1,884.51	271.37	885.67	1,593.84	229.51
113	596.72	1,073.85	154.63	1,037.10	1,866.36	268.76	885.67	1,593.84	229.51
114	596.72	1,073.85	154.63	1,024.71	1,844.06	265.54	885.67	1,593.84	229.51
115	596.72	1,073.85	154.63	1,016.68	1,829.60	263.46	885.67	1,593.84	229.51
116	596.72	1,073.85	154.63	1,011.07	1,819.51	262.01	885.67	1,593.84	229.51
117	559.42	1,006.72	144.97	1,007.03	1,812.25	260.96	885.67	1,593.84	229.51
118	559.42	1,006.72	144.97	974.05	1,752.88	252.42	885.67	1,593.84	229.51
119	559.42	1,006.72	144.97	971.17	1,747.71	251.67	885.67	1,593.84	229.51
120	559.42	1,006.72	144.97	958.90	1,725.64	248.49	885.67	1,593.84	229.51
121	559.42	1,006.72	144.97	953.62	1,716.12	247.12	885.67	1,593.84	229.51
122	559.42	1,006.72	144.97	949.83	1,709.31	246.14	885.67	1,593.84	229.51
123	559.42	1,006.72	144.97	953.96	1,716.73	247.21	885.67	1,593.84	229.51
124	559.42	1,006.72	144.97	943.81	1,698.47	244.58	885.67	1,593.84	229.51
125	559.42	1,006.72	144.97	925.60	1,665.70	239.86	885.67	1,593.84	229.51
126	559.42	1,006.72	144.97	914.18	1,645.15	236.90	885.67	1,593.84	229.51
127	559.42	1,006.72	144.97	903.61	1,626.13	234.16	885.67	1,593.84	229.51
128	559.42	1,006.72	144.97	898.26	1,616.50	232.78	885.67	1,593.84	229.51
129	559.42	1,006.72	144.97	892.91	1,606.87	231.39	885.67	1,593.84	229.51
130	559.42	1,006.72	144.97	880.05	1,583.73	228.06	880.05	1,583.73	228.06
131	559.42	1,006.72	144.97	877.85	1,579.78	227.49	877.85	1,579.78	227.49
132	559.42	1,006.72	144.97	879.63	1,582.97	227.95	879.63	1,582.97	227.95
133	559.42	1,006.72	144.97	883.52	1,589.97	228.96	883.52	1,589.97	228.96
134	559.42	1,006.72	144.97	882.48	1,588.11	228.69	882.48	1,588.11	228.69
135	559.42	1,006.72	144.97	880.56	1,584.65	228.19	880.56	1,584.65	228.19
136	559.42	1,006.72	144.97	880.45	1,584.46	228.16	880.45	1,584.46	228.16
137	559.42	1,006.72	144.97	885.68	1,593.86	229.52	885.67	1,593.84	229.51
138	559.42	1,006.72	144.97	879.76	1,583.20	227.98	879.76	1,583.20	227.98
139	559.42	1,006.72	144.97	878.30	1,580.57	227.60	878.30	1,580.57	227.60
140	563.73	1,014.49	146.09	865.17	1,556.94	224.20	845.22	1,521.06	219.03
141	563.73	1,014.49	146.09	904.28	1,627.34	234.34	845.22	1,521.06	219.03
142	563.73	1,014.49	146.09	931.33	1,676.01	241.35	845.22	1,521.06	219.03
143	563.73	1,014.49	146.09	981.07	1,765.52	254.23	845.22	1,521.06	219.03
144	563.73	1,014.49	146.09	992.82	1,786.67	257.28	845.22	1,521.06	219.03
145	563.73	1,014.49	146.09	1,004.58	1,807.83	260.33	845.22	1,521.06	219.03
146	563.73	1,014.49	146.09	1,001.45	1,802.20	259.52	845.22	1,521.06	219.03
147	563.73	1,014.49	146.09	990.37	1,782.26	256.65	845.22	1,521.06	219.03
148	563.73	1,014.49	146.09	982.33	1,767.80	254.56	845.22	1,521.06	219.03
149	563.73	1,014.49	146.09	979.04	1,761.86	253.71	845.22	1,521.06	219.03
150	563.73	1,014.49	146.09	971.59	1,748.47	251.78	845.22	1,521.06	219.03
151	563.73	1,014.49	146.09	968.21	1,742.38	250.90	845.22	1,521.06	219.03
152	563.73	1,014.49	146.09	959.92	1,727.46	248.75	845.22	1,521.06	219.03
153	563.73	1,014.49	146.09	942.24	1,695.65	244.17	845.22	1,521.06	219.03
154	563.73	1,014.49	146.09	940.72	1,692.91	243.78	845.22	1,521.06	219.03
155	563.73	1,014.49	146.09	921.94	1,659.12	238.91	845.22	1,521.06	219.03
156	563.73	1,014.49	146.09	898.85	1,617.56	232.93	845.22	1,521.06	219.03
157	563.73	1,014.49	146.09	902.23	1,623.65	233.81	845.22	1,521.06	219.03
158	563.73	1,014.49	146.09	896.48	1,613.30	232.32	845.22	1,521.06	219.03
159	563.73	1,014.49	146.09	881.17	1,585.75	228.35	845.22	1,521.06	219.03
160	563.73	1,014.49	146.09	867.98	1,562.00	224.93	845.22	1,521.06	219.03
161	563.73	1,014.49	146.09	855.97	1,540.39	221.82	845.22	1,521.06	219.03
162	563.73	1,014.49	146.09	847.00	1,524.26	219.49	845.22	1,521.06	219.03
163	563.73	1,014.49	146.09	851.48	1,532.32	220.65	845.22	1,521.06	219.03
164	563.73	1,014.49	146.09	887.52	1,597.17	229.99	845.22	1,521.06	219.03
165	563.73	1,014.49	146.09	919.32	1,654.40	238.23	845.22	1,521.06	219.03
166	564.24	1,015.40	146.22	917.37	1,650.90	237.73	821.12	1,477.68	212.79
167	564.24	1,015.40	146.22	964.32	1,735.38	249.89	821.12	1,477.68	212.79
168	564.24	1,015.40	146.22	963.30	1,733.55	249.63	821.12	1,477.68	212.79
169	564.24	1,015.40	146.22	938.27	1,688.50	243.14	821.12	1,477.68	212.79
170	564.24	1,015.40	146.22	928.96	1,671.75	240.73	821.12	1,477.68	212.79

Cultivating time (Hour)	Fish tank inlet carbon dioxide			Fish tank outlet carbon dioxide			Basic respiration		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)	CCO _{2BR} (ppm)	CCO _{2BR} (mg/m ³)	HCO _{2BR} (mg)
171	564.24	1,015.40	146.22	919.07	1,653.94	238.17	821.12	1,477.68	212.79
172	564.24	1,015.40	146.22	927.95	1,669.93	240.47	821.12	1,477.68	212.79
173	564.24	1,015.40	146.22	913.48	1,643.90	236.72	821.12	1,477.68	212.79
174	564.24	1,015.40	146.22	900.80	1,621.06	233.43	821.12	1,477.68	212.79
175	564.24	1,015.40	146.22	883.37	1,589.71	228.92	821.12	1,477.68	212.79
176	564.24	1,015.40	146.22	865.61	1,557.74	224.31	821.12	1,477.68	212.79
177	564.24	1,015.40	146.22	845.22	1,521.06	219.03	821.12	1,477.68	212.79
178	564.24	1,015.40	146.22	833.81	1,500.51	216.07	821.12	1,477.68	212.79
179	564.24	1,015.40	146.22	823.40	1,481.79	213.38	821.12	1,477.68	212.79
180	564.24	1,015.40	146.22	821.12	1,477.68	212.79	821.12	1,477.68	212.79
181	564.24	1,015.40	146.22	836.34	1,505.08	216.73	821.12	1,477.68	212.79
182	564.24	1,015.40	146.22	860.28	1,548.15	222.93	821.12	1,477.68	212.79
183	564.24	1,015.40	146.22	847.17	1,524.56	219.54	821.12	1,477.68	212.79
184	564.24	1,015.40	146.22	836.01	1,504.47	216.64	821.12	1,477.68	212.79
185	564.24	1,015.40	146.22	833.30	1,499.60	215.94	821.12	1,477.68	212.79
186	564.24	1,015.40	146.22	824.84	1,484.37	213.75	821.12	1,477.68	212.79
		Sum	27,327		Sum	45,901		Sum	42,568

Table E2 the concentrations and mass of carbon dioxide in air that flow in/out Fish tank during Nile tilapia cultivation including basic respiration (replication 2).

Cultivating time (Hour)	Fish tank inlet carbon dioxide			Fish tank outlet carbon dioxide			Basic respiration		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)	CCO _{2BR} (ppm)	CCO _{2BR} (mg/m ³)	HCO _{2BR} (mg)
1	552.82	994.85	143.26	843.45	1,517.86	218.57	851.90	1,533.07	218.57
2	552.82	994.85	143.26	925.16	1,664.90	239.75	851.90	1,533.07	220.76
3	552.82	994.85	143.26	936.66	1,685.60	242.73	851.90	1,533.07	220.76
4	552.82	994.85	143.26	965.76	1,737.97	250.27	851.90	1,533.07	220.76
5	552.82	994.85	143.26	973.71	1,752.27	252.33	851.90	1,533.07	220.76
6	552.82	994.85	143.26	944.44	1,699.61	244.74	851.90	1,533.07	220.76
7	552.82	994.85	143.26	930.74	1,674.95	241.19	851.90	1,533.07	220.76
8	552.82	994.85	143.26	919.40	1,654.55	238.26	851.90	1,533.07	220.76
9	552.82	994.85	143.26	905.53	1,629.59	234.66	851.90	1,533.07	220.76
10	552.82	994.85	143.26	883.37	1,589.71	228.92	851.90	1,533.07	220.76
11	552.82	994.85	143.26	871.53	1,568.40	225.85	851.90	1,533.07	220.76
12	552.82	994.85	143.26	867.47	1,561.09	224.80	851.90	1,533.07	220.76
13	552.82	994.85	143.26	856.47	1,541.30	221.95	851.90	1,533.07	220.76
14	552.82	994.85	143.26	865.27	1,557.13	224.23	851.90	1,533.07	220.76
15	552.82	994.85	143.26	872.21	1,569.62	226.02	851.90	1,533.07	220.76
16	552.82	994.85	143.26	860.37	1,548.31	222.96	851.90	1,533.07	220.76
17	552.82	994.85	143.26	851.91	1,533.08	220.76	851.90	1,533.07	220.76
18	552.82	994.85	143.26	859.52	1,546.78	222.74	851.90	1,533.07	220.76
19	552.82	994.85	143.26	863.41	1,553.78	223.75	851.90	1,533.07	220.76
20	552.82	994.85	143.26	867.98	1,562.00	224.93	851.90	1,533.07	220.76
21	552.82	994.85	143.26	895.89	1,612.24	232.16	851.90	1,533.07	220.76
22	555.87	1,000.33	144.05	903.16	1,625.33	234.05	851.90	1,533.07	220.76
23	555.87	1,000.33	144.05	891.49	1,604.32	231.02	851.90	1,533.07	220.76
24	555.87	1,000.33	144.05	1,019.89	1,835.38	264.30	844.00	1,518.85	218.72
25	555.87	1,000.33	144.05	1,084.51	1,951.68	281.04	844.00	1,518.85	218.72
26	555.87	1,000.33	144.05	1,045.60	1,881.66	270.96	844.00	1,518.85	218.72
27	555.87	1,000.33	144.05	1,031.90	1,857.00	267.41	844.00	1,518.85	218.72
28	555.87	1,000.33	144.05	1,041.54	1,874.35	269.91	844.00	1,518.85	218.72
29	555.87	1,000.33	144.05	1,075.55	1,935.54	278.72	844.00	1,518.85	218.72
30	555.87	1,000.33	144.05	1,104.13	1,986.99	286.13	844.00	1,518.85	218.72
31	555.87	1,000.33	144.05	1,121.22	2,017.74	290.55	844.00	1,518.85	218.72
32	555.87	1,000.33	144.05	1,120.88	2,017.13	290.47	844.00	1,518.85	218.72
33	555.87	1,000.33	144.05	1,116.82	2,009.82	289.41	844.00	1,518.85	218.72
34	555.87	1,000.33	144.05	1,094.15	1,969.03	283.54	844.00	1,518.85	218.72

Cultivating time (Hour)	Fish tank inlet carbon dioxide			Fish tank outlet carbon dioxide			Basic respiration		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)	CCO _{2BR} (ppm)	CCO _{2BR} (mg/m ³)	HCO _{2BR} (mg)
35	555.87	1,000.33	144.05	1,062.52	1,912.10	275.34	844.00	1,518.85	218.72
36	555.87	1,000.33	144.05	1,054.23	1,897.18	273.19	844.00	1,518.85	218.72
37	555.87	1,000.33	144.05	1,058.29	1,904.49	274.25	844.00	1,518.85	218.72
38	555.87	1,000.33	144.05	1,051.19	1,891.70	272.41	844.00	1,518.85	218.72
39	555.87	1,000.33	144.05	1,034.95	1,862.48	268.20	844.00	1,518.85	218.72
40	555.87	1,000.33	144.05	1,015.66	1,827.77	263.20	844.00	1,518.85	218.72
41	555.87	1,000.33	144.05	1,035.11	1,862.78	268.24	844.00	1,518.85	218.72
42	555.87	1,000.33	144.05	1,064.89	1,916.36	275.96	844.00	1,518.85	218.72
43	555.87	1,000.33	144.05	1,062.52	1,912.10	275.34	844.00	1,518.85	218.72
44	555.87	1,000.33	144.05	1,076.56	1,937.37	278.98	844.00	1,518.85	218.72
45	555.87	1,000.33	144.05	1,083.16	1,949.24	280.69	844.00	1,518.85	218.72
46	555.87	1,000.33	144.05	1,000.27	1,800.07	259.21	844.00	1,518.85	218.72
47	555.87	1,000.33	144.05	971.85	1,748.93	251.85	844.00	1,518.85	218.72
48	555.87	1,000.33	144.05	918.05	1,652.12	237.90	844.00	1,518.85	218.72
49	555.87	1,000.33	144.05	938.18	1,688.34	243.12	844.00	1,518.85	218.72
50	555.87	1,000.33	144.05	957.81	1,723.66	248.21	844.00	1,518.85	218.72
51	555.87	1,000.33	144.05	938.01	1,688.04	243.08	844.00	1,518.85	218.72
52	555.87	1,000.33	144.05	927.19	1,668.56	240.27	844.00	1,518.85	218.72
53	555.87	1,000.33	144.05	917.04	1,650.29	237.64	844.00	1,518.85	218.72
54	555.87	1,000.33	144.05	885.91	1,594.27	229.58	844.00	1,518.85	218.72
55	555.87	1,000.33	144.05	885.06	1,592.75	229.36	844.00	1,518.85	218.72
56	555.87	1,000.33	144.05	882.19	1,587.58	228.61	844.00	1,518.85	218.72
57	555.87	1,000.33	144.05	881.68	1,586.66	228.48	844.00	1,518.85	218.72
58	555.87	1,000.33	144.05	881.51	1,586.36	228.44	844.00	1,518.85	218.72
59	555.87	1,000.33	144.05	870.01	1,565.66	225.45	844.00	1,518.85	218.72
60	555.87	1,000.33	144.05	851.57	1,532.47	220.68	844.00	1,518.85	218.72
61	555.87	1,000.33	144.05	851.57	1,532.47	220.68	844.00	1,518.85	218.72
62	555.87	1,000.33	144.05	863.92	1,554.70	223.88	844.00	1,518.85	218.72
63	555.87	1,000.33	144.05	862.73	1,552.57	223.57	844.00	1,518.85	218.72
64	555.87	1,000.33	144.05	857.49	1,543.13	222.21	844.00	1,518.85	218.72
65	555.87	1,000.33	144.05	844.63	1,519.99	218.88	844.00	1,518.85	218.72
66	555.87	1,000.33	144.05	844.46	1,519.69	218.84	844.00	1,518.85	218.72
67	555.87	1,000.33	144.05	852.25	1,533.69	220.85	844.00	1,518.85	218.72
68	555.87	1,000.33	144.05	869.67	1,565.05	225.37	844.00	1,518.85	218.72
69	555.87	1,000.33	144.05	909.76	1,637.20	235.76	844.00	1,518.85	218.72
70	566.02	1,018.60	146.68	997.05	1,794.29	258.38	763.09	1,373.25	197.75
71	566.02	1,018.60	146.68	987.24	1,776.63	255.83	763.09	1,373.25	197.75
72	566.02	1,018.60	146.68	963.39	1,733.70	249.65	763.09	1,373.25	197.75
73	566.02	1,018.60	146.68	985.21	1,772.98	255.31	763.09	1,373.25	197.75
74	566.02	1,018.60	146.68	1,001.62	1,802.50	259.56	763.09	1,373.25	197.75
75	566.02	1,018.60	146.68	1,015.66	1,827.77	263.20	763.09	1,373.25	197.75
76	566.02	1,018.60	146.68	1,029.36	1,852.43	266.75	763.09	1,373.25	197.75
77	566.02	1,018.60	146.68	1,061.84	1,910.88	275.17	763.09	1,373.25	197.75
78	566.02	1,018.60	146.68	1,079.94	1,943.46	279.86	763.09	1,373.25	197.75
79	566.02	1,018.60	146.68	1,050.17	1,889.88	272.14	763.09	1,373.25	197.75
80	566.02	1,018.60	146.68	1,032.58	1,858.22	267.58	763.09	1,373.25	197.75
81	566.02	1,018.60	146.68	1,008.56	1,814.99	261.36	763.09	1,373.25	197.75
82	566.02	1,018.60	146.68	992.82	1,786.67	257.28	763.09	1,373.25	197.75
83	566.02	1,018.60	146.68	970.32	1,746.19	251.45	763.09	1,373.25	197.75
84	566.02	1,018.60	146.68	943.60	1,698.09	244.52	763.09	1,373.25	197.75
85	566.02	1,018.60	146.68	927.02	1,668.25	240.23	763.09	1,373.25	197.75
86	566.02	1,018.60	146.68	914.33	1,645.42	236.94	763.09	1,373.25	197.75
87	566.02	1,018.60	146.68	918.90	1,653.64	238.12	763.09	1,373.25	197.75
88	566.02	1,018.60	146.68	914.50	1,645.72	236.98	763.09	1,373.25	197.75
89	566.02	1,018.60	146.68	930.40	1,674.34	241.10	763.09	1,373.25	197.75
90	566.02	1,018.60	146.68	948.33	1,706.61	245.75	763.09	1,373.25	197.75
91	566.02	1,018.60	146.68	926.17	1,666.73	240.01	763.09	1,373.25	197.75
92	566.02	1,018.60	146.68	921.43	1,658.20	238.78	763.09	1,373.25	197.75
93	566.02	1,018.60	146.68	940.38	1,692.30	243.69	763.09	1,373.25	197.75
94	606.62	1,091.66	157.20	896.74	1,613.76	232.38	763.09	1,373.25	197.75

Cultivating time (Hour)	Fish tank inlet carbon dioxide			Fish tank outlet carbon dioxide			Basic respiration		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)	CCO _{2BR} (ppm)	CCO _{2BR} (mg/m ³)	HCO _{2BR} (mg)
95	606.62	1,091.66	157.20	840.57	1,512.69	217.83	763.09	1,373.25	197.75
96	606.62	1,091.66	157.20	806.91	1,452.10	209.10	763.09	1,373.25	197.75
97	606.62	1,091.66	157.20	855.46	1,539.48	221.68	763.09	1,373.25	197.75
98	606.62	1,091.66	157.20	872.55	1,570.22	226.11	763.09	1,373.25	197.75
99	606.62	1,091.66	157.20	879.31	1,582.40	227.87	763.09	1,373.25	197.75
100	606.62	1,091.66	157.20	903.33	1,625.63	234.09	763.09	1,373.25	197.75
101	606.62	1,091.66	157.20	904.01	1,626.85	234.27	763.09	1,373.25	197.75
102	606.62	1,091.66	157.20	891.15	1,603.71	230.93	763.09	1,373.25	197.75
103	606.62	1,091.66	157.20	874.91	1,574.49	226.73	763.09	1,373.25	197.75
104	606.62	1,091.66	157.20	866.29	1,558.96	224.49	763.09	1,373.25	197.75
105	606.62	1,091.66	157.20	857.15	1,542.52	222.12	763.09	1,373.25	197.75
106	606.62	1,091.66	157.20	847.51	1,525.17	219.62	763.09	1,373.25	197.75
107	606.62	1,091.66	157.20	839.05	1,509.95	217.43	763.09	1,373.25	197.75
108	606.62	1,091.66	157.20	833.81	1,500.51	216.07	763.09	1,373.25	197.75
109	606.62	1,091.66	157.20	830.59	1,494.73	215.24	763.09	1,373.25	197.75
110	606.62	1,091.66	157.20	826.53	1,487.42	214.19	763.09	1,373.25	197.75
111	606.62	1,091.66	157.20	814.86	1,466.41	211.16	763.09	1,373.25	197.75
112	606.62	1,091.66	157.20	797.94	1,435.97	206.78	763.09	1,373.25	197.75
113	606.62	1,091.66	157.20	792.36	1,425.92	205.33	763.09	1,373.25	197.75
114	606.62	1,091.66	157.20	775.11	1,394.87	200.86	763.09	1,373.25	197.75
115	606.62	1,091.66	157.20	766.65	1,379.65	198.67	763.09	1,373.25	197.75
116	606.62	1,091.66	157.20	763.09	1,373.26	197.75	763.09	1,373.25	197.75
117	563.99	1,014.94	146.15	760.56	1,368.69	197.09	763.09	1,373.25	197.09
118	563.99	1,014.94	146.15	765.12	1,376.91	198.28	754.77	1,358.28	195.59
119	563.99	1,014.94	146.15	776.63	1,397.61	201.26	754.77	1,358.28	195.59
120	563.99	1,014.94	146.15	776.80	1,397.92	201.30	754.77	1,358.28	195.59
121	563.99	1,014.94	146.15	783.73	1,410.40	203.10	754.77	1,358.28	195.59
122	563.99	1,014.94	146.15	780.01	1,403.70	202.13	754.77	1,358.28	195.59
123	563.99	1,014.94	146.15	775.11	1,394.87	200.86	754.77	1,358.28	195.59
124	563.99	1,014.94	146.15	787.79	1,417.70	204.15	754.77	1,358.28	195.59
125	563.99	1,014.94	146.15	787.12	1,416.49	203.97	754.77	1,358.28	195.59
126	563.99	1,014.94	146.15	780.69	1,404.92	202.31	754.77	1,358.28	195.59
127	563.99	1,014.94	146.15	776.12	1,396.70	201.12	754.77	1,358.28	195.59
128	563.99	1,014.94	146.15	773.75	1,392.44	200.51	754.77	1,358.28	195.59
129	563.99	1,014.94	146.15	774.09	1,393.05	200.60	754.77	1,358.28	195.59
130	563.99	1,014.94	146.15	773.58	1,392.13	200.47	754.77	1,358.28	195.59
131	563.99	1,014.94	146.15	771.21	1,387.87	199.85	754.77	1,358.28	195.59
132	563.99	1,014.94	146.15	775.61	1,395.79	200.99	754.77	1,358.28	195.59
133	563.99	1,014.94	146.15	782.72	1,408.57	202.83	754.77	1,358.28	195.59
134	563.99	1,014.94	146.15	784.07	1,411.01	203.18	754.77	1,358.28	195.59
135	563.99	1,014.94	146.15	782.55	1,408.27	202.79	754.77	1,358.28	195.59
136	563.99	1,014.94	146.15	772.91	1,390.91	200.29	754.77	1,358.28	195.59
137	563.99	1,014.94	146.15	770.88	1,387.26	199.77	754.77	1,358.28	195.59
138	563.99	1,014.94	146.15	762.76	1,372.65	197.66	754.77	1,358.28	195.59
139	563.99	1,014.94	146.15	760.73	1,369.00	197.14	754.77	1,358.28	195.59
140	562.97	1,013.12	145.89	754.13	1,357.12	195.43	754.77	1,358.28	195.43
141	562.97	1,013.12	145.89	767.15	1,380.56	198.80	754.77	1,358.28	195.59
142	562.97	1,013.12	145.89	841.93	1,515.12	218.18	880.00	1,583.64	218.18
143	562.97	1,013.12	145.89	903.67	1,626.24	234.18	880.00	1,583.64	228.04
144	562.97	1,013.12	145.89	1,008.05	1,814.07	261.23	880.00	1,583.64	228.04
145	562.97	1,013.12	145.89	1,038.33	1,868.57	269.07	880.00	1,583.64	228.04
146	562.97	1,013.12	145.89	1,056.26	1,900.84	273.72	880.00	1,583.64	228.04
147	562.97	1,013.12	145.89	1,065.06	1,916.67	276.00	880.00	1,583.64	228.04
148	562.97	1,013.12	145.89	1,076.73	1,937.67	279.02	880.00	1,583.64	228.04
149	562.97	1,013.12	145.89	1,077.91	1,939.80	279.33	880.00	1,583.64	228.04
150	562.97	1,013.12	145.89	1,081.80	1,946.81	280.34	880.00	1,583.64	228.04
151	562.97	1,013.12	145.89	1,074.36	1,933.41	278.41	880.00	1,583.64	228.04
152	562.97	1,013.12	145.89	1,064.89	1,916.36	275.96	880.00	1,583.64	228.04
153	562.97	1,013.12	145.89	1,053.55	1,895.97	273.02	880.00	1,583.64	228.04
154	562.97	1,013.12	145.89	1,048.14	1,886.22	271.62	880.00	1,583.64	228.04

Cultivating time (Hour)	Fish tank inlet carbon dioxide			Fish tank outlet carbon dioxide			Basic respiration		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)	CCO _{2BR} (ppm)	CCO _{2BR} (mg/m ³)	HCO _{2BR} (mg)
155	562.97	1,013.12	145.89	1,043.23	1,877.39	270.34	880.00	1,583.64	228.04
156	562.97	1,013.12	145.89	996.54	1,793.37	258.25	880.00	1,583.64	228.04
157	562.97	1,013.12	145.89	978.95	1,761.71	253.69	880.00	1,583.64	228.04
158	562.97	1,013.12	145.89	983.18	1,769.32	254.78	880.00	1,583.64	228.04
159	562.97	1,013.12	145.89	960.51	1,728.53	248.91	880.00	1,583.64	228.04
160	562.97	1,013.12	145.89	949.01	1,707.83	245.93	880.00	1,583.64	228.04
161	562.97	1,013.12	145.89	923.63	1,662.16	239.35	880.00	1,583.64	228.04
162	562.97	1,013.12	145.89	898.26	1,616.50	232.78	880.00	1,583.64	228.04
163	562.97	1,013.12	145.89	882.86	1,588.79	228.79	880.00	1,583.64	228.04
164	562.97	1,013.12	145.89	880.83	1,585.14	228.26	880.00	1,583.64	228.04
165	562.97	1,013.12	145.89	890.65	1,602.80	230.80	880.00	1,583.64	228.04
166	561.96	1,011.29	145.63	912.81	1,642.68	236.55	830.00	1,493.66	215.09
167	561.96	1,011.29	145.63	916.19	1,648.77	237.42	830.00	1,493.66	215.09
168	561.96	1,011.29	145.63	1,016.17	1,828.69	263.33	830.00	1,493.66	215.09
169	561.96	1,011.29	145.63	990.29	1,782.11	256.62	830.00	1,493.66	215.09
170	561.96	1,011.29	145.63	975.06	1,754.71	252.68	830.00	1,493.66	215.09
171	561.96	1,011.29	145.63	956.11	1,720.61	247.77	830.00	1,493.66	215.09
172	561.96	1,011.29	145.63	975.57	1,755.62	252.81	830.00	1,493.66	215.09
173	561.96	1,011.29	145.63	989.78	1,781.19	256.49	830.00	1,493.66	215.09
174	561.96	1,011.29	145.63	965.93	1,738.27	250.31	830.00	1,493.66	215.09
175	561.96	1,011.29	145.63	951.55	1,712.39	246.58	830.00	1,493.66	215.09
176	561.96	1,011.29	145.63	923.46	1,661.86	239.31	830.00	1,493.66	215.09
177	561.96	1,011.29	145.63	892.51	1,606.15	231.29	830.00	1,493.66	215.09
178	561.96	1,011.29	145.63	873.90	1,572.66	226.46	830.00	1,493.66	215.09
179	561.96	1,011.29	145.63	859.52	1,546.78	222.74	830.00	1,493.66	215.09
180	561.96	1,011.29	145.63	844.29	1,519.38	218.79	830.00	1,493.66	215.09
181	561.96	1,011.29	145.63	838.37	1,508.73	217.26	830.00	1,493.66	215.09
182	561.96	1,011.29	145.63	882.86	1,588.79	228.79	830.00	1,493.66	215.09
183	561.96	1,011.29	145.63	913.82	1,644.51	236.81	830.00	1,493.66	215.09
184	561.96	1,011.29	145.63	884.56	1,591.84	229.22	830.00	1,493.66	215.09
185	561.96	1,011.29	145.63	869.33	1,564.44	225.28	830.00	1,493.66	215.09
186	561.96	1,011.29	145.63	854.11	1,537.04	221.33	830.00	1,493.66	215.09
	Sum		27,271	Sum		44,233	Sum		39,302

Table E3 the amount of carbon dioxide from Fish cultivating unit during Nile tilapia cultivation

CO ₂ parameter	Replication 1	Replication 2
Amount of inlet CO ₂ (g)	27.33	27.27
Amount of outlet CO ₂ (g)	45.90	44.23
Amount of gained CO ₂ (g)	18.58	16.96
Basic respiration (g)	15.24	12.03
Feed utilization (g)	3.33	4.93

Table E4 Cell number density of *Scenedesmus armatus* during the cultivation in 1-L photobioreactor with 4 conditions (C: Ambient air + Original BG-11, T1: Outlet air from aquaculture unit + Original BG-11, T2: Ambient air + Modified BG-11 and T3: Outlet air from aquaculture unit + Modified BG-11).

Cultivating time (days)	Cell number density of C (10^4 cells / mL)				
	1	2	3	Average	S.D.
0	27.06	22.00	15.83	21.63	5.62
1	46.67	51.33	36.83	44.94	7.40
2	176.67	117.08	112.08	135.28	35.93
3	207.50	235.42	202.92	215.28	17.59
4	241.67	372.50	454.17	356.11	107.19
5	379.17	468.75	529.17	459.03	75.47
6	376.39	904.17	920.83	733.80	309.64
7	454.17	1,054.17	841.67	783.33	304.22
8	404.17	1,195.83	658.33	752.78	404.20
Cultivating time (days)	Cell number density of T1 (10^4 cells / mL)				
	1	2	3	Average	S.D.
0	20.28	26.17	18.10	21.51	4.17
1	50.33	45.17	43.67	46.39	3.50
2	123.33	123.33	152.50	133.06	16.84
3	233.33	161.67	271.67	222.22	55.84
4	393.75	210.83	395.83	333.47	106.21
5	829.17	602.08	666.67	699.31	117.01
6	925.00	887.50	933.33	915.28	24.41
7	1,133.33	912.50	1,050.00	1,031.94	111.52
8	1,500.00	1,000.00	1,104.17	1,201.39	263.80
Cultivating time (days)	Cell number density of T2 (10^4 cells / mL)				
	1	2	3	Average	S.D.
0	21.06	22.72	19.17	20.98	1.78
1	47.00	36.00	26.50	36.50	10.26
2	159.17	49.67	48.33	85.72	63.61
3	183.33	142.50	75.42	133.75	54.49
4	268.06	148.33	203.33	206.57	59.93
5	402.08	151.67	202.50	252.08	132.37
6	379.17	154.17	207.50	246.94	117.57
7	418.75	270.83	283.33	324.31	82.03
8	454.17	425.00	363.89	414.35	46.07

Cultivating time (days)	Cell number density of T3 (10 ⁴ cells / mL)				
	1	2	3	Average	S.D.
0	17.17	20.39	20.94	19.50	2.04
1	35.33	44.17	34.33	37.94	5.41
2	73.75	97.92	111.67	94.44	19.20
3	88.75	165.83	81.83	112.14	46.63
4	239.17	413.89	297.22	316.76	88.98
5	406.94	566.67	322.22	431.94	124.13
6	566.67	729.17	416.67	570.83	156.29
7	750.00	945.83	556.25	750.69	194.79
8	1,266.67	1,158.33	693.75	1,039.58	304.36

Table E5 Biomass of *Scenedesmus armatus* during the cultivation in 1-L photobioreactor with 4 conditions (C: Ambient air + Original BG-11, T1: Outlet air from aquaculture unit + Original BG-11, T2: Ambient air + Modified BG-11 and T3: Outlet air from aquaculture unit + Modified BG-11).

Cultivating time (days)	Biomass of C (mg / L)				
	1	2	3	Average	S.D.
0	41.33	34.67	48.00	41.33	6.67
2	113.33	143.59	146.67	134.53	18.42
4	255.56	333.33	393.33	327.41	69.08
6	421.43	511.11	700.00	605.56	142.20
8	550.00	901.67	798.33	750.00	180.75
Cultivating time (days)	Biomass of T1 (mg / L)				
	1	2	3	Average	S.D.
0	40.00	53.33	61.33	51.56	10.78
2	108.89	124.44	144.44	125.93	17.82
4	324.44	282.22	368.89	325.19	43.34
6	664.44	491.11	640.00	598.52	93.82
8	1,003.33	841.67	901.67	915.56	81.72
Cultivating time (days)	Biomass of T2 (mg / L)				
	1	2	3	Average	S.D.
0	27.78	58.67	29.33	43.22	17.40
2	88.89	91.11	66.67	90.00	13.52
4	271.11	246.67	206.67	258.89	32.53
6	633.33	262.22	215.56	447.78	228.92

8	836.67	561.67	491.67	699.17	182.37
Cultivating time (days)	Biomass of T3 (mg / L)				
	1	2	3	Average	S.D.
0	32.00	40.00	33.33	35.11	4.29
2	35.56	64.44	64.44	54.81	16.68
4	202.22	364.44	217.78	261.48	89.51
6	524.44	597.78	544.44	555.56	37.91
8	820.00	1,011.67	803.33	878.33	115.77

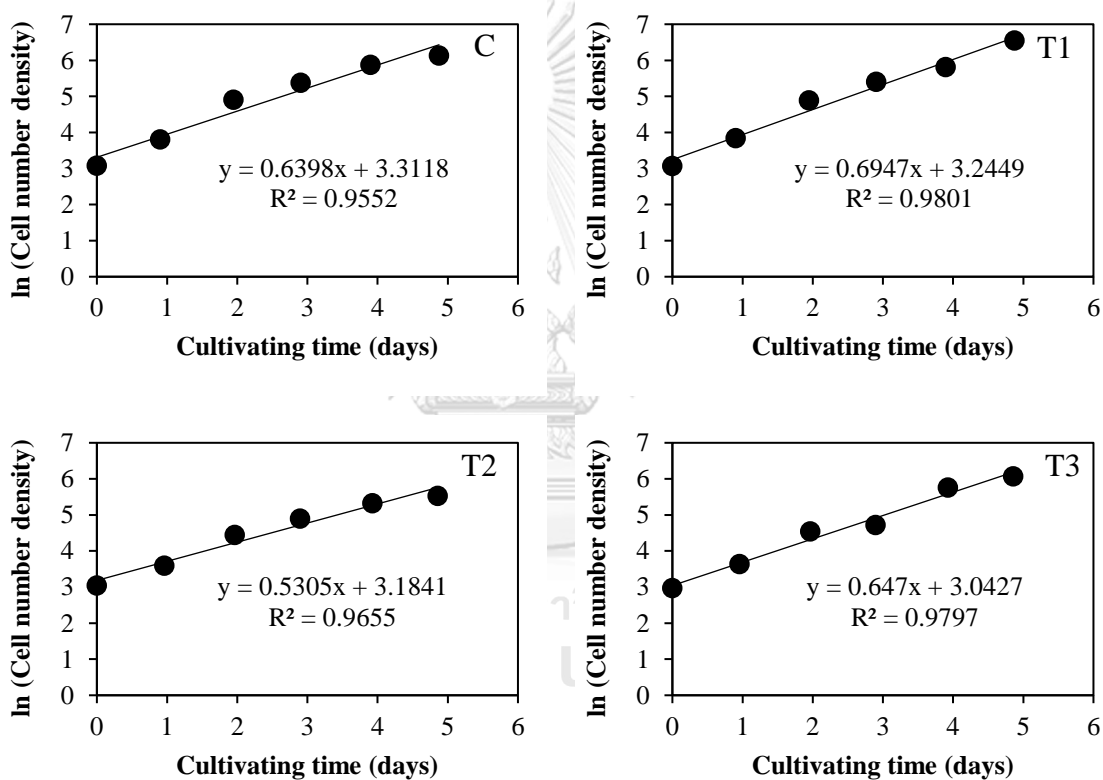


Figure E1 Maximum specific growth rate of *Scenedesmus armatus* during the cultivation in 1-L photobioreactor with 4 conditions (C: Ambient air + Original BG-11, T1: Outlet air from aquaculture unit + Original BG-11, T2: Ambient air + Modified BG-11 and T3: Outlet air from aquaculture unit + Modified BG-11).

Table E6 Nitrate concentrations of *Scenedesmus armatus* during the cultivation in 1-L photobioreactor with 4 conditions (C: Ambient air + Original BG-11, T1: Outlet air from aquaculture unit + Original BG-11, T2: Ambient air + Modified BG-11 and T3: Outlet air from aquaculture unit + Modified BG-11).

Cultivating time (days)	Nitrate concentrations of C (mg N/ L)				
	1	2	3	Average	S.D.
0	255.06	251.91	251.15	252.71	2.08
2	253.82	237.77	249.52	247.04	8.31
4	244.74	255.93	242.74	247.80	7.11
6	229.65	224.92	223.44	226.00	3.24
8	186.38	214.86	227.58	209.61	21.10
Cultivating time (days)	Nitrate concentrations of T1 (mg N/ L)				
	1	2	3	Average	S.D.
0	271.98	259.84	265.58	265.80	6.07
2	259.17	223.24	257.74	246.72	20.34
4	247.80	260.23	250.10	252.71	6.61
6	246.21	178.59	257.64	227.48	42.72
8	183.32	196.63	206.58	195.51	11.67
Cultivating time (days)	Nitrate concentrations of T2 (mg N/ L)				
	1	2	3	Average	S.D.
0	237.00	220.76	206.80	221.52	15.11
2	233.56	221.71	227.64	227.64	5.93
4	266.63	190.46	225.25	227.45	38.13
6	229.65	208.95	217.52	218.71	10.40
8	207.18	182.34	189.63	193.05	12.77
Cultivating time (days)	Nitrate concentrations of T3 (mg N/ L)				
	1	2	3	Average	S.D.
0	239.58	230.03	236.91	235.51	4.93
2	222.38	231.94	228.12	227.48	4.81
4	215.60	201.64	223.34	213.53	10.99
6	179.38	142.42	182.34	168.05	22.24
8	172.38	199.88	193.48	188.58	14.39

Table E7 Phosphate concentrations of *Scenedesmus armatus* during the cultivation in 1-L photobioreactor with 4 conditions (C: Ambient air + Original BG-11, T1: Outlet air from aquaculture unit + Original BG-11, T2: Ambient air + Modified BG-11 and T3: Outlet air from aquaculture unit + Modified BG-11).

Cultivating time (days)	Phosphate concentrations of C (mg P/ L)				
	1	2	3	Average	S.D.
0	9.00	8.93	7.75	8.56	0.05
2	6.70	8.46	7.68	7.61	0.88
4	1.73	3.34	1.82	2.30	0.91
6	1.06	3.67	7.70	4.14	3.35
8	1.99	2.18	1.08	1.75	0.59
Cultivating time (days)	Phosphate concentrations of T1 (mg P/ L)				
	1	2	3	Average	S.D.
0	9.42	8.78	8.30	8.83	0.45
2	8.04	6.41	5.95	6.80	1.10
4	3.27	2.84	2.17	2.76	0.55
6	3.55	2.74	4.22	3.50	0.74
8	2.39	2.34	2.85	2.53	0.28
Cultivating time (days)	Phosphate concentrations of T2 (mg P/ L)				
	1	2	3	Average	S.D.
0	6.28	6.91	7.47	6.89	0.59
2	5.93	6.35	6.93	6.40	0.50
4	1.92	0.52	1.34	1.26	0.71
6	1.34	0.32	0.40	0.69	0.56
8	0.00	0.35	0.40	0.25	0.22
Cultivating time (days)	Phosphate concentrations of T3 (mg P/ L)				
	1	2	3	Average	S.D.
0	8.25	5.48	7.76	7.16	1.48
2	6.61	6.86	7.39	6.95	0.40
4	5.20	2.12	4.67	4.00	1.65
6	0.26	0.10	0.33	0.23	0.12
8	0.21	0.26	0.25	0.24	0.03

Table E8 pH of *Scenedesmus armatus* during the cultivation in 1-L photobioreactor with 4 conditions (C: Ambient air + Original BG-11, T1: Outlet air from aquaculture unit + Original BG-11, T2: Ambient air + Modified BG-11 and T3: Outlet air from aquaculture unit + Modified BG-11).

Cultivating time (days)	pH			
	C	T1	T2	T3
0	7.9 ± 0.2	8.0 ± 0	8.1 ± 0.6	7.8 ± 0
2	8.8 ± 0.4	8.9 ± 0.7	8.7 ± 0.1	8.3 ± 0.2
4	10.7 ± 0	10.9 ± 0.3	10.6 ± 0.2	9.8 ± 0.8
6	10.8 ± 0.5	10.3 ± 0.2	10.4 ± 0.2	10.4 ± 0.4
8	11.0 ± 0.4	10.6 ± 0.3	10.5 ± 0.2	10.3 ± 0.4

Table E9 Total alkalinity of *Scenedesmus armatus* during the cultivation in 1-L photobioreactor with 4 conditions (C: Ambient air + Original BG-11, T1: Outlet air from aquaculture unit + Original BG-11, T2: Ambient air + Modified BG-11 and T3: Outlet air from aquaculture unit + Modified BG-11)

Cultivating time (days)	Total alkalinity (mg CaCO ₃ / L)			
	C	T1	T2	T3
0	60.00 ± 0.00	60.00 ± 0.00	50.00 ± 14.14	40.00 ± 0.00
2	100.00 ± 0.00	100.00 ± 0.00	70.00 ± 14.14	86.67 ± 11.55
4	133.33 ± 11.55	133.33 ± 11.55	100.00 ± 28.28	120.00 ± 20.00
6	146.67 ± 23.09	173.33 ± 46.19	130.00 ± 42.43	140.00 ± 34.64
8	220.00 ± 52.92	286.67 ± 23.09	210.00 ± 70.71	266.67 ± 30.55

Table E10 Dissolved inorganic nitrogen concentrations of Fish tank (FT) and Solid separating (SU)

Cultivating time (days)	Total ammonia nitrogen concentrations (mg N / L)		Nitrite concentrations (mg N / L)		Nitrate concentrations (mg N / L)	
	FT	SU	FT	SU	FT	SU
0	0.10 ± 0.01	0.16 ± 0.03	0.03 ± 0.02	0.06 ± 0.02	47.93 ± 1.74	50.32 ± 1.37
2	0.12 ± 0.02	0.21 ± 0.02	0.22 ± 0.11	0.06 ± 0.03	49.50 ± 0.73	52.42 ± 3.48
4	0.10 ± 0.01	0.19 ± 0.03	0.06 ± 0.02	0.03 ± 0.01	49.07 ± 2.78	55.39 ± 2.20
6	0.05 ± 0.00	0.08 ± 0.05	0.04 ± 0.01	0.02 ± 0.01	47.69 ± 0.88	46.69 ± 4.79
8	0.06 ± 0.01	0.09 ± 0.04	0.03 ± 0.00	0.03 ± 0.00	54.94 ± 0.95	55.19 ± 1.40

Table E11 Dissolved oxygen, pH, temperature and total alkalinity of Fish tank (FT) and Solid separating unit (SU).

Cultivating time (days)	Dissolved oxygen (mg / L)		pH		Temperature (°C)	
	FT	SU	FT	SU	FT	SU
0	7.1	7.6	8.2	8.3	29.3	29.3
2	6.5	6.8	8.2	8.4	30.3	30.6
4	6.4	6.7	8.1	8.2	30.7	31.2
6	5.3	5.9	8.1	8.2	31.4	31.6
8	7.0	6.7	8.1	8.1	31.3	31.2

Table E12 Pigments content in *Scenedesmus armatus* at the end of cultivation on day 9.

Pigment	Pigment content (mg/g)									
	C					T1				
	1	2	3	Average	S.D.	1	2	3	Average	S.D.
Chlorophyll a	3.66	6.27	2.83	4.25	1.80	3.55	3.59	3.57	3.57	0.02
Total carotenoids	1.32	2.19	1.21	1.58	0.54	1.45	1.20	2.05	1.57	0.44
Lutein	0.64	1.04	0.76	0.81	0.21	0.73	0.61	0.65	0.67	0.06
Pigment	Pigment content (mg/g)									
	T2					T3				
	1	2	3	Average	S.D.	1	2	3	Average	S.D.
Chlorophyll a	4.16	3.27	10.71	6.05	4.07	9.06	6.79	5.82	7.23	1.66
Total carotenoids	1.54	1.05	1.62	1.41	0.31	2.46	2.35	1.69	2.17	0.41
Lutein	0.71	0.66	0.64	0.67	0.03	1.10	0.85	0.87	0.94	0.14

Appendix F

Table F1 Dissolved oxygen, pH, temperature and alkalinity of RAS during Nile tilapia cultivations with 3 kg/m³ fish density (FT: Fish tank, SU: Solid separating unit)

Cultivating time (days)	Dissolved oxygen (mg / L)		pH		Temperature (°C)		Total alkalinity (mg CaCO ₃ / L)	
	FT	SU	FT	SU	FT	SU	FT	SU
0	6.8	6.6	7.5	7.7	30.60	30.85	160.0	150.0
2	6.8	7.1	8.0	8.1	31.10	31.25	160.0	170.0
4	6.9	7.2	7.9	8.0	30.20	30.30	140.0	160.0
6	6.0	7.3	7.6	7.9	30.80	30.90	160.0	160.0
8	6.7	7.3	7.9	8.1	29.70	30.25	140.0	150.0

Table F2 Dissolved oxygen, pH, temperature and alkalinity of RAS during Nile tilapia cultivations with 5 kg/m³ fish density (FT: Fish tank, SU: Solid separating unit)

Cultivating time (days)	Dissolved oxygen (mg / L)		pH		Temperature		Total alkalinity (mg CaCO ₃ / L)	
	FT	SU	FT	SU	FT	SU	FT	SU
0	6.1	7.5	8.3	8.3	29.30	29.25	180.0	200.0
2	6.4	6.7	7.9	7.9	29.20	30.10	180.0	193.3
4	7.1	6.9	8.2	8.1	30.00	30.00	200.0	200.0
6	7.0	7.5	8.2	8.2	29.40	28.00	200.0	173.3
8	6.6	7.5	8.2	8.3	30.30	29.30	180.0	153.3

Table F3 Dissolved oxygen, pH, temperature and alkalinity of RAS during Nile tilapia cultivations with 10 kg/m³ fish density (FT: Fish tank, SU: Solid separating unit)

Cultivating time (days)	Dissolved oxygen (mg / L)		pH		Temperature		Total alkalinity (mg CaCO ₃ / L)	
	FT	SU	FT	SU	FT	SU	FT	SU
0	6.6	8.2	8.0	8.3	29.60	29.65	160.0	140.0
2	6.4	7.7	8.0	8.0	30.30	30.30	160.0	160.0
4	6.1	7.6	8.1	8.0	30.40	30.40	180.0	180.0
6	6.9	8.2	8.0	8.0	30.50	30.45	180.0	180.0

Cultivating time (days)	Dissolved oxygen (mg / L)		pH		Temperature		Total alkalinity (mg CaCO ₃ / L)	
	FT	SU	FT	SU	FT	SU	FT	SU
8	5.6	6.8	8.1	8.2	30.80	31.05	200.0	200.0

Table F4 Dissolved inorganic nitrogen concentrations of RAS during Nile tilapia cultivations with 3 kg/m³ fish density (FT: Fish tank, SU: Solid separating unit)

Cultivating time (days)	Total ammonia nitrogen concentrations (mg N/L)		Nitrite concentrations (mg N / L)		Nitrate concentrations (mg N / L)	
	FT	SU	FT	SU	FT	SU
0	0.11	0.13	0.01	0.02	39.53	39.85
2	0.06	0.26	0.01	0.07	39.37	43.23
4	0.08	0.13	0.03	0.04	37.18	37.86
6	0.12	0.12	0.07	0.02	36.72	41.32
8	0.10	0.27	0.07	0.05	35.53	38.00

Table F5 Dissolved inorganic nitrogen concentrations of RAS during Nile tilapia cultivations with 5 kg/m³ fish density (FT: Fish tank, SU: Solid separating unit)

Cultivating time (days)	Total ammonia nitrogen concentrations (mg N/L)		Nitrite concentrations (mg N / L)		Nitrate concentrations (mg N / L)	
	FT	SU	FT	SU	FT	SU
0	0.16	0.17	0.03	0.04	31.82	32.34
2	0.20	0.33	0.05	0.07	32.07	36.72
4	0.21	0.37	0.07	0.09	34.10	39.03
6	0.59	0.79	0.30	0.11	36.81	39.64
8	0.23	0.29	0.08	0.09	31.08	30.85

Table F6 Dissolved inorganic nitrogen concentrations of RAS during Nile tilapia cultivations with 10 kg/m³ fish density (FT: Fish tank, SU: Solid separating unit)

Cultivating time (days)	Total ammonia nitrogen concentrations (mg N / L)		Nitrite concentrations (mg N / L)		Nitrate concentrations (mg N / L)	
	FT	SU	FT	SU	FT	SU
0	0.49	0.51	0.04	0.04	43.04	46.88
2	0.57	0.51	0.56	0.79	43.75	47.84
4	0.41	0.56	0.20	0.33	39.72	46.43
6	0.32	0.38	0.10	0.29	42.74	44.15

Cultivating time (days)	Total ammonia nitrogen concentrations (mg N / L)		Nitrite concentrations (mg N / L)		Nitrate concentrations (mg N / L)	
	FT	SU	FT	SU	FT	SU
8	0.35	0.55	0.23	0.57	41.32	41.34

Table F7 Total suspended solids concentration of RAS during Nile tilapia cultivations with 3, 5 and 10 kg/m³ fish density (FT: The concentration of Fish tank, SU1: The concentration of the 1st chamber of Solid separating unit and SU2: The concentration of the 2nd chamber of Solid separating unit).

Cultivating time (days)	Total suspended solids concentration (mg / L)								
	Fish density 3 kg / m ³			Fish density 5 kg / m ³			Fish density 10 kg / m ³		
	FT	SU1	SU2	FT	SU1	SU2	FT	SU	SU2
0	60.00	388.00	221.33	50.00	332.22	110.00	103.33	166.67	106.67
2	36.00	385.33	260.00	67.78	273.33	122.22	82.22	158.89	296.67
4	57.33	624.00	129.33	66.67	466.67	120.00	78.89	238.89	207.78
6	88.00	618.67	99.56	105.56	508.15	176.30	40.00	391.11	328.15
8	77.33	643.56	201.78	54.44	562.96	99.26	91.11	454.81	173.33

Table F8 Total suspended solids and the ratio of suspended solids in each part of RAS during Nile tilapia cultivations with 3, 5 and 10 kg/m³ fish density (FU: The concentration of Fish cultivating unit, SU1: The concentration of the 1st chamber of Solid separating unit and SU2: The concentration of the 2nd chamber of Solid separating unit).

Cultivating time (days)	Total suspended solids (g)								
	Ratio of suspended solids in each part (%)								
	Fish density 3 kg / m ³			Fish density 5 kg / m ³			Fish density 10 kg / m ³		
	FU	SU1	SU2	FU	SU1	SU2	FU	SU	SU2
0	3.24	10.48	5.98	2.70	8.97	2.97	5.58	4.50	2.88
	16.45%	53.20%	30.35%	18.44%	61.27%	20.29%	43.06%	34.72%	22.22%
2	1.94	10.40	7.02	3.66	7.38	3.30	4.44	4.29	8.01
	10.04%	53.72%	36.25%	25.52%	51.46%	23.01%	26.52%	25.63%	47.85%
4	3.10	16.85	3.49	3.60	12.60	3.24	4.26	6.45	5.61
	13.21%	71.89%	14.90%	18.52%	64.81%	16.67%	26.10%	39.52%	34.38%
6	4.75	16.70	2.69	5.70	13.72	4.76	2.16	10.56	8.86
	19.68%	69.18%	11.13%	23.57%	56.74%	19.69%	10.01%	48.93%	41.06%

Cultivating time (days)	Total suspended solids (g)								
	Ratio of suspended solids in each part (%)								
	Fish density 3 kg / m ³			Fish density 5 kg / m ³			Fish density 10 kg / m ³		
8	4.18	17.38	5.45	2.94	15.20	2.68	4.92	12.28	4.68
	15.47%	64.36%	20.18%	14.12%	73.01%	12.87%	22.49%	56.12%	21.39%



Table F9 the concentrations and mass of carbon dioxide in air that flow in/out Fish tank during Nile tilapia cultivation with 3 kg/m³ fish density including basic respiration.

Cultivating time (Hour)	Fish tank inlet carbon dioxide			Fish tank outlet carbon dioxide			Basic respiration		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)	CCO _{2BR} (ppm)	CCO _{2BR} (mg/m ³)	HCO _{2BR} (mg)
1	535.57	963.80	138.79	767.15	1,380.56	198.80	767.15	1,380.56	198.80
2	535.57	963.80	138.79	868.49	1,562.92	225.06	804.54	1,447.84	208.49
3	535.57	963.80	138.79	862.23	1,551.65	223.44	804.54	1,447.84	208.49
4	535.57	963.80	138.79	835.67	1,503.86	216.56	804.54	1,447.84	208.49
5	535.57	963.80	138.79	827.89	1,489.85	214.54	804.54	1,447.84	208.49
6	535.57	963.80	138.79	855.97	1,540.39	221.82	804.54	1,447.84	208.49
7	535.57	963.80	138.79	888.28	1,598.54	230.19	804.54	1,447.84	208.49
8	535.57	963.80	138.79	910.95	1,639.33	236.06	804.54	1,447.84	208.49
9	535.57	963.80	138.79	919.24	1,654.25	238.21	804.54	1,447.84	208.49
10	535.57	963.80	138.79	917.37	1,650.90	237.73	804.54	1,447.84	208.49
11	535.57	963.80	138.79	905.70	1,629.89	234.70	804.54	1,447.84	208.49
12	535.57	963.80	138.79	873.90	1,572.66	226.46	804.54	1,447.84	208.49
13	535.57	963.80	138.79	851.91	1,533.08	220.76	804.54	1,447.84	208.49
14	535.57	963.80	138.79	853.94	1,536.74	221.29	804.54	1,447.84	208.49
15	535.57	963.80	138.79	851.91	1,533.08	220.76	804.54	1,447.84	208.49
16	535.57	963.80	138.79	842.10	1,515.43	218.22	804.54	1,447.84	208.49
17	535.57	963.80	138.79	822.81	1,480.72	213.22	804.54	1,447.84	208.49
18	535.57	963.80	138.79	814.18	1,465.20	210.99	804.54	1,447.84	208.49
19	535.57	963.80	138.79	850.89	1,531.26	220.50	804.54	1,447.84	208.49
20	566.02	1,018.60	146.68	861.72	1,550.74	223.31	848.19	1,526.39	219.80
21	566.02	1,018.60	146.68	865.27	1,557.13	224.23	848.19	1,526.39	219.80
22	566.02	1,018.60	146.68	877.96	1,579.97	227.52	848.19	1,526.39	219.80
23	566.02	1,018.60	146.68	843.28	1,517.56	218.53	843.28	1,517.56	218.53
24	566.02	1,018.60	146.68	862.23	1,551.65	223.44	848.19	1,526.39	219.80
25	566.02	1,018.60	146.68	885.91	1,594.27	229.58	848.19	1,526.39	219.80
26	566.02	1,018.60	146.68	870.68	1,566.88	225.63	848.19	1,526.39	219.80
27	566.02	1,018.60	146.68	929.05	1,671.90	240.75	848.19	1,526.39	219.80
28	566.02	1,018.60	146.68	939.03	1,689.87	243.34	848.19	1,526.39	219.80
29	566.02	1,018.60	146.68	970.15	1,745.88	251.41	848.19	1,526.39	219.80
30	566.02	1,018.60	146.68	970.83	1,747.10	251.58	848.19	1,526.39	219.80
31	566.02	1,018.60	146.68	940.89	1,693.21	243.82	848.19	1,526.39	219.80
32	566.02	1,018.60	146.68	929.39	1,672.51	240.84	848.19	1,526.39	219.80
33	566.02	1,018.60	146.68	916.87	1,649.99	237.60	848.19	1,526.39	219.80
34	566.02	1,018.60	146.68	902.83	1,624.72	233.96	848.19	1,526.39	219.80
35	566.02	1,018.60	146.68	880.16	1,583.92	228.08	848.19	1,526.39	219.80
36	566.02	1,018.60	146.68	870.01	1,565.66	225.45	848.19	1,526.39	219.80
37	566.02	1,018.60	146.68	866.12	1,558.66	224.45	848.19	1,526.39	219.80
38	566.02	1,018.60	146.68	856.81	1,541.91	222.04	848.19	1,526.39	219.80
39	566.02	1,018.60	146.68	867.64	1,561.40	224.84	848.19	1,526.39	219.80
40	566.02	1,018.60	146.68	870.52	1,566.57	225.59	848.19	1,526.39	219.80
41	566.02	1,018.60	146.68	858.84	1,545.57	222.56	848.19	1,526.39	219.80
42	566.02	1,018.60	146.68	850.89	1,531.26	220.50	848.19	1,526.39	219.80
43	566.02	1,018.60	146.68	862.56	1,552.26	223.53	848.19	1,526.39	219.80
44	566.02	1,018.60	146.68	862.23	1,551.65	223.44	848.19	1,526.39	219.80
45	566.02	1,018.60	146.68	704.39	1,267.62	182.54	704.39	1,267.62	182.54
46	566.02	1,018.60	146.68	792.53	1,426.23	205.38	792.53	1,426.23	205.38
47	549.27	988.46	142.34	947.32	1,704.78	245.49	780.18	1,404.00	202.18
48	549.27	988.46	142.34	965.25	1,737.05	250.14	780.18	1,404.00	202.18
49	549.27	988.46	142.34	928.03	1,670.08	240.49	780.18	1,404.00	202.18
50	549.27	988.46	142.34	908.92	1,635.68	235.54	780.18	1,404.00	202.18
51	549.27	988.46	142.34	881.68	1,586.66	228.48	780.18	1,404.00	202.18
52	549.27	988.46	142.34	861.89	1,551.04	223.35	780.18	1,404.00	202.18
53	549.27	988.46	142.34	879.31	1,582.40	227.87	780.18	1,404.00	202.18
54	549.27	988.46	142.34	903.50	1,625.93	234.13	780.18	1,404.00	202.18
55	549.27	988.46	142.34	888.28	1,598.54	230.19	780.18	1,404.00	202.18
56	549.27	988.46	142.34	867.81	1,561.70	224.88	780.18	1,404.00	202.18

Cultivating time (Hour)	Fish tank inlet carbon dioxide			Fish tank outlet carbon dioxide			Basic respiration		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)	CCO _{2BR} (ppm)	CCO _{2BR} (mg/m ³)	HCO _{2BR} (mg)
57	549.27	988.46	142.34	846.49	1,523.34	219.36	780.18	1,404.00	202.18
58	549.27	988.46	142.34	832.11	1,497.47	215.63	780.18	1,404.00	202.18
59	549.27	988.46	142.34	819.77	1,475.24	212.43	780.18	1,404.00	202.18
60	549.27	988.46	142.34	815.87	1,468.24	211.43	780.18	1,404.00	202.18
61	549.27	988.46	142.34	804.03	1,446.93	208.36	780.18	1,404.00	202.18
62	549.27	988.46	142.34	789.48	1,420.75	204.59	780.18	1,404.00	202.18
63	549.27	988.46	142.34	800.31	1,440.23	207.39	780.18	1,404.00	202.18
64	549.27	988.46	142.34	792.02	1,425.32	205.25	780.18	1,404.00	202.18
65	549.27	988.46	142.34	783.56	1,410.09	203.05	780.18	1,404.00	202.18
66	549.27	988.46	142.34	801.66	1,442.67	207.74	780.18	1,404.00	202.18
67	549.27	988.46	142.34	783.39	1,409.79	203.01	780.18	1,404.00	202.18
68	569.06	1,024.08	147.47	760.56	1,368.69	197.09	760.56	1,368.69	197.09
69	569.06	1,024.08	147.47	849.20	1,528.21	220.06	771.05	1,387.57	199.81
70	569.06	1,024.08	147.47	895.89	1,612.24	232.16	771.05	1,387.57	199.81
71	569.06	1,024.08	147.47	927.02	1,668.25	240.23	771.05	1,387.57	199.81
72	569.06	1,024.08	147.47	925.83	1,666.12	239.92	771.05	1,387.57	199.81
73	569.06	1,024.08	147.47	901.81	1,622.89	233.70	771.05	1,387.57	199.81
74	569.06	1,024.08	147.47	884.22	1,591.23	229.14	771.05	1,387.57	199.81
75	569.06	1,024.08	147.47	887.77	1,597.62	230.06	771.05	1,387.57	199.81
76	569.06	1,024.08	147.47	948.33	1,706.61	245.75	771.05	1,387.57	199.81
77	569.06	1,024.08	147.47	998.41	1,796.72	258.73	771.05	1,387.57	199.81
78	569.06	1,024.08	147.47	1,051.69	1,892.62	272.54	771.05	1,387.57	199.81
79	569.06	1,024.08	147.47	1,068.44	1,922.76	276.88	771.05	1,387.57	199.81
80	569.06	1,024.08	147.47	1,058.97	1,905.71	274.42	771.05	1,387.57	199.81
81	569.06	1,024.08	147.47	1,053.72	1,896.27	273.06	771.05	1,387.57	199.81
82	569.06	1,024.08	147.47	1,055.08	1,898.70	273.41	771.05	1,387.57	199.81
83	569.06	1,024.08	147.47	1,052.71	1,894.44	272.80	771.05	1,387.57	199.81
84	569.06	1,024.08	147.47	1,049.83	1,889.27	272.05	771.05	1,387.57	199.81
85	569.06	1,024.08	147.47	900.29	1,620.15	233.30	771.05	1,387.57	199.81
86	569.06	1,024.08	147.47	828.73	1,491.38	214.76	771.05	1,387.57	199.81
87	569.06	1,024.08	147.47	807.42	1,453.02	209.23	771.05	1,387.57	199.81
88	569.06	1,024.08	147.47	791.68	1,424.71	205.16	771.05	1,387.57	199.81
89	569.06	1,024.08	147.47	775.44	1,395.48	200.95	771.05	1,387.57	199.81
90	569.06	1,024.08	147.47	769.35	1,384.52	199.37	769.35	1,384.52	199.37
91	569.06	1,024.08	147.47	709.98	1,277.67	183.98	709.98	1,277.67	183.98
92	569.06	1,024.08	147.47	807.75	1,453.63	209.32	771.05	1,387.57	199.81
93	569.06	1,024.08	147.47	920.59	1,656.68	238.56	771.05	1,387.57	199.81
94	569.06	1,024.08	147.47	906.38	1,631.11	234.88	771.05	1,387.57	199.81
95	569.06	1,024.08	147.47	854.11	1,537.04	221.33	771.05	1,387.57	199.81
96	580.23	1,044.17	150.36	836.01	1,504.47	216.64	780.18	1,404.00	202.18
97	580.23	1,044.17	150.36	842.43	1,516.04	218.31	780.18	1,404.00	202.18
98	580.23	1,044.17	150.36	858.34	1,544.65	222.43	780.18	1,404.00	202.18
99	580.23	1,044.17	150.36	858.50	1,544.96	222.47	780.18	1,404.00	202.18
100	580.23	1,044.17	150.36	863.07	1,553.18	223.66	780.18	1,404.00	202.18
101	580.23	1,044.17	150.36	873.05	1,571.14	226.24	780.18	1,404.00	202.18
102	580.23	1,044.17	150.36	880.83	1,585.14	228.26	780.18	1,404.00	202.18
103	580.23	1,044.17	150.36	882.36	1,587.88	228.65	780.18	1,404.00	202.18
104	580.23	1,044.17	150.36	884.05	1,590.93	229.09	780.18	1,404.00	202.18
105	580.23	1,044.17	150.36	880.33	1,584.23	228.13	780.18	1,404.00	202.18
106	580.23	1,044.17	150.36	866.12	1,558.66	224.45	780.18	1,404.00	202.18
107	580.23	1,044.17	150.36	855.97	1,540.39	221.82	780.18	1,404.00	202.18
108	580.23	1,044.17	150.36	847.68	1,525.47	219.67	780.18	1,404.00	202.18
109	580.23	1,044.17	150.36	833.81	1,500.51	216.07	780.18	1,404.00	202.18
110	580.23	1,044.17	150.36	812.15	1,461.54	210.46	780.18	1,404.00	202.18
111	580.23	1,044.17	150.36	799.63	1,439.01	207.22	780.18	1,404.00	202.18
112	580.23	1,044.17	150.36	792.19	1,425.62	205.29	780.18	1,404.00	202.18
113	580.23	1,044.17	150.36	782.55	1,408.27	202.79	780.18	1,404.00	202.18
114	580.23	1,044.17	150.36	780.69	1,404.92	202.31	780.18	1,404.00	202.18
115	580.23	1,044.17	150.36	788.13	1,418.31	204.24	780.18	1,404.00	202.18
116	580.23	1,044.17	150.36	722.16	1,299.59	187.14	722.16	1,299.59	187.14

Cultivating time (Hour)	Fish tank inlet carbon dioxide			Fish tank outlet carbon dioxide			Basic respiration		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)	CCO _{2BR} (ppm)	CCO _{2BR} (mg/m ³)	HCO _{2BR} (mg)
117	560.94	1,009.46	145.36	676.31	1,217.08	175.26	651.28	1,172.03	168.77
118	560.94	1,009.46	145.36	684.43	1,231.70	177.36	651.28	1,172.03	168.77
119	560.94	1,009.46	145.36	695.77	1,252.09	180.30	651.28	1,172.03	168.77
120	560.94	1,009.46	145.36	702.87	1,264.88	182.14	651.28	1,172.03	168.77
121	560.94	1,009.46	145.36	694.41	1,249.66	179.95	651.28	1,172.03	168.77
122	560.94	1,009.46	145.36	692.55	1,246.31	179.47	651.28	1,172.03	168.77
123	560.94	1,009.46	145.36	692.21	1,245.70	179.38	651.28	1,172.03	168.77
124	560.94	1,009.46	145.36	683.42	1,229.87	177.10	651.28	1,172.03	168.77
125	560.94	1,009.46	145.36	669.21	1,204.30	173.42	651.28	1,172.03	168.77
126	560.94	1,009.46	145.36	663.12	1,193.34	171.84	651.28	1,172.03	168.77
127	560.94	1,009.46	145.36	662.10	1,191.51	171.58	651.28	1,172.03	168.77
128	560.94	1,009.46	145.36	662.95	1,193.03	171.80	651.28	1,172.03	168.77
129	560.94	1,009.46	145.36	666.33	1,199.12	172.67	651.28	1,172.03	168.77
130	560.94	1,009.46	145.36	670.56	1,206.73	173.77	651.28	1,172.03	168.77
131	560.94	1,009.46	145.36	674.11	1,213.13	174.69	651.28	1,172.03	168.77
132	560.94	1,009.46	145.36	662.78	1,192.73	171.75	651.28	1,172.03	168.77
133	560.94	1,009.46	145.36	662.95	1,193.03	171.80	651.28	1,172.03	168.77
134	560.94	1,009.46	145.36	660.24	1,188.16	171.10	651.28	1,172.03	168.77
135	560.94	1,009.46	145.36	656.18	1,180.86	170.04	651.28	1,172.03	168.77
136	560.94	1,009.46	145.36	651.44	1,172.33	168.82	651.28	1,172.03	168.77
137	560.94	1,009.46	145.36	647.72	1,165.64	167.85	647.72	1,165.64	167.85
138	560.94	1,009.46	145.36	645.69	1,161.98	167.33	645.69	1,161.98	167.33
139	560.94	1,009.46	145.36	866.46	1,559.26	224.53	651.28	1,172.03	168.77
140	566.90	1,020.19	146.91	753.45	1,355.90	195.25	753.45	1,355.90	195.25
141	566.90	1,020.19	146.91	975.74	1,755.93	252.85	920.25	1,656.07	238.47
142	566.90	1,020.19	146.91	1,038.67	1,869.18	269.16	920.25	1,656.07	238.47
143	566.90	1,020.19	146.91	1,075.21	1,934.93	278.63	920.25	1,656.07	238.47
144	566.90	1,020.19	146.91	1,099.91	1,979.38	285.03	920.25	1,656.07	238.47
145	566.90	1,020.19	146.91	1,105.32	1,989.12	286.43	920.25	1,656.07	238.47
146	566.90	1,020.19	146.91	1,116.15	2,008.60	289.24	920.25	1,656.07	238.47
147	566.90	1,020.19	146.91	1,115.30	2,007.08	289.02	920.25	1,656.07	238.47
148	566.90	1,020.19	146.91	1,107.69	1,993.38	287.05	920.25	1,656.07	238.47
149	566.90	1,020.19	146.91	1,100.58	1,980.60	285.21	920.25	1,656.07	238.47
150	566.90	1,020.19	146.91	1,079.61	1,942.85	279.77	920.25	1,656.07	238.47
151	566.90	1,020.19	146.91	1,054.91	1,898.40	273.37	920.25	1,656.07	238.47
152	566.90	1,020.19	146.91	1,025.98	1,846.34	265.87	920.25	1,656.07	238.47
153	566.90	1,020.19	146.91	1,009.23	1,816.20	261.53	920.25	1,656.07	238.47
154	566.90	1,020.19	146.91	971.85	1,748.93	251.85	920.25	1,656.07	238.47
155	566.90	1,020.19	146.91	942.24	1,695.65	244.17	920.25	1,656.07	238.47
156	566.90	1,020.19	146.91	947.49	1,705.09	245.53	920.25	1,656.07	238.47
157	566.90	1,020.19	146.91	948.33	1,706.61	245.75	920.25	1,656.07	238.47
158	566.90	1,020.19	146.91	937.84	1,687.73	243.03	920.25	1,656.07	238.47
159	566.90	1,020.19	146.91	924.14	1,663.08	239.48	920.25	1,656.07	238.47
160	566.90	1,020.19	146.91	912.64	1,642.37	236.50	912.64	1,642.37	236.50
161	566.90	1,020.19	146.91	914.67	1,646.03	237.03	914.67	1,646.03	237.03
162	566.90	1,020.19	146.91	923.46	1,661.86	239.31	920.25	1,656.07	238.47
163	566.90	1,020.19	146.91	931.42	1,676.17	241.37	920.25	1,656.07	238.47
164	592.41	1,066.09	153.52	1,061.17	1,909.66	274.99	877.62	1,579.36	227.43
165	592.41	1,066.09	153.52	1,119.70	2,015.00	290.16	877.62	1,579.36	227.43
166	592.41	1,066.09	153.52	1,132.89	2,038.74	293.58	877.62	1,579.36	227.43
167	592.41	1,066.09	153.52	1,152.52	2,074.06	298.66	877.62	1,579.36	227.43
168	592.41	1,066.09	153.52	1,149.81	2,069.19	297.96	877.62	1,579.36	227.43
169	592.41	1,066.09	153.52	1,153.36	2,075.58	298.88	877.62	1,579.36	227.43
170	592.41	1,066.09	153.52	1,149.13	2,067.97	297.79	877.62	1,579.36	227.43
171	592.41	1,066.09	153.52	1,132.05	2,037.22	293.36	877.62	1,579.36	227.43
172	592.41	1,066.09	153.52	1,107.01	1,992.17	286.87	877.62	1,579.36	227.43
173	592.41	1,066.09	153.52	1,083.50	1,949.85	280.78	877.62	1,579.36	227.43
174	592.41	1,066.09	153.52	1,068.27	1,922.45	276.83	877.62	1,579.36	227.43
175	592.41	1,066.09	153.52	1,041.71	1,874.65	269.95	877.62	1,579.36	227.43
176	592.41	1,066.09	153.52	1,006.86	1,811.94	260.92	877.62	1,579.36	227.43

Cultivating time (Hour)	Fish tank inlet carbon dioxide			Fish tank outlet carbon dioxide			Basic respiration		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)	CCO _{2BR} (ppm)	CCO _{2BR} (mg/m ³)	HCO _{2BR} (mg)
177	592.41	1,066.09	153.52	978.27	1,760.49	253.51	877.62	1,579.36	227.43
178	592.41	1,066.09	153.52	954.08	1,716.96	247.24	877.62	1,579.36	227.43
179	592.41	1,066.09	153.52	932.43	1,677.99	241.63	877.62	1,579.36	227.43
180	592.41	1,066.09	153.52	906.38	1,631.11	234.88	877.62	1,579.36	227.43
181	592.41	1,066.09	153.52	892.34	1,605.84	231.24	877.62	1,579.36	227.43
182	592.41	1,066.09	153.52	882.70	1,588.49	228.74	877.62	1,579.36	227.43
183	592.41	1,066.09	153.52	890.48	1,602.49	230.76	877.62	1,579.36	227.43
184	592.41	1,066.09	153.52	883.03	1,589.10	228.83	877.62	1,579.36	227.43
185	592.41	1,066.09	153.52	876.77	1,577.83	227.21	876.77	1,577.83	227.21
186	592.41	1,066.09	153.52	876.27	1,576.92	227.08	876.27	1,576.92	227.08
187	592.41	1,066.09	153.52	877.62	1,579.36	227.43	877.62	1,579.36	227.43
		Sum	27,427		Sum	42,726		Sum	38,899

Table F10 the concentrations and mass of carbon dioxide in air that flow in/out Solid separating unit during Nile tilapia cultivation with 3 kg/m³ fish density.

Cultivating time (Hour)	Solid separating unit inlet carbon dioxide			Solid separating unit outlet carbon dioxide		
	CCO _{2SI} (ppm)	CCO _{2SI} (mg/m ³)	HCO _{2SI} (g)	CCO _{2SO} (ppm)	CCO _{2SO} (mg/m ³)	HCO _{2SO} (g)
1-19	535.57	963.80	10,987	745.67	1,341.90	15,298
20-46	566.02	1,018.60	16,501	680.71	1,225.00	19,845
47-67	549.27	988.46	12,454	692.89	1,246.92	15,711
68-95	569.06	1,024.08	17,205	746.69	1,343.73	22,575
96-116	580.23	1,044.17	13,157	754.81	1,358.34	17,115
117-139	560.94	1,009.46	13,931	646.20	1,162.90	16,048
140-163	566.90	1,020.19	14,691	705.70	1,269.98	18,288
164-187	592.41	1,066.09	15,352	708.12	1,274.32	18,350
		Sum	114,277		Sum	42,726

Table F11 the concentrations and mass of carbon dioxide in air that flow in/out Fish tank during Nile tilapia cultivation with 5 kg/m³ fish density including basic respiration.

Cultivating time (Hour)	Fish tank inlet carbon dioxide			Fish tank outlet carbon dioxide			Basic respiration		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)	CCO _{2BR} (ppm)	CCO _{2BR} (mg/m ³)	HCO _{2BR} (mg)
1	537.09	966.54	139.18	866.62	1,559.57	224.58	808.60	1,455.15	209.54
2	537.09	966.54	139.18	884.56	1,591.84	229.22	808.60	1,455.15	209.54
3	537.09	966.54	139.18	872.71	1,570.53	226.16	808.60	1,455.15	209.54
4	537.09	966.54	139.18	864.76	1,556.22	224.10	808.60	1,455.15	209.54
5	537.09	966.54	139.18	867.30	1,560.79	224.75	808.60	1,455.15	209.54
6	537.09	966.54	139.18	867.98	1,562.00	224.93	808.60	1,455.15	209.54
7	537.09	966.54	139.18	870.18	1,565.96	225.50	808.60	1,455.15	209.54
8	537.09	966.54	139.18	873.73	1,572.36	226.42	808.60	1,455.15	209.54
9	537.09	966.54	139.18	873.39	1,571.75	226.33	808.60	1,455.15	209.54
10	537.09	966.54	139.18	869.67	1,565.05	225.37	808.60	1,455.15	209.54
11	537.09	966.54	139.18	863.41	1,553.78	223.75	808.60	1,455.15	209.54
12	537.09	966.54	139.18	853.26	1,535.52	221.11	808.60	1,455.15	209.54
13	537.09	966.54	139.18	844.46	1,519.69	218.84	808.60	1,455.15	209.54
14	537.09	966.54	139.18	834.31	1,501.42	216.20	808.60	1,455.15	209.54
15	537.09	966.54	139.18	823.66	1,482.24	213.44	808.60	1,455.15	209.54
16	537.09	966.54	139.18	818.41	1,472.81	212.08	808.60	1,455.15	209.54
17	537.09	966.54	139.18	817.90	1,471.89	211.95	808.60	1,455.15	209.54

Cultivating time (Hour)	Fish tank inlet carbon dioxide			Fish tank outlet carbon dioxide			Basic respiration		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)	CCO _{2BR} (ppm)	CCO _{2BR} (mg/m ³)	HCO _{2BR} (mg)
18	537.09	966.54	139.18	812.32	1,461.85	210.51	808.60	1,455.15	209.54
19	537.09	966.54	139.18	781.53	1,406.44	202.53	781.53	1,406.44	139.18
20	531.51	956.49	137.73	850.72	1,530.95	220.46	848.19	1,526.39	219.80
21	531.51	956.49	137.73	968.80	1,743.45	251.06	848.19	1,526.39	219.80
22	531.51	956.49	137.73	955.94	1,720.31	247.72	848.19	1,526.39	219.80
23	531.51	956.49	137.73	942.41	1,695.95	244.22	848.19	1,526.39	219.80
24	531.51	956.49	137.73	935.14	1,682.86	242.33	848.19	1,526.39	219.80
25	531.51	956.49	137.73	930.91	1,675.25	241.24	848.19	1,526.39	219.80
26	531.51	956.49	137.73	933.78	1,680.43	241.98	848.19	1,526.39	219.80
27	531.51	956.49	137.73	936.49	1,685.30	242.68	848.19	1,526.39	219.80
28	531.51	956.49	137.73	935.81	1,684.08	242.51	848.19	1,526.39	219.80
29	531.51	956.49	137.73	930.40	1,674.34	241.10	848.19	1,526.39	219.80
30	531.51	956.49	137.73	912.30	1,641.77	236.41	848.19	1,526.39	219.80
31	531.51	956.49	137.73	906.38	1,631.11	234.88	848.19	1,526.39	219.80
32	531.51	956.49	137.73	892.68	1,606.45	231.33	848.19	1,526.39	219.80
33	531.51	956.49	137.73	877.28	1,578.75	227.34	848.19	1,526.39	219.80
34	531.51	956.49	137.73	878.13	1,580.27	227.56	848.19	1,526.39	219.80
35	531.51	956.49	137.73	882.70	1,588.49	228.74	848.19	1,526.39	219.80
36	531.51	956.49	137.73	877.96	1,579.97	227.52	848.19	1,526.39	219.80
37	531.51	956.49	137.73	863.24	1,553.48	223.70	848.19	1,526.39	219.80
38	531.51	956.49	137.73	852.08	1,533.39	220.81	848.19	1,526.39	219.80
39	531.51	956.49	137.73	848.02	1,526.08	219.76	848.02	1,526.08	219.76
40	531.51	956.49	137.73	840.40	1,512.38	217.78	840.40	1,512.38	217.78
41	531.51	956.49	137.73	852.25	1,533.69	220.85	848.19	1,526.39	219.80
42	531.51	956.49	137.73	848.52	1,526.99	219.89	848.19	1,526.39	219.80
43	531.51	956.49	137.73	712.01	1,281.32	184.51	712.01	1,281.32	184.51
44	531.51	956.49	137.73	798.62	1,437.19	206.96	798.62	1,437.19	206.96
45	490.91	883.43	127.21	1,028.52	1,850.91	266.53	840.07	1,511.77	217.70
46	490.91	883.43	127.21	1,048.82	1,887.44	271.79	840.07	1,511.77	217.70
47	490.91	883.43	127.21	1,027.67	1,849.39	266.31	840.07	1,511.77	217.70
48	490.91	883.43	127.21	1,021.24	1,837.82	264.65	840.07	1,511.77	217.70
49	490.91	883.43	127.21	1,006.53	1,811.33	260.83	840.07	1,511.77	217.70
50	490.91	883.43	127.21	994.85	1,790.33	257.81	840.07	1,511.77	217.70
51	490.91	883.43	127.21	996.38	1,793.07	258.20	840.07	1,511.77	217.70
52	490.91	883.43	127.21	987.24	1,776.63	255.83	840.07	1,511.77	217.70
53	490.91	883.43	127.21	978.44	1,760.80	253.55	840.07	1,511.77	217.70
54	490.91	883.43	127.21	968.12	1,742.23	250.88	840.07	1,511.77	217.70
55	490.91	883.43	127.21	957.64	1,723.35	248.16	840.07	1,511.77	217.70
56	490.91	883.43	127.21	933.45	1,679.82	241.89	840.07	1,511.77	217.70
57	490.91	883.43	127.21	922.45	1,660.03	239.04	840.07	1,511.77	217.70
58	490.91	883.43	127.21	912.64	1,642.37	236.50	840.07	1,511.77	217.70
59	490.91	883.43	127.21	906.89	1,632.02	235.01	840.07	1,511.77	217.70
60	490.91	883.43	127.21	898.43	1,616.80	232.82	840.07	1,511.77	217.70
61	490.91	883.43	127.21	879.31	1,582.40	227.87	840.07	1,511.77	217.70
62	490.91	883.43	127.21	863.24	1,553.48	223.70	840.07	1,511.77	217.70
63	490.91	883.43	127.21	851.06	1,531.56	220.54	840.07	1,511.77	217.70
64	490.91	883.43	127.21	846.83	1,523.95	219.45	840.07	1,511.77	217.70
65	490.91	883.43	127.21	845.31	1,521.21	219.05	840.07	1,511.77	217.70
66	490.91	883.43	127.21	854.95	1,538.56	221.55	840.07	1,511.77	217.70
67	490.91	883.43	127.21	861.38	1,550.13	223.22	840.07	1,511.77	217.70
68	490.91	883.43	127.21	858.67	1,545.26	222.52	840.07	1,511.77	217.70
69	490.91	883.43	127.21	842.10	1,515.43	218.22	840.07	1,511.77	217.70
70	490.91	883.43	127.21	744.66	1,340.07	192.97	744.66	1,340.07	192.97
71	538.61	969.28	139.58	948.50	1,706.91	245.80	882.70	1,588.49	228.74
72	538.61	969.28	139.58	971.34	1,748.01	251.71	882.70	1,588.49	228.74
73	538.61	969.28	139.58	972.52	1,750.14	252.02	882.70	1,588.49	228.74
74	538.61	969.28	139.58	954.08	1,716.96	247.24	882.70	1,588.49	228.74
75	538.61	969.28	139.58	945.29	1,701.13	244.96	882.70	1,588.49	228.74
76	538.61	969.28	139.58	936.83	1,685.91	242.77	882.70	1,588.49	228.74
77	538.61	969.28	139.58	925.66	1,665.82	239.88	882.70	1,588.49	228.74

Cultivating time (Hour)	Fish tank inlet carbon dioxide			Fish tank outlet carbon dioxide			Basic respiration		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)	CCO _{2BR} (ppm)	CCO _{2BR} (mg/m ³)	HCO _{2BR} (mg)
78	538.61	969.28	139.58	928.88	1,671.60	240.71	882.70	1,588.49	228.74
79	538.61	969.28	139.58	927.19	1,668.56	240.27	882.70	1,588.49	228.74
80	538.61	969.28	139.58	935.48	1,683.47	242.42	882.70	1,588.49	228.74
81	538.61	969.28	139.58	932.43	1,677.99	241.63	882.70	1,588.49	228.74
82	538.61	969.28	139.58	932.60	1,678.30	241.67	882.70	1,588.49	228.74
83	538.61	969.28	139.58	913.15	1,643.29	236.63	882.70	1,588.49	228.74
84	538.61	969.28	139.58	911.62	1,640.55	236.24	882.70	1,588.49	228.74
85	538.61	969.28	139.58	894.88	1,610.41	231.90	882.70	1,588.49	228.74
86	538.61	969.28	139.58	886.92	1,596.10	229.84	882.70	1,588.49	228.74
87	538.61	969.28	139.58	859.52	1,546.78	222.74	859.52	1,546.78	222.74
88	538.61	969.28	139.58	845.31	1,521.21	219.05	845.31	1,521.21	219.05
89	545.72	982.06	141.42	763.94	1,374.78	197.97	763.94	1,374.78	197.97
90	545.72	982.06	141.42	940.38	1,692.30	243.69	940.38	1,692.30	243.69
91	545.72	982.06	141.42	1,195.99	2,152.30	309.93	948.67	1,707.22	245.84
92	545.72	982.06	141.42	1,159.45	2,086.54	300.46	948.67	1,707.22	245.84
93	545.72	982.06	141.42	1,137.80	2,047.57	294.85	948.67	1,707.22	245.84
94	545.72	982.06	141.42	1,128.33	2,030.52	292.40	948.67	1,707.22	245.84
95	545.72	982.06	141.42	1,130.02	2,033.57	292.83	948.67	1,707.22	245.84
96	545.72	982.06	141.42	1,117.67	2,011.34	289.63	948.67	1,707.22	245.84
97	545.72	982.06	141.42	1,115.64	2,007.69	289.11	948.67	1,707.22	245.84
98	545.72	982.06	141.42	1,132.72	2,038.44	293.54	948.67	1,707.22	245.84
99	545.72	982.06	141.42	1,120.88	2,017.13	290.47	948.67	1,707.22	245.84
100	545.72	982.06	141.42	1,102.10	1,983.34	285.60	948.67	1,707.22	245.84
101	545.72	982.06	141.42	1,093.14	1,967.20	283.28	948.67	1,707.22	245.84
102	545.72	982.06	141.42	1,068.27	1,922.45	276.83	948.67	1,707.22	245.84
103	545.72	982.06	141.42	1,059.81	1,907.23	274.64	948.67	1,707.22	245.84
104	545.72	982.06	141.42	1,052.03	1,893.23	272.62	948.67	1,707.22	245.84
105	545.72	982.06	141.42	1,030.38	1,854.26	267.01	948.67	1,707.22	245.84
106	545.72	982.06	141.42	1,022.77	1,840.56	265.04	948.67	1,707.22	245.84
107	545.72	982.06	141.42	1,013.12	1,823.21	262.54	948.67	1,707.22	245.84
108	545.72	982.06	141.42	1,006.53	1,811.33	260.83	948.67	1,707.22	245.84
109	545.72	982.06	141.42	1,000.27	1,800.07	259.21	948.67	1,707.22	245.84
110	545.72	982.06	141.42	993.67	1,788.20	257.50	948.67	1,707.22	245.84
111	545.72	982.06	141.42	996.88	1,793.98	258.33	948.67	1,707.22	245.84
112	545.72	982.06	141.42	982.33	1,767.80	254.56	948.67	1,707.22	245.84
113	545.72	982.06	141.42	964.57	1,735.83	249.96	948.67	1,707.22	245.84
114	545.72	982.06	141.42	957.13	1,722.44	248.03	948.67	1,707.22	245.84
115	566.02	1,018.60	146.68	781.20	1,405.83	202.44	781.20	1,405.83	202.44
116	566.02	1,018.60	146.68	1,063.20	1,913.32	275.52	948.67	1,707.22	245.84
117	566.02	1,018.60	146.68	1,411.00	2,539.23	365.65	911.12	1,639.63	236.11
118	566.02	1,018.60	146.68	1,496.60	2,693.27	387.83	911.12	1,639.63	236.11
119	566.02	1,018.60	146.68	1,458.03	2,623.86	377.84	911.12	1,639.63	236.11
120	566.02	1,018.60	146.68	1,419.80	2,555.06	367.93	911.12	1,639.63	236.11
121	566.02	1,018.60	146.68	1,395.10	2,510.61	361.53	911.12	1,639.63	236.11
122	566.02	1,018.60	146.68	1,425.21	2,564.80	369.33	911.12	1,639.63	236.11
123	566.02	1,018.60	146.68	1,444.16	2,598.90	374.24	911.12	1,639.63	236.11
124	566.02	1,018.60	146.68	1,416.92	2,549.88	367.18	911.12	1,639.63	236.11
125	566.02	1,018.60	146.68	1,418.28	2,552.32	367.53	911.12	1,639.63	236.11
126	566.02	1,018.60	146.68	1,433.16	2,579.11	371.39	911.12	1,639.63	236.11
127	566.02	1,018.60	146.68	1,421.66	2,558.41	368.41	911.12	1,639.63	236.11
128	566.02	1,018.60	146.68	1,385.63	2,493.56	359.07	911.12	1,639.63	236.11
129	566.02	1,018.60	146.68	1,378.69	2,481.08	357.28	911.12	1,639.63	236.11
130	566.02	1,018.60	146.68	1,364.82	2,456.12	353.68	911.12	1,639.63	236.11
131	566.02	1,018.60	146.68	1,372.09	2,469.21	355.57	911.12	1,639.63	236.11
132	566.02	1,018.60	146.68	1,337.92	2,407.71	346.71	911.12	1,639.63	236.11
133	566.02	1,018.60	146.68	1,313.06	2,362.96	340.27	911.12	1,639.63	236.11
134	566.02	1,018.60	146.68	1,290.22	2,321.86	334.35	911.12	1,639.63	236.11
135	566.02	1,018.60	146.68	1,270.26	2,285.94	329.18	911.12	1,639.63	236.11
136	566.02	1,018.60	146.68	924.48	1,663.68	239.57	911.12	1,639.63	236.11
137	572.11	1,029.56	148.26	1,120.04	2,015.61	290.25	911.12	1,639.63	236.11

Cultivating time (Hour)	Fish tank inlet carbon dioxide			Fish tank outlet carbon dioxide			Basic respiration		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)	CCO _{2BR} (ppm)	CCO _{2BR} (mg/m ³)	HCO _{2BR} (mg)
138	572.11	1,029.56	148.26	1,153.70	2,076.19	298.97	911.12	1,639.63	236.11
139	572.11	1,029.56	148.26	1,127.31	2,028.70	292.13	911.12	1,639.63	236.11
140	572.11	1,029.56	148.26	1,101.09	1,981.51	285.34	911.12	1,639.63	236.11
141	572.11	1,029.56	148.26	1,088.40	1,958.68	282.05	911.12	1,639.63	236.11
142	572.11	1,029.56	148.26	1,062.01	1,911.19	275.21	911.12	1,639.63	236.11
143	572.11	1,029.56	148.26	1,042.05	1,875.26	270.04	911.12	1,639.63	236.11
144	572.11	1,029.56	148.26	1,031.56	1,856.39	267.32	911.12	1,639.63	236.11
145	572.11	1,029.56	148.26	1,019.72	1,835.08	264.25	911.12	1,639.63	236.11
146	572.11	1,029.56	148.26	1,000.27	1,800.07	259.21	911.12	1,639.63	236.11
147	572.11	1,029.56	148.26	999.59	1,798.85	259.03	911.12	1,639.63	236.11
148	572.11	1,029.56	148.26	984.70	1,772.06	255.18	911.12	1,639.63	236.11
149	572.11	1,029.56	148.26	966.43	1,739.18	250.44	911.12	1,639.63	236.11
150	572.11	1,029.56	148.26	956.62	1,721.53	247.90	911.12	1,639.63	236.11
151	572.11	1,029.56	148.26	943.43	1,697.78	244.48	911.12	1,639.63	236.11
152	572.11	1,029.56	148.26	940.04	1,691.69	243.60	911.12	1,639.63	236.11
153	572.11	1,029.56	148.26	941.57	1,694.43	244.00	911.12	1,639.63	236.11
154	572.11	1,029.56	148.26	932.43	1,677.99	241.63	911.12	1,639.63	236.11
155	572.11	1,029.56	148.26	927.02	1,668.25	240.23	911.12	1,639.63	236.11
156	572.11	1,029.56	148.26	924.14	1,663.08	239.48	911.12	1,639.63	236.11
157	572.11	1,029.56	148.26	919.57	1,654.86	238.30	911.12	1,639.63	236.11
158	572.11	1,029.56	148.26	913.48	1,643.90	236.72	911.12	1,639.63	236.11
159	572.11	1,029.56	148.26	908.24	1,634.46	235.36	908.24	1,634.46	235.36
160	572.11	1,029.56	148.26	911.79	1,640.85	236.28	911.12	1,639.63	236.11
161	572.11	1,029.56	148.26	917.37	1,650.90	237.73	911.12	1,639.63	236.11
162	572.11	1,029.56	148.26	830.25	1,494.12	215.15	830.25	1,494.12	215.15
163	588.35	1,058.78	152.46	888.95	1,599.75	230.36	888.95	1,599.75	230.36
164	588.35	1,058.78	152.46	1,028.69	1,851.21	266.57	897.92	1,615.89	232.69
165	588.35	1,058.78	152.46	1,043.07	1,877.09	270.30	897.92	1,615.89	232.69
166	588.35	1,058.78	152.46	1,032.75	1,858.52	267.63	897.92	1,615.89	232.69
167	588.35	1,058.78	152.46	1,024.46	1,843.60	265.48	897.92	1,615.89	232.69
168	588.35	1,058.78	152.46	1,008.72	1,815.29	261.40	897.92	1,615.89	232.69
169	588.35	1,058.78	152.46	996.71	1,793.68	258.29	897.92	1,615.89	232.69
170	588.35	1,058.78	152.46	989.78	1,781.19	256.49	897.92	1,615.89	232.69
171	588.35	1,058.78	152.46	1,001.96	1,803.11	259.65	897.92	1,615.89	232.69
172	588.35	1,058.78	152.46	991.13	1,783.63	256.84	897.92	1,615.89	232.69
173	588.35	1,058.78	152.46	970.32	1,746.19	251.45	897.92	1,615.89	232.69
174	588.35	1,058.78	152.46	952.22	1,713.61	246.76	897.92	1,615.89	232.69
175	588.35	1,058.78	152.46	936.15	1,684.69	242.60	897.92	1,615.89	232.69
176	588.35	1,058.78	152.46	929.72	1,673.12	240.93	897.92	1,615.89	232.69
177	588.35	1,058.78	152.46	923.97	1,662.77	239.44	897.92	1,615.89	232.69
178	588.35	1,058.78	152.46	911.62	1,640.55	236.24	897.92	1,615.89	232.69
179	588.35	1,058.78	152.46	904.18	1,627.15	234.31	897.92	1,615.89	232.69
180	588.35	1,058.78	152.46	904.86	1,628.37	234.49	897.92	1,615.89	232.69
181	588.35	1,058.78	152.46	900.12	1,619.85	233.26	897.92	1,615.89	232.69
182	588.35	1,058.78	152.46	895.38	1,611.32	232.03	895.38	1,611.32	232.03
183	588.35	1,058.78	152.46	890.48	1,602.49	230.76	890.48	1,602.49	230.76
184	588.35	1,058.78	152.46	883.03	1,589.10	228.83	883.03	1,589.10	228.83
		Sum	26,020		Sum	47,205		Sum	41,832

Table F12 the concentrations and mass of carbon dioxide in air that flow in/out Solid separating unit during Nile tilapia cultivation with 5 kg/m³ fish density.

Cultivating time (Hour)	Solid separating unit inlet carbon dioxide			Solid separating unit outlet carbon dioxide		
	CCO _{2SI} (ppm)	CCO _{2SI} (mg/m ³)	HCO _{2SI} (mg)	CCO _{2SO} (ppm)	CCO _{2SO} (mg/m ³)	HCO _{2SO} (mg)
1-19	537.09	966.54	11,018	793.88	1,428.66	16,287

Cultivating time (Hour)	Solid separating unit inlet carbon dioxide			Solid separating unit outlet carbon dioxide		
	CCO _{2SI} (ppm)	CCO _{2SI} (mg/m ³)	HCO _{2SI} (mg)	CCO _{2SO} (ppm)	CCO _{2SO} (mg/m ³)	HCO _{2SO} (mg)
20-44	531.51	956.49	14,347	815.20	1,467.02	22,005
45-70	490.91	883.43	13,782	786.27	1,414.96	22,073
71-88	538.61	969.28	10,468	790.84	1,423.18	15,370
89-114	545.72	982.06	15,320	825.86	1,486.20	23,185
115-136	566.02	1,018.60	13,446	827.89	1,489.85	19,666
137-162	572.11	1,029.56	16,061	764.96	1,376.61	21,475
163-184	588.35	1,058.78	13,976	887.77	1,597.62	21,089
	Sum			Sum		
	108,418			161,150		

Table F13 the concentrations and mass of carbon dioxide in air that flow in/out Fish tank during Nile tilapia cultivation with 10 kg/m³ fish density including basic respiration.

Cultivating time (Hour)	Fish tank inlet carbon dioxide			Fish tank outlet carbon dioxide			Basic respiration		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)	CCO _{2BR} (ppm)	CCO _{2BR} (mg/m ³)	HCO _{2BR} (mg)
1	583.27	1,049.65	151.15	1,253.68	2,256.11	324.88	1,253.68	2,256.11	324.88
2	583.27	1,049.65	151.15	1,355.85	2,439.98	351.36	1,288.70	2,319.12	333.95
3	583.27	1,049.65	151.15	1,361.94	2,450.94	352.94	1,288.70	2,319.12	333.95
4	583.27	1,049.65	151.15	1,341.64	2,414.41	347.68	1,288.70	2,319.12	333.95
5	583.27	1,049.65	151.15	1,316.95	2,369.96	341.27	1,288.70	2,319.12	333.95
6	583.27	1,049.65	151.15	1,334.71	2,401.93	345.88	1,288.70	2,319.12	333.95
7	583.27	1,049.65	151.15	1,383.43	2,489.61	358.50	1,288.70	2,319.12	333.95
8	583.27	1,049.65	151.15	1,330.31	2,394.01	344.74	1,288.70	2,319.12	333.95
9	583.27	1,049.65	151.15	1,318.47	2,372.70	341.67	1,288.70	2,319.12	333.95
10	583.27	1,049.65	151.15	1,289.03	2,319.73	334.04	1,288.70	2,319.12	333.95
11	583.27	1,049.65	151.15	1,322.19	2,379.40	342.63	1,288.70	2,319.12	333.95
12	583.27	1,049.65	151.15	1,414.22	2,545.01	366.48	1,288.70	2,319.12	333.95
13	583.27	1,049.65	151.15	1,440.95	2,593.11	373.41	1,288.70	2,319.12	333.95
14	583.27	1,049.65	151.15	1,441.96	2,594.94	373.67	1,288.70	2,319.12	333.95
15	583.27	1,049.65	151.15	1,443.14	2,597.07	373.98	1,288.70	2,319.12	333.95
16	583.27	1,049.65	151.15	1,484.25	2,671.05	384.63	1,288.70	2,319.12	333.95
17	583.27	1,049.65	151.15	1,542.95	2,776.68	399.84	1,288.70	2,319.12	333.95
18	583.27	1,049.65	151.15	1,564.94	2,816.26	405.54	1,288.70	2,319.12	333.95
19	583.27	1,049.65	151.15	1,585.92	2,854.01	410.98	1,288.70	2,319.12	333.95
20	590.38	1,062.43	152.99	582.26	1,047.82	150.89	582.26	1,047.82	150.89
21	590.38	1,062.43	152.99	1,468.86	2,643.34	380.64	1,468.86	2,643.34	380.64
22	590.38	1,062.43	152.99	1,968.91	3,543.24	510.23	1,486.62	2,675.31	385.24
23	590.38	1,062.43	152.99	2,137.91	3,847.37	554.02	1,486.62	2,675.31	385.24
24	590.38	1,062.43	152.99	2,113.89	3,804.14	547.80	1,486.62	2,675.31	385.24
25	590.38	1,062.43	152.99	2,088.68	3,758.78	541.26	1,486.62	2,675.31	385.24
26	590.38	1,062.43	152.99	2,068.05	3,721.64	535.92	1,486.62	2,675.31	385.24
27	590.38	1,062.43	152.99	2,003.09	3,604.74	519.08	1,486.62	2,675.31	385.24
28	590.38	1,062.43	152.99	1,991.58	3,584.03	516.10	1,486.62	2,675.31	385.24
29	590.38	1,062.43	152.99	1,937.79	3,487.22	502.16	1,486.62	2,675.31	385.24
30	590.38	1,062.43	152.99	1,873.50	3,371.54	485.50	1,486.62	2,675.31	385.24
31	590.38	1,062.43	152.99	1,808.04	3,253.73	468.54	1,486.62	2,675.31	385.24
32	590.38	1,062.43	152.99	1,760.84	3,168.79	456.31	1,486.62	2,675.31	385.24
33	590.38	1,062.43	152.99	1,686.24	3,034.54	436.97	1,486.62	2,675.31	385.24
34	590.38	1,062.43	152.99	1,623.14	2,920.98	420.62	1,486.62	2,675.31	385.24
35	590.38	1,062.43	152.99	1,563.59	2,813.82	405.19	1,486.62	2,675.31	385.24
36	590.38	1,062.43	152.99	1,543.80	2,778.21	400.06	1,486.62	2,675.31	385.24
37	590.38	1,062.43	152.99	1,536.02	2,764.20	398.05	1,486.62	2,675.31	385.24
38	590.38	1,062.43	152.99	1,528.57	2,750.81	396.12	1,486.62	2,675.31	385.24
39	590.38	1,062.43	152.99	1,506.75	2,711.54	390.46	1,486.62	2,675.31	385.24

Cultivating time (Hour)	Fish tank inlet carbon dioxide			Fish tank outlet carbon dioxide			Basic respiration		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)	CCO _{2BR} (ppm)	CCO _{2BR} (mg/m ³)	HCO _{2BR} (mg)
40	590.38	1,062.43	152.99	1,517.24	2,730.41	393.18	1,486.62	2,675.31	385.24
41	590.38	1,062.43	152.99	1,522.15	2,739.24	394.45	1,486.62	2,675.31	385.24
42	590.38	1,062.43	152.99	1,497.79	2,695.40	388.14	1,486.62	2,675.31	385.24
43	586.32	1,055.13	151.94	856.47	1,541.30	221.95	856.47	1,541.30	221.95
44	586.32	1,055.13	151.94	1,337.92	2,407.71	346.71	1,301.89	2,342.87	337.37
45	586.32	1,055.13	151.94	1,929.16	3,471.70	499.92	1,301.89	2,342.87	337.37
46	586.32	1,055.13	151.94	1,880.78	3,384.63	487.39	1,301.89	2,342.87	337.37
47	586.32	1,055.13	151.94	1,819.03	3,273.51	471.39	1,301.89	2,342.87	337.37
48	586.32	1,055.13	151.94	1,780.80	3,204.71	461.48	1,301.89	2,342.87	337.37
49	586.32	1,055.13	151.94	1,765.91	3,177.92	457.62	1,301.89	2,342.87	337.37
50	586.32	1,055.13	151.94	1,752.38	3,153.57	454.11	1,301.89	2,342.87	337.37
51	586.32	1,055.13	151.94	1,700.28	3,059.80	440.61	1,301.89	2,342.87	337.37
52	586.32	1,055.13	151.94	1,638.36	2,948.38	424.57	1,301.89	2,342.87	337.37
53	586.32	1,055.13	151.94	1,575.26	2,834.83	408.22	1,301.89	2,342.87	337.37
54	586.32	1,055.13	151.94	1,529.93	2,753.24	396.47	1,301.89	2,342.87	337.37
55	586.32	1,055.13	151.94	1,496.77	2,693.57	387.87	1,301.89	2,342.87	337.37
56	586.32	1,055.13	151.94	1,447.71	2,605.29	375.16	1,301.89	2,342.87	337.37
57	586.32	1,055.13	151.94	1,414.89	2,546.23	366.66	1,301.89	2,342.87	337.37
58	586.32	1,055.13	151.94	1,393.24	2,507.26	361.05	1,301.89	2,342.87	337.37
59	586.32	1,055.13	151.94	1,359.41	2,446.38	352.28	1,301.89	2,342.87	337.37
60	586.32	1,055.13	151.94	1,331.49	2,396.14	345.04	1,301.89	2,342.87	337.37
61	586.32	1,055.13	151.94	1,343.51	2,417.76	348.16	1,301.89	2,342.87	337.37
62	586.32	1,055.13	151.94	1,329.97	2,393.41	344.65	1,301.89	2,342.87	337.37
63	586.32	1,055.13	151.94	1,311.53	2,360.22	339.87	1,301.89	2,342.87	337.37
64	586.32	1,055.13	151.94	1,300.54	2,340.43	337.02	1,300.54	2,340.43	337.02
65	586.32	1,055.13	151.94	1,290.05	2,321.56	334.30	1,290.05	2,321.56	334.30
66	583.27	1,049.65	151.15	924.48	1,663.68	239.57	924.48	1,663.68	239.57
67	583.27	1,049.65	151.15	878.80	1,581.49	227.73	878.80	1,581.49	227.73
68	583.27	1,049.65	151.15	1,799.58	3,238.50	466.34	1,280.58	2,304.51	331.85
69	583.27	1,049.65	151.15	1,766.59	3,179.14	457.80	1,280.58	2,304.51	331.85
70	583.27	1,049.65	151.15	1,738.17	3,128.00	450.43	1,280.58	2,304.51	331.85
71	583.27	1,049.65	151.15	1,746.29	3,142.61	452.54	1,280.58	2,304.51	331.85
72	583.27	1,049.65	151.15	1,761.52	3,170.01	456.48	1,280.58	2,304.51	331.85
73	583.27	1,049.65	151.15	1,762.87	3,172.44	456.83	1,280.58	2,304.51	331.85
74	583.27	1,049.65	151.15	1,737.83	3,127.39	450.34	1,280.58	2,304.51	331.85
75	583.27	1,049.65	151.15	1,688.77	3,039.10	437.63	1,280.58	2,304.51	331.85
76	583.27	1,049.65	151.15	1,621.62	2,918.24	420.23	1,280.58	2,304.51	331.85
77	583.27	1,049.65	151.15	1,572.56	2,829.96	407.51	1,280.58	2,304.51	331.85
78	583.27	1,049.65	151.15	1,515.04	2,726.45	392.61	1,280.58	2,304.51	331.85
79	583.27	1,049.65	151.15	1,460.06	2,627.51	378.36	1,280.58	2,304.51	331.85
80	583.27	1,049.65	151.15	1,424.71	2,563.89	369.20	1,280.58	2,304.51	331.85
81	583.27	1,049.65	151.15	1,404.74	2,527.96	364.03	1,280.58	2,304.51	331.85
82	583.27	1,049.65	151.15	1,360.59	2,448.51	352.59	1,280.58	2,304.51	331.85
83	583.27	1,049.65	151.15	1,333.36	2,399.49	345.53	1,280.58	2,304.51	331.85
84	583.27	1,049.65	151.15	1,321.34	2,377.88	342.41	1,280.58	2,304.51	331.85
85	583.27	1,049.65	151.15	1,318.13	2,372.09	341.58	1,280.58	2,304.51	331.85
86	583.27	1,049.65	151.15	1,304.43	2,347.44	338.03	1,280.58	2,304.51	331.85
87	583.27	1,049.65	151.15	1,293.26	2,327.34	335.14	1,280.58	2,304.51	331.85
88	583.27	1,049.65	151.15	1,312.72	2,362.35	340.18	1,280.58	2,304.51	331.85
89	583.27	1,049.65	151.15	1,307.64	2,353.22	338.86	1,280.58	2,304.51	331.85
90	583.27	1,049.65	151.15	1,265.18	2,276.81	327.86	1,265.18	2,276.81	327.86
91	602.56	1,084.35	156.15	875.59	1,575.70	226.90	875.59	1,575.70	226.90
92	602.56	1,084.35	156.15	790.84	1,423.18	204.94	790.84	1,423.18	204.94
93	602.56	1,084.35	156.15	1,573.57	2,831.79	407.78	1,293.77	2,328.26	335.27
94	602.56	1,084.35	156.15	1,693.34	3,047.32	438.81	1,293.77	2,328.26	335.27
95	602.56	1,084.35	156.15	1,637.69	2,947.16	424.39	1,293.77	2,328.26	335.27
96	602.56	1,084.35	156.15	1,621.62	2,918.24	420.23	1,293.77	2,328.26	335.27
97	602.56	1,084.35	156.15	1,603.68	2,885.97	415.58	1,293.77	2,328.26	335.27
98	602.56	1,084.35	156.15	1,585.08	2,852.49	410.76	1,293.77	2,328.26	335.27
99	602.56	1,084.35	156.15	1,565.11	2,816.56	405.59	1,293.77	2,328.26	335.27

Cultivating time (Hour)	Fish tank inlet carbon dioxide			Fish tank outlet carbon dioxide			Basic respiration		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)	CCO _{2BR} (ppm)	CCO _{2BR} (mg/m ³)	HCO _{2BR} (mg)
100	602.56	1,084.35	156.15	1,580.00	2,843.35	409.44	1,293.77	2,328.26	335.27
101	602.56	1,084.35	156.15	1,622.12	2,919.16	420.36	1,293.77	2,328.26	335.27
102	602.56	1,084.35	156.15	1,601.32	2,881.71	414.97	1,293.77	2,328.26	335.27
103	602.56	1,084.35	156.15	1,542.61	2,776.07	399.75	1,293.77	2,328.26	335.27
104	602.56	1,084.35	156.15	1,485.10	2,672.57	384.85	1,293.77	2,328.26	335.27
105	602.56	1,084.35	156.15	1,425.38	2,565.10	369.37	1,293.77	2,328.26	335.27
106	602.56	1,084.35	156.15	1,375.65	2,475.60	356.49	1,293.77	2,328.26	335.27
107	602.56	1,084.35	156.15	1,339.95	2,411.37	347.24	1,293.77	2,328.26	335.27
108	602.56	1,084.35	156.15	1,323.21	2,381.23	342.90	1,293.77	2,328.26	335.27
109	602.56	1,084.35	156.15	1,327.94	2,389.75	344.12	1,293.77	2,328.26	335.27
110	602.56	1,084.35	156.15	1,308.15	2,354.13	339.00	1,293.77	2,328.26	335.27
111	602.56	1,084.35	156.15	1,292.76	2,326.43	335.01	1,292.76	2,326.43	335.01
112	602.56	1,084.35	156.15	1,274.15	2,292.94	330.18	1,274.15	2,292.94	330.18
113	602.56	1,084.35	156.15	1,275.50	2,295.38	330.53	1,275.50	2,295.38	330.53
114	602.56	1,084.35	156.15	1,274.15	2,292.94	330.18	1,274.15	2,292.94	330.18
115	602.56	1,084.35	156.15	1,001.28	1,801.90	259.47	1,001.28	1,801.90	259.47
116	622.86	1,120.88	161.41	705.07	1,268.84	182.71	705.07	1,268.84	182.71
117	622.86	1,120.88	161.41	1,481.04	2,665.26	383.80	1,334.37	2,401.32	345.79
118	622.86	1,120.88	161.41	1,689.45	3,040.32	437.81	1,334.37	2,401.32	345.79
119	622.86	1,120.88	161.41	1,712.80	3,082.33	443.86	1,334.37	2,401.32	345.79
120	622.86	1,120.88	161.41	1,720.41	3,096.03	445.83	1,334.37	2,401.32	345.79
121	622.86	1,120.88	161.41	1,709.58	3,076.55	443.02	1,334.37	2,401.32	345.79
122	622.86	1,120.88	161.41	1,713.81	3,084.16	444.12	1,334.37	2,401.32	345.79
123	622.86	1,120.88	161.41	1,727.18	3,108.21	447.58	1,334.37	2,401.32	345.79
124	622.86	1,120.88	161.41	1,701.80	3,062.54	441.01	1,334.37	2,401.32	345.79
125	622.86	1,120.88	161.41	1,641.07	2,953.25	425.27	1,334.37	2,401.32	345.79
126	622.86	1,120.88	161.41	1,578.14	2,840.01	408.96	1,334.37	2,401.32	345.79
127	622.86	1,120.88	161.41	1,530.94	2,755.07	396.73	1,334.37	2,401.32	345.79
128	622.86	1,120.88	161.41	1,489.33	2,680.18	385.95	1,334.37	2,401.32	345.79
129	622.86	1,120.88	161.41	1,464.97	2,636.34	379.63	1,334.37	2,401.32	345.79
130	622.86	1,120.88	161.41	1,444.16	2,598.90	374.24	1,334.37	2,401.32	345.79
131	622.86	1,120.88	161.41	1,419.80	2,555.06	367.93	1,334.37	2,401.32	345.79
132	622.86	1,120.88	161.41	1,394.26	2,509.09	361.31	1,334.37	2,401.32	345.79
133	622.86	1,120.88	161.41	1,388.33	2,498.43	359.77	1,334.37	2,401.32	345.79
134	622.86	1,120.88	161.41	1,372.60	2,470.12	355.70	1,334.37	2,401.32	345.79
135	622.86	1,120.88	161.41	1,351.46	2,432.07	350.22	1,334.37	2,401.32	345.79
136	622.86	1,120.88	161.41	1,345.20	2,420.80	348.60	1,334.37	2,401.32	345.79
137	622.86	1,120.88	161.41	1,369.05	2,463.73	354.78	1,334.37	2,401.32	345.79
138	622.86	1,120.88	161.41	1,347.40	2,424.76	349.17	1,334.37	2,401.32	345.79
139	622.86	1,120.88	161.41	1,338.26	2,408.32	346.80	1,334.37	2,401.32	345.79
140	622.86	1,120.88	161.41	1,074.02	1,932.80	278.32	1,074.02	1,932.80	278.32
141	654.32	1,177.51	169.56	893.86	1,608.58	231.64	893.86	1,608.58	231.64
142	654.32	1,177.51	169.56	1,801.27	3,241.55	466.78	1,740.37	3,131.95	451.00
143	654.32	1,177.51	169.56	1,790.27	3,221.76	463.93	1,740.37	3,131.95	451.00
144	654.32	1,177.51	169.56	1,773.87	3,192.23	459.68	1,740.37	3,131.95	451.00
145	654.32	1,177.51	169.56	1,806.01	3,250.07	468.01	1,740.37	3,131.95	451.00
146	654.32	1,177.51	169.56	1,860.82	3,348.71	482.21	1,740.37	3,131.95	451.00
147	654.32	1,177.51	169.56	1,981.77	3,566.38	513.56	1,740.37	3,131.95	451.00
148	654.32	1,177.51	169.56	2,054.68	3,697.59	532.45	1,740.37	3,131.95	451.00
149	654.32	1,177.51	169.56	2,068.55	3,722.55	536.05	1,740.37	3,131.95	451.00
150	654.32	1,177.51	169.56	2,138.42	3,848.28	554.15	1,740.37	3,131.95	451.00
151	654.32	1,177.51	169.56	2,171.75	3,908.25	562.79	1,740.37	3,131.95	451.00
152	654.32	1,177.51	169.56	2,129.79	3,832.75	551.92	1,740.37	3,131.95	451.00
153	654.32	1,177.51	169.56	2,061.79	3,710.37	534.29	1,740.37	3,131.95	451.00
154	654.32	1,177.51	169.56	2,001.56	3,602.00	518.69	1,740.37	3,131.95	451.00
155	654.32	1,177.51	169.56	1,980.59	3,564.25	513.25	1,740.37	3,131.95	451.00
156	654.32	1,177.51	169.56	1,931.36	3,475.66	500.49	1,740.37	3,131.95	451.00
157	654.32	1,177.51	169.56	1,925.44	3,465.00	498.96	1,740.37	3,131.95	451.00
158	654.32	1,177.51	169.56	1,916.47	3,448.87	496.64	1,740.37	3,131.95	451.00
159	654.32	1,177.51	169.56	1,890.25	3,401.68	489.84	1,740.37	3,131.95	451.00

Cultivating time (Hour)	Fish tank inlet carbon dioxide			Fish tank outlet carbon dioxide			Basic respiration		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)	CCO _{2BR} (ppm)	CCO _{2BR} (mg/m ³)	HCO _{2BR} (mg)
160	654.32	1,177.51	169.56	1,913.94	3,444.30	495.98	1,740.37	3,131.95	451.00
161	654.32	1,177.51	169.56	1,906.83	3,431.51	494.14	1,740.37	3,131.95	451.00
162	654.32	1,177.51	169.56	1,903.95	3,426.34	493.39	1,740.37	3,131.95	451.00
163	654.32	1,177.51	169.56	1,725.15	3,104.56	447.06	1,725.15	3,104.56	447.06
164	654.32	1,177.51	169.56	1,518.09	2,731.93	393.40	1,518.09	2,731.93	393.40
165	490.91	883.43	127.21	821.63	1,478.59	212.92	821.63	1,478.59	212.92
166	490.91	883.43	127.21	635.88	1,144.33	164.78	635.88	1,144.33	164.78
167	490.91	883.43	127.21	894.54	1,609.80	231.81	894.54	1,609.80	231.81
168	490.91	883.43	127.21	2,525.81	4,545.43	654.54	2,093.59	3,767.61	542.54
169	490.91	883.43	127.21	2,693.29	4,846.81	697.94	2,093.59	3,767.61	542.54
170	490.91	883.43	127.21	2,688.38	4,837.98	696.67	2,093.59	3,767.61	542.54
171	490.91	883.43	127.21	2,640.68	4,752.13	684.31	2,093.59	3,767.61	542.54
172	490.91	883.43	127.21	2,632.56	4,737.52	682.20	2,093.59	3,767.61	542.54
173	490.91	883.43	127.21	2,609.72	4,696.42	676.29	2,093.59	3,767.61	542.54
174	490.91	883.43	127.21	2,587.73	4,656.85	670.59	2,093.59	3,767.61	542.54
175	490.91	883.43	127.21	2,516.17	4,528.07	652.04	2,093.59	3,767.61	542.54
176	490.91	883.43	127.21	2,463.05	4,432.48	638.28	2,093.59	3,767.61	542.54
177	490.91	883.43	127.21	2,389.46	4,300.06	619.21	2,093.59	3,767.61	542.54
178	490.91	883.43	127.21	2,295.91	4,131.70	594.97	2,093.59	3,767.61	542.54
179	490.91	883.43	127.21	2,240.93	4,032.76	580.72	2,093.59	3,767.61	542.54
180	490.91	883.43	127.21	2,205.07	3,968.23	571.42	2,093.59	3,767.61	542.54
181	490.91	883.43	127.21	2,187.65	3,936.87	566.91	2,093.59	3,767.61	542.54
182	490.91	883.43	127.21	2,140.45	3,851.93	554.68	2,093.59	3,767.61	542.54
183	490.91	883.43	127.21	2,113.38	3,803.22	547.66	2,093.59	3,767.61	542.54
184	490.91	883.43	127.21	2,091.22	3,763.34	541.92	2,091.22	3,763.34	541.92
185	490.91	883.43	127.21	2,091.05	3,763.04	541.88	2,091.05	3,763.04	541.88
		Sum	28,344		Sum	77,581		Sum	68,078

Table F14 the concentrations and mass of carbon dioxide in air that flow in/out Solid separating unit during Nile tilapia cultivation with 10 kg/m³ fish density.

Cultivating time (Hour)	Solid separating unit inlet carbon dioxide			Solid separating unit outlet carbon dioxide		
	CCO _{2SI} (ppm)	CCO _{2SI} (mg/m ³)	HCO _{2SI} (mg)	CCO _{2SO} (ppm)	CCO _{2SO} (mg/m ³)	HCO _{2SO} (mg)
1-19	583.27	1,049.65	11,966	752.78	1,354.69	15,443
20-42	590.38	1,062.43	14,662	908.07	1,634.15	22,551
43-65	586.32	1,055.13	14,561	829.92	1,493.51	20,610
66-90	583.27	1,049.65	15,745	991.30	1,783.93	26,759
91-115	602.56	1,084.35	16,265	887.77	1,597.62	23,964
116-140	622.86	1,120.88	16,813	1062.35	1,911.80	28,677
141-164	654.32	1,177.51	16,956	950.70	1,710.87	24,636
165-185	490.91	883.43	11,131	885.74	1,593.97	20,084
		Sum	118,099		Sum	182,726

Table F15 the amount of carbon dioxide from RAS during the cultivations with fish density of 3, 5 and 10 kg/m³ (FT: Fish tank, SU: Solid separating unit).

Fish density (kg / m ³)	Inlet CO ₂ from FT (g)	Outlet CO ₂ from FT (g)	Gained CO ₂ from FT (g)	Basic respiration CO ₂ (g)	Feed utilization CO ₂ (g)	Inlet CO ₂ from SU (g)	Outlet CO ₂ from SU (g)	Gained CO ₂ from SU (g)	Gained CO ₂ from RAS (g)
	3	27.43	42.73	15.30 100%	11.47 74.99%	3.83 25.01%	114.28	143.23	28.95
5	26.02	47.21	21.18 100%	15.81 74.64%	5.37 25.36%	108.42	161.15	52.73	73.92
10	28.34	77.58	49.24 100%	39.73 80.70%	9.50 19.30%	118.10	182.73	64.63	113.86

Table F16 the average oxygen of inlet air (Ambient air), outlet air from Fish tank (Outlet FT), outlet air from Solid separating unit (Outlet SU) and outlet air from microalgal cultivation during the cultivations with fish density of 3, 5 and 10 kg/m³.

Replication	Oxygen concentration (%)											
	Fish density 3 kg / m ³				Fish density 5 kg / m ³				Fish density 10 kg / m ³			
	Ambient air	Outlet FT	Outlet SU	Outlet MC	Ambient air	Outlet FT	Outlet SU	Outlet MC	Ambient air	Outlet FT	Outlet SU	Outlet MC
1	20.90	20.90	20.90	20.90	20.60	20.60	20.60	20.60	20.86	20.67	20.97	20.73
2	20.80	20.53	20.70	20.80	20.40	20.60	20.45	20.45	20.26	20.40	20.33	20.23
3	20.80	20.50	20.67	20.80	20.65	20.60	20.60	20.75	20.80	20.70	20.80	20.70
4	20.80	20.50	20.70	20.57	20.20	20.50	20.50	20.50	20.90	20.73	20.90	20.90
5	19.93	19.57	19.83	19.80	20.45	20.50	20.35	20.50	20.57	20.53	20.53	20.53
6	20.40	20.30	20.90	20.00	20.40	20.30	20.50	20.30	20.60	20.60	20.57	20.70
7	20.30	20.30	20.90	20.10	20.30	20.10	20.30	20.20	20.60	20.60	20.60	20.50
8	20.40	20.30	20.30	20.20	20.50	20.00	20.30	20.30	20.90	20.90	20.90	20.80
9	20.60	20.50	20.60	20.50	20.30	20.10	20.30	20.30	21.30	21.30	21.30	21.20
Average	20.55	20.38	20.61	20.41	20.42	20.37	20.43	20.43	20.75	20.71	20.77	20.70
S.D.	0.32	0.36	0.35	0.40	0.15	0.24	0.13	0.18	0.29	0.26	0.29	0.27

Table F17 the cells number density of *Scenedesmus armatus* when was cultivated with effluent from Solids separating unit. Control-3 was the cultivation was aerated with ambient air and CO2-3 was the cultivation that was aerated outlet air from Fish cultivating unit during the Nile tilapia cultivations with fish density of 3 kg/m³.

Cultivating time (days)	Cell number density (10 ⁴ cells / mL)									
	Control-3					CO2-3				
	1	2	3	Average	S.D.	1	2	3	Average	S.D.
0	19.44	12.11	32.44	21.33	10.30	28.33	23.61	31.53	25.97	3.34
1	61.17	44.17	50.17	51.83	8.62	64.83	48.67	76.17	56.75	11.43
2	201.67	153.33	204.17	186.39	28.65	194.17	151.67	140.00	172.92	30.05

Cultivating time (days)	Cell number density (10^4 cells / mL)									
	Control-3					CO2-3				
	1	2	3	Average	S.D.	1	2	3	Average	S.D.
3	201.67	197.50	227.50	208.89	16.25	195.00	118.33	157.50	156.67	54.21
4	185.83	178.33	197.50	187.22	9.66	332.50	170.00	187.50	251.25	114.90
5	134.17	278.33	200.00	204.17	72.17	320.00	170.00	170.83	245.00	106.07
6	207.50	271.67	216.67	231.94	34.70	304.17	180.00	185.00	242.08	87.80
7	210.00	262.50	211.67	228.06	29.84	327.50	168.33	192.50	247.92	112.55
8	212.50	254.17	206.67	224.44	25.90	350.83	155.83	200.00	253.33	137.89

Table F18 the cells number density of *Scenedesmus armatus* when was cultivated with effluent from Solid separating unit. Control-5 was the cultivation was aerated with ambient air and CO2-5 was the cultivation that was aerated outlet air from Fish cultivating unit during the Nile tilapia cultivations with fish density of 5 kg/m^3 .

Cultivating time (days)	Cell number density (10^4 cells / mL)									
	Control-5					CO2-5				
	1	2	3	Average	S.D.	1	2	3	Average	S.D.
0	15.94	19.78	17.72	17.81	1.92	15.67	16.33	19.94	16.00	0.47
1	55.00	68.00	58.50	60.50	6.73	67.67	91.33	54.50	79.50	16.73
2	186.67	293.33	216.67	232.22	55.01	186.67	269.17	193.33	227.92	58.34
3	261.67	204.17	215.83	227.22	30.39	222.50	272.50	231.67	247.50	35.36
4	265.00	252.50	205.83	241.11	31.18	200.00	318.33	280.83	259.17	83.67
5	270.83	288.89	284.72	281.48	9.45	287.50	291.67	287.50	289.58	2.95
6	350.00	306.94	233.33	296.76	59.00	311.11	475.00	281.94	393.06	115.89
7	406.94	365.28	207.50	326.57	105.20	420.83	520.83	326.39	470.83	70.71
8	458.33	415.28	241.67	371.76	114.70	529.17	484.72	311.11	506.94	31.43

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Table F19 the cells number density of *Scenedesmus armatus* when was cultivated with effluent from Solid separating unit. Control-10 was the cultivation was aerated with ambient air and CO2-10 was the cultivation that was aerated outlet air from Fish cultivating unit during the Nile tilapia cultivations with fish density of 10 kg/m^3 .

Cultivating time (days)	Cell number density (10^4 cells / mL)									
	Control-10					CO2-10				
	1	2	3	Average	S.D.	1	2	3	Average	S.D.
0	13.10	15.97	11.63	13.57	2.20	13.90	12.70	17.47	13.30	0.85
1	48.33	55.83	60.33	54.83	6.06	97.83	101.67	96.83	99.75	2.71
2	250.83	250.00	307.50	269.44	32.96	342.50	447.50	416.67	395.00	74.25
3	272.50	260.00	275.83	269.44	8.35	501.39	540.28	475.00	520.83	27.50
4	298.33	230.83	244.17	257.78	35.75	461.11	515.28	552.78	488.19	38.30

Cultivating time (days)	Cell number density (10^4 cells / mL)									
	Control-10					CO2-10				
	1	2	3	Average	S.D.	1	2	3	Average	S.D.
5	222.50	245.83	322.22	263.52	52.16	558.33	477.08	483.33	517.71	57.45
6	266.67	270.00	265.28	267.31	2.43	470.83	512.50	450.00	491.67	29.46
7	315.83	221.67	279.17	272.22	47.47	450.00	522.92	368.75	486.46	51.56
8	266.67	185.00	295.83	249.17	57.45	537.50	568.75	414.58	553.13	22.10

Table F20 the biomass of *Scenedesmus armatus* when was cultivated with effluent from Solids separating unit. Control was the cultivation was aerated with ambient air and CO2 was the cultivation that was aerated outlet air from Fish cultivating unit during the Nile tilapia cultivations with fish density of 3, 5 and 10 kg/m³.

Cultivating time (days)	Biomass during FISH-3 (mg / L)									
	Control-3					CO2-3				
	1	2	3	Average	S.D.	1	2	3	Average	S.D.
0	73.33	53.33	64.00	63.56	10.01	61.33	54.67	50.67	55.56	5.39
2	102.22	162.22	180.00	148.15	40.75	184.44	146.67	140.00	157.04	23.97
4	202.22	251.11	204.44	219.26	27.61	273.33	211.11	208.89	231.11	36.58
6	86.67	262.22	180.00	176.30	87.84	215.56	195.56	197.78	202.96	10.96
8	216.67	245.33	188.00	216.67	28.67	224.00	250.67	238.67	237.78	13.36
Cultivating time (days)	Biomass during FISH-5 (mg / L)									
	Control-5					CO2-5				
	1	2	3	Average	S.D.	1	2	3	Average	S.D.
0	15.56	42.67	42.67	33.63	15.65	28.00	49.33	50.67	42.67	12.72
2	275.56	253.33	304.44	277.78	25.63	226.67	171.11	237.78	211.85	35.72
4	282.22	224.44	282.22	262.96	33.36	193.33	371.11	322.22	295.56	91.84
6	364.44	304.44	237.78	302.22	63.36	328.89	386.67	297.78	337.78	45.11
8	390.00	351.67	306.67	349.44	41.71	375.00	425.00	261.67	353.89	83.69
Cultivating time (days)	Biomass during FISH-10 (mg / L)									
	Control-10					CO2-10				
	1	2	3	Average	S.D.	1	2	3	Average	S.D.
0	68.00	65.33	40.00	57.78	15.45	58.67	48.00	34.67	47.11	12.02
2	175.56	137.78	182.22	165.19	23.97	228.89	253.33	215.56	232.59	19.16
4	262.22	171.11	268.89	234.07	54.63	386.67	431.11	411.11	409.63	22.26
6	235.56	151.11	208.89	198.52	43.17	422.22	426.67	362.22	403.70	35.99
8	211.67	108.33	146.67	155.56	52.24	391.67	445.00	365.00	400.56	40.73

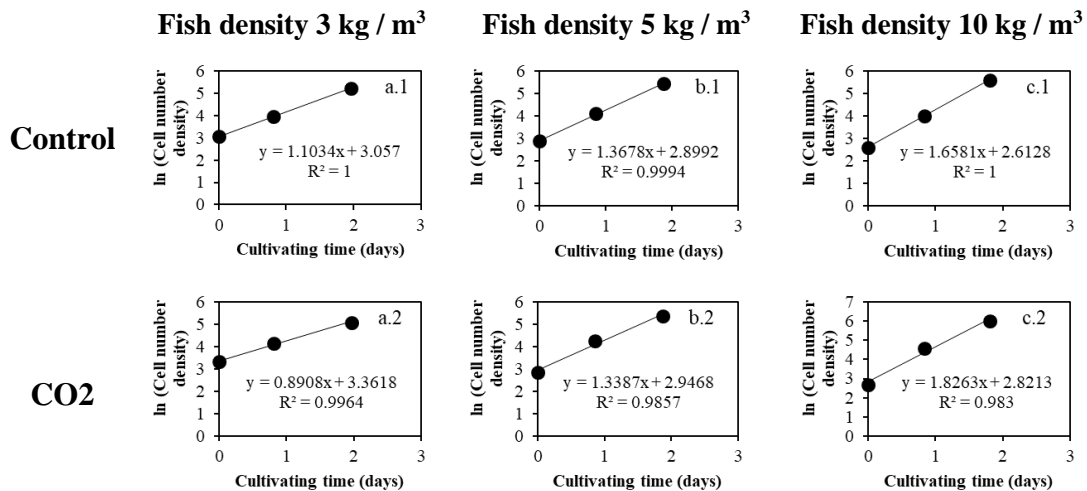


Figure E3 the specific growth rate of *Scenedesmus armatus* when was cultivated with effluent from Solid separating unit. Control was the cultivation was aerated with ambient air, CO2 was the cultivation that was aerated outlet air from Fish cultivating unit during the Nile tilapia cultivations with fish density of 3, 5 and 10 kg/m³.

Table F21 Dissolved inorganic nitrogen concentration the biomass of *Scenedesmus armatus* when was cultivated with effluent from Solid separating unit. Control-3, 5 and 10 was the cultivation was aerated with ambient air and CO2-3, 5 and 10 was the cultivation that was aerated outlet air from Fish cultivating unit during the Nile tilapia cultivations with fish density of 3, 5 and 10 kg/m³.

Cultivating time (days)	Dissolved inorganic nitrogen concentration of control-3 (mg N / L)														
	Total ammonia nitrogen					Nitrite					Nitrate				
	1	2	3	Average	S.D.	1	2	3	Average	S.D.	1	2	3	Average	S.D.
0	0.00	0.00	0.00	0.00	0.00	0.66	0.43	0.45	0.51	0.13	39.01	42.55	43.25	41.61	2.78
2	0.03	0.03	0.03	0.03	0.00	0.33	0.69	0.43	0.48	0.19	24.69	34.98	36.62	32.10	6.46
4	0.05	0.07	0.04	0.05	0.02	0.46	0.39	0.60	0.49	0.11	31.17	28.44	31.67	30.43	1.74
6	0.05	0.10	0.03	0.06	0.03	0.43	0.33	0.38	0.38	0.05	30.06	26.87	29.55	28.83	1.71
8	0.06	0.08	0.05	0.06	0.02	0.37	0.34	0.35	0.35	0.01	30.85	24.90	28.78	28.18	3.02
Cultivating time (days)	Dissolved inorganic nitrogen concentration of CO2-3 (mg N / L)														
	Total ammonia nitrogen					Nitrite					Nitrate				
	1	2	3	Average	S.D.	1	2	3	Average	S.D.	1	2	3	Average	S.D.
0	0.00	0.01	0.00	0.00	0.01	0.48	0.31	0.24	0.34	0.12	46.50	39.77	45.11	41.61	2.78
2	0.01	0.06	0.01	0.03	0.03	0.39	0.16	0.22	0.26	0.12	33.10	27.75	29.80	30.22	3.55
4	0.11	0.12	0.20	0.14	0.05	0.33	0.16	0.21	0.23	0.09	29.85	24.31	28.10	27.42	2.70
6	0.12	0.10	0.08	0.10	0.02	0.28	0.17	0.16	0.20	0.07	25.65	23.18	22.06	23.63	1.84
8	0.09	0.11	0.09	0.10	0.01	0.27	0.13	0.14	0.18	0.08	30.67	20.76	22.91	24.78	5.22
Cultivating time (days)	Dissolved inorganic nitrogen concentration of control-5 (mg N / L)														
	Total ammonia nitrogen					Nitrite					Nitrate				
	1	2	3	Average	S.D.	1	2	3	Average	S.D.	1	2	3	Average	S.D.
0	0.00	0.04	0.02	0.02	0.02	0.09	0.10	0.08	0.09	0.01	37.69	35.80	35.80	36.43	1.09
2	0.47	0.40	0.72	0.53	0.17	0.06	0.11	0.13	0.10	0.04	11.54	13.40	7.00	10.65	3.29
4	0.50	0.41	0.51	0.47	0.06	0.09	0.07	0.03	0.07	0.03	11.66	11.24	6.25	9.72	3.01
6	0.38	0.42	1.54	0.78	0.66	0.08	0.11	0.06	0.08	0.03	11.67	10.92	5.83	9.47	3.17
8	0.23	0.27	0.31	0.27	0.04	0.08	0.13	0.09	0.10	0.03	7.10	6.78	5.13	6.33	1.06

Cultivating time (days)	Dissolved inorganic nitrogen concentration of CO2-5 (mg N / L)														
	Total ammonia nitrogen					Nitrite					Nitrate				
	1	2	3	Average	S.D.	1	2	3	Average	S.D.	1	2	3	Average	S.D.
0	0.05	0.09	0.06	0.06	0.02	0.10	0.13	0.11	0.11	0.02	33.91	33.91	32.59	33.47	0.76
2	0.66	1.15	0.53	0.78	0.33	0.04	0.03	0.05	0.04	0.01	13.05	15.33	9.52	12.63	2.93
4	0.61	0.77	0.72	0.70	0.08	0.02	0.03	0.04	0.03	0.01	9.94	13.95	8.64	10.84	2.77
6	0.46	0.38	1.16	0.67	0.43	0.02	0.02	0.06	0.03	0.02	12.67	10.41	8.02	10.37	2.33
8	0.18	0.29	0.24	0.24	0.06	0.02	0.01	0.05	0.03	0.03	7.21	3.17	5.93	5.44	2.07
Cultivating time (days)	Dissolved inorganic nitrogen concentration of control-10 (mg N / L)														
	Total ammonia nitrogen					Nitrite					Nitrate				
	1	2	3	Average	S.D.	1	2	3	Average	S.D.	1	2	3	Average	S.D.
0	0.00	0.04	0.02	0.02	0.02	0.09	0.10	0.08	0.09	0.01	37.69	35.80	35.80	36.43	1.09
2	0.47	0.40	0.72	0.53	0.17	0.06	0.11	0.13	0.10	0.04	11.54	13.40	7.00	10.65	3.29
4	0.50	0.41	0.51	0.47	0.06	0.09	0.07	0.03	0.07	0.03	11.66	11.24	6.25	9.72	3.01
6	0.38	0.42	1.54	0.78	0.66	0.08	0.11	0.06	0.08	0.03	11.67	10.92	5.83	9.47	3.17
8	0.23	0.27	0.31	0.27	0.04	0.08	0.13	0.09	0.10	0.03	7.10	6.78	5.13	6.33	1.06
Cultivating time (days)	Dissolved inorganic nitrogen concentration of CO2-10 (mg N / L)														
	Total ammonia nitrogen					Nitrite					Nitrate				
	1	2	3	Average	S.D.	1	2	3	Average	S.D.	1	2	3	Average	S.D.
0	0.05	0.09	0.06	0.06	0.02	0.10	0.13	0.11	0.11	0.02	33.91	33.91	32.59	33.47	0.76
2	0.66	1.15	0.53	0.78	0.33	0.04	0.03	0.05	0.04	0.01	13.05	15.33	9.52	12.63	2.93
4	0.61	0.77	0.72	0.70	0.08	0.02	0.03	0.04	0.03	0.01	9.94	13.95	8.64	10.84	2.77
6	0.46	0.38	1.16	0.67	0.43	0.02	0.02	0.06	0.03	0.02	12.67	10.41	8.02	10.37	2.33
8	0.18	0.29	0.24	0.24	0.06	0.02	0.01	0.05	0.03	0.03	7.21	3.17	5.93	5.44	2.07

Table F22 Phosphate concentration of *Scenedesmus armatus* when was cultivated with effluent from Solid separating unit. Control-3, 5 and 10 was the cultivation was aerated with ambient air and CO2-3, 5 and 10 was the cultivation that was aerated outlet air from Fish cultivating unit during the Nile tilapia cultivations with fish density of 3, 5 and 10 kg/m³.

Cultivating time (days)	Phosphate concentration (mg P / L)									
	Control-3					CO2-3				
	1	2	3	Average	S.D.	1	2	3	Average	S.D.
0	4.15	7.83	5.64	5.99	2.60	6.10	4.84	6.85	5.47	0.89
2	2.29	4.21	3.11	3.20	0.97	3.46	4.32	5.46	4.41	1.00
4	3.44	3.17	3.83	3.48	0.33	5.41	4.42	6.80	5.55	1.20
6	2.68	3.59	2.91	3.06	0.48	4.41	3.80	4.13	4.11	0.31
8	2.84	3.42	3.17	3.14	0.29	3.87	3.95	5.10	4.31	0.69
Cultivating time (days)	Phosphate concentration (mg P / L)									
	Control-5					CO2-5				
	1	2	3	Average	S.D.	1	2	3	Average	S.D.
0	8.33	7.91	0.00	5.41	4.69	8.26	7.79	0.00	5.35	4.64
2	5.06	5.69	5.05	5.26	0.37	5.05	5.64	5.01	5.23	0.35
4	5.86	6.26	6.10	6.07	0.20	5.81	6.19	6.04	6.01	0.19
6	4.43	4.61	4.38	4.47	0.12	4.41	4.60	4.36	4.46	0.12
8	3.17	3.29	3.33	3.26	0.08	3.15	3.26	3.32	3.24	0.09

Cultivating time (days)	Phosphate concentration (mg P / L)									
	Control-10					CO2-10				
	1	2	3	Average	S.D.	1	2	3	Average	S.D.
0	4.61	5.88	6.40	5.63	0.92	6.59	7.77	7.82	7.39	0.70
2	2.29	5.35	1.17	2.93	2.16	5.27	5.65	5.98	5.64	0.35
4	3.00	5.29	1.65	3.31	1.84	4.81	4.39	5.20	4.80	0.41
6	1.78	2.80	2.32	2.30	0.51	2.69	2.58	3.31	2.86	0.40
8	1.60	3.86	2.32	2.59	1.16	2.68	2.92	2.94	2.85	0.14

Table F23 the concentrations and mass of carbon dioxide in air that flow in/out microalgal cultivation during Nile tilapia cultivation with 3 kg/m³ fish density.

Cultivating time (Hour)	Microalgal cultivation inlet carbon dioxide			Microalgal cultivation outlet carbon dioxide		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)
1	767.15	1,380.56	198.80	581.24	1,045.99	150.62
2	868.49	1,562.92	225.06	581.24	1,045.99	150.62
3	862.23	1,551.65	223.44	581.24	1,045.99	150.62
4	835.67	1,503.86	216.56	581.24	1,045.99	150.62
5	827.89	1,489.85	214.54	581.24	1,045.99	150.62
6	855.97	1,540.39	221.82	581.24	1,045.99	150.62
7	888.28	1,598.54	230.19	581.24	1,045.99	150.62
8	910.95	1,639.33	236.06	581.24	1,045.99	150.62
9	919.24	1,654.25	238.21	581.24	1,045.99	150.62
10	917.37	1,650.90	237.73	575.15	1,035.03	149.05
11	905.70	1,629.89	234.70	575.15	1,035.03	149.05
12	873.90	1,572.66	226.46	575.15	1,035.03	149.05
13	851.91	1,533.08	220.76	575.15	1,035.03	149.05
14	853.94	1,536.74	221.29	575.15	1,035.03	149.05
15	851.91	1,533.08	220.76	575.15	1,035.03	149.05
16	842.10	1,515.43	218.22	575.15	1,035.03	149.05
17	822.81	1,480.72	213.22	575.15	1,035.03	149.05
18	814.18	1,465.20	210.99	575.15	1,035.03	149.05
19	850.89	1,531.26	220.50	575.15	1,035.03	149.05
20	861.72	1,550.74	223.31	578.20	1,040.51	149.83
21	865.27	1,557.13	224.23	578.20	1,040.51	149.83
22	877.96	1,579.97	227.52	578.20	1,040.51	149.83
23	843.28	1,517.56	218.53	578.20	1,040.51	149.83
24	862.23	1,551.65	223.44	578.20	1,040.51	149.83
25	885.91	1,594.27	229.58	578.20	1,040.51	149.83
26	870.68	1,566.88	225.63	578.20	1,040.51	149.83
27	929.05	1,671.90	240.75	578.20	1,040.51	149.83
28	939.03	1,689.87	243.34	578.20	1,040.51	149.83
29	970.15	1,745.88	251.41	578.20	1,040.51	149.83
30	970.83	1,747.10	251.58	578.20	1,040.51	149.83
31	940.89	1,693.21	243.82	578.20	1,040.51	149.83
32	929.39	1,672.51	240.84	578.20	1,040.51	149.83
33	916.87	1,649.99	237.60	578.20	1,040.51	149.83
34	902.83	1,624.72	233.96	578.20	1,040.51	149.83
35	880.16	1,583.92	228.08	578.20	1,040.51	149.83
36	870.01	1,565.66	225.45	578.20	1,040.51	149.83
37	866.12	1,558.66	224.45	578.20	1,040.51	149.83
38	856.81	1,541.91	222.04	578.20	1,040.51	149.83
39	867.64	1,561.40	224.84	578.20	1,040.51	149.83
40	870.52	1,566.57	225.59	578.20	1,040.51	149.83
41	858.84	1,545.57	222.56	578.20	1,040.51	149.83

Cultivating time (Hour)	Microalgal cultivation inlet carbon dioxide			Microalgal cultivation outlet carbon dioxide		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)
42	850.89	1,531.26	220.50	578.20	1,040.51	149.83
43	862.56	1,552.26	223.53	578.20	1,040.51	149.83
44	862.23	1,551.65	223.44	578.20	1,040.51	149.83
45	704.39	1,267.62	182.54	578.20	1,040.51	149.83
46	792.53	1,426.23	205.38	578.20	1,040.51	149.83
47	947.32	1,704.78	245.49	569.06	1,024.08	147.47
48	965.25	1,737.05	250.14	569.06	1,024.08	147.47
49	928.03	1,670.08	240.49	569.06	1,024.08	147.47
50	908.92	1,635.68	235.54	569.06	1,024.08	147.47
51	881.68	1,586.66	228.48	569.06	1,024.08	147.47
52	861.89	1,551.04	223.35	569.06	1,024.08	147.47
53	879.31	1,582.40	227.87	569.06	1,024.08	147.47
54	903.50	1,625.93	234.13	569.06	1,024.08	147.47
55	888.28	1,598.54	230.19	569.06	1,024.08	147.47
56	867.81	1,561.70	224.88	569.06	1,024.08	147.47
57	846.49	1,523.34	219.36	569.06	1,024.08	147.47
58	832.11	1,497.47	215.63	569.06	1,024.08	147.47
59	819.77	1,475.24	212.43	569.06	1,024.08	147.47
60	815.87	1,468.24	211.43	569.06	1,024.08	147.47
61	804.03	1,446.93	208.36	569.06	1,024.08	147.47
62	789.48	1,420.75	204.59	569.06	1,024.08	147.47
63	800.31	1,440.23	207.39	569.06	1,024.08	147.47
64	792.02	1,425.32	205.25	569.06	1,024.08	147.47
65	783.56	1,410.09	203.05	569.06	1,024.08	147.47
66	801.66	1,442.67	207.74	569.06	1,024.08	147.47
67	783.39	1,409.79	203.01	569.06	1,024.08	147.47
68	760.56	1,368.69	197.09	637.07	1,146.46	165.09
69	849.20	1,528.21	220.06	637.07	1,146.46	165.09
70	895.89	1,612.24	232.16	637.07	1,146.46	165.09
71	927.02	1,668.25	240.23	637.07	1,146.46	165.09
72	925.83	1,666.12	239.92	637.07	1,146.46	165.09
73	901.81	1,622.89	233.70	637.07	1,146.46	165.09
74	884.22	1,591.23	229.14	637.07	1,146.46	165.09
75	887.77	1,597.62	230.06	637.07	1,146.46	165.09
76	948.33	1,706.61	245.75	637.07	1,146.46	165.09
77	998.41	1,796.72	258.73	637.07	1,146.46	165.09
78	1,051.69	1,892.62	272.54	637.07	1,146.46	165.09
79	1,068.44	1,922.76	276.88	637.07	1,146.46	165.09
80	1,058.97	1,905.71	274.42	637.07	1,146.46	165.09
81	1,053.72	1,896.27	273.06	637.07	1,146.46	165.09
82	1,055.08	1,898.70	273.41	637.07	1,146.46	165.09
83	1,052.71	1,894.44	272.80	637.07	1,146.46	165.09
84	1,049.83	1,889.27	272.05	637.07	1,146.46	165.09
85	900.29	1,620.15	233.30	637.07	1,146.46	165.09
86	828.73	1,491.38	214.76	637.07	1,146.46	165.09
87	807.42	1,453.02	209.23	637.07	1,146.46	165.09
88	791.68	1,424.71	205.16	637.07	1,146.46	165.09
89	775.44	1,395.48	200.95	637.07	1,146.46	165.09
90	769.35	1,384.52	199.37	637.07	1,146.46	165.09
91	709.98	1,277.67	183.98	637.07	1,146.46	165.09
92	807.75	1,453.63	209.32	637.07	1,146.46	165.09
93	920.59	1,656.68	238.56	637.07	1,146.46	165.09
94	906.38	1,631.11	234.88	637.07	1,146.46	165.09
95	854.11	1,537.04	221.33	637.07	1,146.46	165.09
96	836.01	1,504.47	216.64	639.10	1,150.11	165.62
97	842.43	1,516.04	218.31	639.10	1,150.11	165.62
98	858.34	1,544.65	222.43	639.10	1,150.11	165.62
99	858.50	1,544.96	222.47	639.10	1,150.11	165.62
100	863.07	1,553.18	223.66	639.10	1,150.11	165.62
101	873.05	1,571.14	226.24	639.10	1,150.11	165.62

Cultivating time (Hour)	Microalgal cultivation inlet carbon dioxide			Microalgal cultivation outlet carbon dioxide		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)
102	880.83	1,585.14	228.26	639.10	1,150.11	165.62
103	882.36	1,587.88	228.65	639.10	1,150.11	165.62
104	884.05	1,590.93	229.09	639.10	1,150.11	165.62
105	880.33	1,584.23	228.13	639.10	1,150.11	165.62
106	866.12	1,558.66	224.45	639.10	1,150.11	165.62
107	855.97	1,540.39	221.82	639.10	1,150.11	165.62
108	847.68	1,525.47	219.67	639.10	1,150.11	165.62
109	833.81	1,500.51	216.07	639.10	1,150.11	165.62
110	812.15	1,461.54	210.46	639.10	1,150.11	165.62
111	799.63	1,439.01	207.22	639.10	1,150.11	165.62
112	792.19	1,425.62	205.29	639.10	1,150.11	165.62
113	782.55	1,408.27	202.79	639.10	1,150.11	165.62
114	780.69	1,404.92	202.31	639.10	1,150.11	165.62
115	788.13	1,418.31	204.24	639.10	1,150.11	165.62
116	722.16	1,299.59	187.14	639.10	1,150.11	165.62
117	676.31	1,217.08	175.26	588.35	1,058.78	152.46
118	684.43	1,231.70	177.36	588.35	1,058.78	152.46
119	695.77	1,252.09	180.30	588.35	1,058.78	152.46
120	702.87	1,264.88	182.14	588.35	1,058.78	152.46
121	694.41	1,249.66	179.95	588.35	1,058.78	152.46
122	692.55	1,246.31	179.47	588.35	1,058.78	152.46
123	692.21	1,245.70	179.38	588.35	1,058.78	152.46
124	683.42	1,229.87	177.10	588.35	1,058.78	152.46
125	669.21	1,204.30	173.42	588.35	1,058.78	152.46
126	663.12	1,193.34	171.84	588.35	1,058.78	152.46
127	662.10	1,191.51	171.58	588.35	1,058.78	152.46
128	662.95	1,193.03	171.80	588.35	1,058.78	152.46
129	666.33	1,199.12	172.67	588.35	1,058.78	152.46
130	670.56	1,206.73	173.77	588.35	1,058.78	152.46
131	674.11	1,213.13	174.69	588.35	1,058.78	152.46
132	662.78	1,192.73	171.75	588.35	1,058.78	152.46
133	662.95	1,193.03	171.80	588.35	1,058.78	152.46
134	660.24	1,188.16	171.10	588.35	1,058.78	152.46
135	656.18	1,180.86	170.04	588.35	1,058.78	152.46
136	651.44	1,172.33	168.82	588.35	1,058.78	152.46
137	647.72	1,165.64	167.85	588.35	1,058.78	152.46
138	645.69	1,161.98	167.33	588.35	1,058.78	152.46
139	866.46	1,559.26	224.53	588.35	1,058.78	152.46
140	753.45	1,355.90	195.25	594.18	1,069.28	153.98
141	975.74	1,755.93	252.85	594.18	1,069.28	153.98
142	1,038.67	1,869.18	269.16	594.18	1,069.28	153.98
143	1,075.21	1,934.93	278.63	594.18	1,069.28	153.98
144	1,099.91	1,979.38	285.03	594.18	1,069.28	153.98
145	1,105.32	1,989.12	286.43	594.18	1,069.28	153.98
146	1,116.15	2,008.60	289.24	594.18	1,069.28	153.98
147	1,115.30	2,007.08	289.02	594.18	1,069.28	153.98
148	1,107.69	1,993.38	287.05	594.18	1,069.28	153.98
149	1,100.58	1,980.60	285.21	594.18	1,069.28	153.98
150	1,079.61	1,942.85	279.77	594.18	1,069.28	153.98
151	1,054.91	1,898.40	273.37	594.18	1,069.28	153.98
152	1,025.98	1,846.34	265.87	594.18	1,069.28	153.98
153	1,009.23	1,816.20	261.53	594.18	1,069.28	153.98
154	971.85	1,748.93	251.85	594.18	1,069.28	153.98
155	942.24	1,695.65	244.17	594.18	1,069.28	153.98
156	947.49	1,705.09	245.53	594.18	1,069.28	153.98
157	948.33	1,706.61	245.75	594.18	1,069.28	153.98
158	937.84	1,687.73	243.03	594.18	1,069.28	153.98
159	924.14	1,663.08	239.48	594.18	1,069.28	153.98
160	912.64	1,642.37	236.50	594.18	1,069.28	153.98
161	914.67	1,646.03	237.03	594.18	1,069.28	153.98

Cultivating time (Hour)	Microalgal cultivation inlet carbon dioxide			Microalgal cultivation outlet carbon dioxide		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)
162	923.46	1,661.86	239.31	594.18	1,069.28	153.98
163	931.42	1,676.17	241.37	594.18	1,069.28	153.98
164	1,061.17	1,909.66	274.99	585.30	1,053.30	151.68
165	1,119.70	2,015.00	290.16	585.30	1,053.30	151.68
166	1,132.89	2,038.74	293.58	585.30	1,053.30	151.68
167	1,152.52	2,074.06	298.66	585.30	1,053.30	151.68
168	1,149.81	2,069.19	297.96	585.30	1,053.30	151.68
169	1,153.36	2,075.58	298.88	585.30	1,053.30	151.68
170	1,149.13	2,067.97	297.79	585.30	1,053.30	151.68
171	1,132.05	2,037.22	293.36	585.30	1,053.30	151.68
172	1,107.01	1,992.17	286.87	585.30	1,053.30	151.68
173	1,083.50	1,949.85	280.78	585.30	1,053.30	151.68
174	1,068.27	1,922.45	276.83	585.30	1,053.30	151.68
175	1,041.71	1,874.65	269.95	585.30	1,053.30	151.68
176	1,006.86	1,811.94	260.92	585.30	1,053.30	151.68
177	978.27	1,760.49	253.51	585.30	1,053.30	151.68
178	954.08	1,716.96	247.24	585.30	1,053.30	151.68
179	932.43	1,677.99	241.63	585.30	1,053.30	151.68
180	906.38	1,631.11	234.88	585.30	1,053.30	151.68
181	892.34	1,605.84	231.24	585.30	1,053.30	151.68
182	882.70	1,588.49	228.74	585.30	1,053.30	151.68
183	890.48	1,602.49	230.76	585.30	1,053.30	151.68
184	883.03	1,589.10	228.83	585.30	1,053.30	151.68
185	876.77	1,577.83	227.21	585.30	1,053.30	151.68
186	876.27	1,576.92	227.08	585.30	1,053.30	151.68
187	877.62	1,579.36	227.43	585.30	1,053.30	151.68
	Sum		42,726	Sum		28,931

Table F24 the concentrations and mass of carbon dioxide in air that flow in/out microalgal cultivation during Nile tilapia cultivation with 5 kg/m³ fish density.

Cultivating time (Hour)	Microalgal cultivation inlet carbon dioxide			Microalgal cultivation outlet carbon dioxide		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)
1	866.62	1,559.57	224.58	558.91	1,005.81	144.84
2	884.56	1,591.84	229.22	558.91	1,005.81	144.84
3	872.71	1,570.53	226.16	558.91	1,005.81	144.84
4	864.76	1,556.22	224.10	558.91	1,005.81	144.84
5	867.30	1,560.79	224.75	558.91	1,005.81	144.84
6	867.98	1,562.00	224.93	558.91	1,005.81	144.84
7	870.18	1,565.96	225.50	558.91	1,005.81	144.84
8	873.73	1,572.36	226.42	558.91	1,005.81	144.84
9	873.39	1,571.75	226.33	558.91	1,005.81	144.84
10	869.67	1,565.05	225.37	558.91	1,005.81	144.84
11	863.41	1,553.78	223.75	558.91	1,005.81	144.84
12	853.26	1,535.52	221.11	558.91	1,005.81	144.84
13	844.46	1,519.69	218.84	558.91	1,005.81	144.84
14	834.31	1,501.42	216.20	558.91	1,005.81	144.84
15	823.66	1,482.24	213.44	558.91	1,005.81	144.84
16	818.41	1,472.81	212.08	558.91	1,005.81	144.84
17	817.90	1,471.89	211.95	558.91	1,005.81	144.84
18	812.32	1,461.85	210.51	558.91	1,005.81	144.84
19	781.53	1,406.44	202.53	558.91	1,005.81	144.84
20	850.72	1,530.95	220.46	555.36	999.42	143.92
21	968.80	1,743.45	251.06	555.36	999.42	143.92
22	955.94	1,720.31	247.72	555.36	999.42	143.92
23	942.41	1,695.95	244.22	555.36	999.42	143.92
24	935.14	1,682.86	242.33	555.36	999.42	143.92

Cultivating time (Hour)	Microalgal cultivation inlet carbon dioxide			Microalgal cultivation outlet carbon dioxide		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)
25	930.91	1,675.25	241.24	555.36	999.42	143.92
26	933.78	1,680.43	241.98	555.36	999.42	143.92
27	936.49	1,685.30	242.68	555.36	999.42	143.92
28	935.81	1,684.08	242.51	555.36	999.42	143.92
29	930.40	1,674.34	241.10	555.36	999.42	143.92
30	912.30	1,641.77	236.41	555.36	999.42	143.92
31	906.38	1,631.11	234.88	555.36	999.42	143.92
32	892.68	1,606.45	231.33	555.36	999.42	143.92
33	877.28	1,578.75	227.34	555.36	999.42	143.92
34	878.13	1,580.27	227.56	555.36	999.42	143.92
35	882.70	1,588.49	228.74	555.36	999.42	143.92
36	877.96	1,579.97	227.52	555.36	999.42	143.92
37	863.24	1,553.48	223.70	555.36	999.42	143.92
38	852.08	1,533.39	220.81	555.36	999.42	143.92
39	848.02	1,526.08	219.76	555.36	999.42	143.92
40	840.40	1,512.38	217.78	555.36	999.42	143.92
41	852.25	1,533.69	220.85	555.36	999.42	143.92
42	848.52	1,526.99	219.89	555.36	999.42	143.92
43	712.01	1,281.32	184.51	555.36	999.42	143.92
44	798.62	1,437.19	206.96	555.36	999.42	143.92
45	1,028.52	1,850.91	266.53	624.89	1,124.54	161.93
46	1,048.82	1,887.44	271.79	624.89	1,124.54	161.93
47	1,027.67	1,849.39	266.31	624.89	1,124.54	161.93
48	1,021.24	1,837.82	264.65	624.89	1,124.54	161.93
49	1,006.53	1,811.33	260.83	624.89	1,124.54	161.93
50	994.85	1,790.33	257.81	624.89	1,124.54	161.93
51	996.38	1,793.07	258.20	624.89	1,124.54	161.93
52	987.24	1,776.63	255.83	624.89	1,124.54	161.93
53	978.44	1,760.80	253.55	624.89	1,124.54	161.93
54	968.12	1,742.23	250.88	624.89	1,124.54	161.93
55	957.64	1,723.35	248.16	624.89	1,124.54	161.93
56	933.45	1,679.82	241.89	624.89	1,124.54	161.93
57	922.45	1,660.03	239.04	624.89	1,124.54	161.93
58	912.64	1,642.37	236.50	624.89	1,124.54	161.93
59	906.89	1,632.02	235.01	624.89	1,124.54	161.93
60	898.43	1,616.80	232.82	624.89	1,124.54	161.93
61	879.31	1,582.40	227.87	624.89	1,124.54	161.93
62	863.24	1,553.48	223.70	624.89	1,124.54	161.93
63	851.06	1,531.56	220.54	624.89	1,124.54	161.93
64	846.83	1,523.95	219.45	624.89	1,124.54	161.93
65	845.31	1,521.21	219.05	624.89	1,124.54	161.93
66	854.95	1,538.56	221.55	624.89	1,124.54	161.93
67	861.38	1,550.13	223.22	624.89	1,124.54	161.93
68	858.67	1,545.26	222.52	624.89	1,124.54	161.93
69	842.10	1,515.43	218.22	624.89	1,124.54	161.93
70	744.66	1,340.07	192.97	624.89	1,124.54	161.93
71	948.50	1,706.91	245.80	596.97	1,074.31	154.70
72	971.34	1,748.01	251.71	596.97	1,074.31	154.70
73	972.52	1,750.14	252.02	596.97	1,074.31	154.70
74	954.08	1,716.96	247.24	596.97	1,074.31	154.70
75	945.29	1,701.13	244.96	596.97	1,074.31	154.70
76	936.83	1,685.91	242.77	596.97	1,074.31	154.70
77	925.66	1,665.82	239.88	596.97	1,074.31	154.70
78	928.88	1,671.60	240.71	596.97	1,074.31	154.70
79	927.19	1,668.56	240.27	596.97	1,074.31	154.70
80	935.48	1,683.47	242.42	596.97	1,074.31	154.70
81	932.43	1,677.99	241.63	596.97	1,074.31	154.70
82	932.60	1,678.30	241.67	596.97	1,074.31	154.70
83	913.15	1,643.29	236.63	596.97	1,074.31	154.70
84	911.62	1,640.55	236.24	596.97	1,074.31	154.70

Cultivating time (Hour)	Microalgal cultivation inlet carbon dioxide			Microalgal cultivation outlet carbon dioxide		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)
85	894.88	1,610.41	231.90	596.97	1,074.31	154.70
86	886.92	1,596.10	229.84	596.97	1,074.31	154.70
87	859.52	1,546.78	222.74	596.97	1,074.31	154.70
88	845.31	1,521.21	219.05	596.97	1,074.31	154.70
89	763.94	1,374.78	197.97	556.88	1,002.16	144.31
90	940.38	1,692.30	243.69	556.88	1,002.16	144.31
91	1,195.99	2,152.30	309.93	556.88	1,002.16	144.31
92	1,159.45	2,086.54	300.46	556.88	1,002.16	144.31
93	1,137.80	2,047.57	294.85	556.88	1,002.16	144.31
94	1,128.33	2,030.52	292.40	556.88	1,002.16	144.31
95	1,130.02	2,033.57	292.83	556.88	1,002.16	144.31
96	1,117.67	2,011.34	289.63	556.88	1,002.16	144.31
97	1,115.64	2,007.69	289.11	556.88	1,002.16	144.31
98	1,132.72	2,038.44	293.54	556.88	1,002.16	144.31
99	1,120.88	2,017.13	290.47	556.88	1,002.16	144.31
100	1,102.10	1,983.34	285.60	556.88	1,002.16	144.31
101	1,093.14	1,967.20	283.28	556.88	1,002.16	144.31
102	1,068.27	1,922.45	276.83	556.88	1,002.16	144.31
103	1,059.81	1,907.23	274.64	556.88	1,002.16	144.31
104	1,052.03	1,893.23	272.62	556.88	1,002.16	144.31
105	1,030.38	1,854.26	267.01	556.88	1,002.16	144.31
106	1,022.77	1,840.56	265.04	556.88	1,002.16	144.31
107	1,013.12	1,823.21	262.54	556.88	1,002.16	144.31
108	1,006.53	1,811.33	260.83	556.88	1,002.16	144.31
109	1,000.27	1,800.07	259.21	556.88	1,002.16	144.31
110	993.67	1,788.20	257.50	556.88	1,002.16	144.31
111	996.88	1,793.98	258.33	556.88	1,002.16	144.31
112	982.33	1,767.80	254.56	556.88	1,002.16	144.31
113	964.57	1,735.83	249.96	556.88	1,002.16	144.31
114	957.13	1,722.44	248.03	556.88	1,002.16	144.31
115	781.20	1,405.83	202.44	586.32	1,055.13	151.94
116	1,063.20	1,913.32	275.52	586.32	1,055.13	151.94
117	1,411.00	2,539.23	365.65	586.32	1,055.13	151.94
118	1,496.60	2,693.27	387.83	586.32	1,055.13	151.94
119	1,458.03	2,623.86	377.84	586.32	1,055.13	151.94
120	1,419.80	2,555.06	367.93	586.32	1,055.13	151.94
121	1,395.10	2,510.61	361.53	586.32	1,055.13	151.94
122	1,425.21	2,564.80	369.33	586.32	1,055.13	151.94
123	1,444.16	2,598.90	374.24	586.32	1,055.13	151.94
124	1,416.92	2,549.88	367.18	586.32	1,055.13	151.94
125	1,418.28	2,552.32	367.53	586.32	1,055.13	151.94
126	1,433.16	2,579.11	371.39	586.32	1,055.13	151.94
127	1,421.66	2,558.41	368.41	586.32	1,055.13	151.94
128	1,385.63	2,493.56	359.07	586.32	1,055.13	151.94
129	1,378.69	2,481.08	357.28	586.32	1,055.13	151.94
130	1,364.82	2,456.12	353.68	586.32	1,055.13	151.94
131	1,372.09	2,469.21	355.57	586.32	1,055.13	151.94
132	1,337.92	2,407.71	346.71	586.32	1,055.13	151.94
133	1,313.06	2,362.96	340.27	586.32	1,055.13	151.94
134	1,290.22	2,321.86	334.35	586.32	1,055.13	151.94
135	1,270.26	2,285.94	329.18	586.32	1,055.13	151.94
136	924.48	1,663.68	239.57	586.32	1,055.13	151.94
137	1,120.04	2,015.61	290.25	630.98	1,135.50	163.51
138	1,153.70	2,076.19	298.97	630.98	1,135.50	163.51
139	1,127.31	2,028.70	292.13	630.98	1,135.50	163.51
140	1,101.09	1,981.51	285.34	630.98	1,135.50	163.51
141	1,088.40	1,958.68	282.05	630.98	1,135.50	163.51
142	1,062.01	1,911.19	275.21	630.98	1,135.50	163.51
143	1,042.05	1,875.26	270.04	630.98	1,135.50	163.51
144	1,031.56	1,856.39	267.32	630.98	1,135.50	163.51

Cultivating time (Hour)	Microalgal cultivation inlet carbon dioxide			Microalgal cultivation outlet carbon dioxide		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)
145	1,019.72	1,835.08	264.25	630.98	1,135.50	163.51
146	1,000.27	1,800.07	259.21	630.98	1,135.50	163.51
147	999.59	1,798.85	259.03	630.98	1,135.50	163.51
148	984.70	1,772.06	255.18	630.98	1,135.50	163.51
149	966.43	1,739.18	250.44	630.98	1,135.50	163.51
150	956.62	1,721.53	247.90	630.98	1,135.50	163.51
151	943.43	1,697.78	244.48	630.98	1,135.50	163.51
152	940.04	1,691.69	243.60	630.98	1,135.50	163.51
153	941.57	1,694.43	244.00	630.98	1,135.50	163.51
154	932.43	1,677.99	241.63	630.98	1,135.50	163.51
155	927.02	1,668.25	240.23	630.98	1,135.50	163.51
156	924.14	1,663.08	239.48	630.98	1,135.50	163.51
157	919.57	1,654.86	238.30	630.98	1,135.50	163.51
158	913.48	1,643.90	236.72	630.98	1,135.50	163.51
159	908.24	1,634.46	235.36	630.98	1,135.50	163.51
160	911.79	1,640.85	236.28	630.98	1,135.50	163.51
161	917.37	1,650.90	237.73	630.98	1,135.50	163.51
162	830.25	1,494.12	215.15	630.98	1,135.50	163.51
163	888.95	1,599.75	230.36	593.42	1,067.91	153.78
164	1,028.69	1,851.21	266.57	593.42	1,067.91	153.78
165	1,043.07	1,877.09	270.30	593.42	1,067.91	153.78
166	1,032.75	1,858.52	267.63	593.42	1,067.91	153.78
167	1,024.46	1,843.60	265.48	593.42	1,067.91	153.78
168	1,008.72	1,815.29	261.40	593.42	1,067.91	153.78
169	996.71	1,793.68	258.29	593.42	1,067.91	153.78
170	989.78	1,781.19	256.49	593.42	1,067.91	153.78
171	1,001.96	1,803.11	259.65	593.42	1,067.91	153.78
172	991.13	1,783.63	256.84	593.42	1,067.91	153.78
173	970.32	1,746.19	251.45	593.42	1,067.91	153.78
174	952.22	1,713.61	246.76	593.42	1,067.91	153.78
175	936.15	1,684.69	242.60	593.42	1,067.91	153.78
176	929.72	1,673.12	240.93	593.42	1,067.91	153.78
177	923.97	1,662.77	239.44	593.42	1,067.91	153.78
178	911.62	1,640.55	236.24	593.42	1,067.91	153.78
179	904.18	1,627.15	234.31	593.42	1,067.91	153.78
180	904.86	1,628.37	234.49	593.42	1,067.91	153.78
181	900.12	1,619.85	233.26	593.42	1,067.91	153.78
182	895.38	1,611.32	232.03	593.42	1,067.91	153.78
183	890.48	1,602.49	230.76	593.42	1,067.91	153.78
184	883.03	1,589.10	228.83	593.42	1,067.91	153.78
		Sum	47,205		Sum	28,074

Table F25 the concentrations and mass of carbon dioxide in air that flow in/out microalgal cultivation during Nile tilapia cultivation with 5 kg/m³ fish density.

Cultivating time (Hour)	Microalgal cultivation inlet carbon dioxide			Microalgal cultivation outlet carbon dioxide		
	CCO _{2FI} (ppm)	CCO _{2FI} (mg/m ³)	HCO _{2FI} (mg)	CCO _{2FO} (ppm)	CCO _{2FO} (mg/m ³)	HCO _{2FO} (mg)
1	1,253.68	2,256.11	324.88	559.93	1,007.64	145.10
2	1,355.85	2,439.98	351.36	559.93	1,007.64	145.10
3	1,361.94	2,450.94	352.94	559.93	1,007.64	145.10
4	1,341.64	2,414.41	347.68	559.93	1,007.64	145.10
5	1,316.95	2,369.96	341.27	559.93	1,007.64	145.10
6	1,334.71	2,401.93	345.88	559.93	1,007.64	145.10
7	1,383.43	2,489.61	358.50	559.93	1,007.64	145.10
8	1,330.31	2,394.01	344.74	559.93	1,007.64	145.10

Cultivating time (Hour)	Microalgal cultivation inlet carbon dioxide			Microalgal cultivation outlet carbon dioxide		
	CCO ₂ F _I (ppm)	CCO ₂ F _I (mg/m ³)	HCO ₂ F _I (mg)	CCO ₂ F _O (ppm)	CCO ₂ F _O (mg/m ³)	HCO ₂ F _O (mg)
9	1,318.47	2,372.70	341.67	559.93	1,007.64	145.10
10	1,289.03	2,319.73	334.04	559.93	1,007.64	145.10
11	1,322.19	2,379.40	342.63	559.93	1,007.64	145.10
12	1,414.22	2,545.01	366.48	559.93	1,007.64	145.10
13	1,440.95	2,593.11	373.41	559.93	1,007.64	145.10
14	1,441.96	2,594.94	373.67	559.93	1,007.64	145.10
15	1,443.14	2,597.07	373.98	559.93	1,007.64	145.10
16	1,484.25	2,671.05	384.63	559.93	1,007.64	145.10
17	1,542.95	2,776.68	399.84	559.93	1,007.64	145.10
18	1,564.94	2,816.26	405.54	559.93	1,007.64	145.10
19	1,585.92	2,854.01	410.98	559.93	1,007.64	145.10
20	582.26	1,047.82	150.89	602.56	1,084.35	156.15
21	1,468.86	2,643.34	380.64	602.56	1,084.35	156.15
22	1,968.91	3,543.24	510.23	602.56	1,084.35	156.15
23	2,137.91	3,847.37	554.02	602.56	1,084.35	156.15
24	2,113.89	3,804.14	547.80	602.56	1,084.35	156.15
25	2,088.68	3,758.78	541.26	602.56	1,084.35	156.15
26	2,068.05	3,721.64	535.92	602.56	1,084.35	156.15
27	2,003.09	3,604.74	519.08	602.56	1,084.35	156.15
28	1,991.58	3,584.03	516.10	602.56	1,084.35	156.15
29	1,937.79	3,487.22	502.16	602.56	1,084.35	156.15
30	1,873.50	3,371.54	485.50	602.56	1,084.35	156.15
31	1,808.04	3,253.73	468.54	602.56	1,084.35	156.15
32	1,760.84	3,168.79	456.31	602.56	1,084.35	156.15
33	1,686.24	3,034.54	436.97	602.56	1,084.35	156.15
34	1,623.14	2,920.98	420.62	602.56	1,084.35	156.15
35	1,563.59	2,813.82	405.19	602.56	1,084.35	156.15
36	1,543.80	2,778.21	400.06	602.56	1,084.35	156.15
37	1,536.02	2,764.20	398.05	602.56	1,084.35	156.15
38	1,528.57	2,750.81	396.12	602.56	1,084.35	156.15
39	1,506.75	2,711.54	390.46	602.56	1,084.35	156.15
40	1,517.24	2,730.41	393.18	602.56	1,084.35	156.15
41	1,522.15	2,739.24	394.45	602.56	1,084.35	156.15
42	1,497.79	2,695.40	388.14	602.56	1,084.35	156.15
43	856.47	1,541.30	221.95	572.11	1,029.56	148.26
44	1,337.92	2,407.71	346.71	572.11	1,029.56	148.26
45	1,929.16	3,471.70	499.92	572.11	1,029.56	148.26
46	1,880.78	3,384.63	487.39	572.11	1,029.56	148.26
47	1,819.03	3,273.51	471.39	572.11	1,029.56	148.26
48	1,780.80	3,204.71	461.48	572.11	1,029.56	148.26
49	1,765.91	3,177.92	457.62	572.11	1,029.56	148.26
50	1,752.38	3,153.57	454.11	572.11	1,029.56	148.26
51	1,700.28	3,059.80	440.61	572.11	1,029.56	148.26
52	1,638.36	2,948.38	424.57	572.11	1,029.56	148.26
53	1,575.26	2,834.83	408.22	572.11	1,029.56	148.26
54	1,529.93	2,753.24	396.47	572.11	1,029.56	148.26
55	1,496.77	2,693.57	387.87	572.11	1,029.56	148.26
56	1,447.71	2,605.29	375.16	572.11	1,029.56	148.26
57	1,414.89	2,546.23	366.66	572.11	1,029.56	148.26
58	1,393.24	2,507.26	361.05	572.11	1,029.56	148.26
59	1,359.41	2,446.38	352.28	572.11	1,029.56	148.26
60	1,331.49	2,396.14	345.04	572.11	1,029.56	148.26
61	1,343.51	2,417.76	348.16	572.11	1,029.56	148.26
62	1,329.97	2,393.41	344.65	572.11	1,029.56	148.26
63	1,311.53	2,360.22	339.87	572.11	1,029.56	148.26
64	1,300.54	2,340.43	337.02	572.11	1,029.56	148.26
65	1,290.05	2,321.56	334.30	572.11	1,029.56	148.26
66	924.48	1,663.68	239.57	613.72	1,104.44	159.04
67	878.80	1,581.49	227.73	613.72	1,104.44	159.04
68	1,799.58	3,238.50	466.34	613.72	1,104.44	159.04

Cultivating time (Hour)	Microalgal cultivation inlet carbon dioxide			Microalgal cultivation outlet carbon dioxide		
	CCO ₂ F _I (ppm)	CCO ₂ F _I (mg/m ³)	HCO ₂ F _I (mg)	CCO ₂ F _O (ppm)	CCO ₂ F _O (mg/m ³)	HCO ₂ F _O (mg)
69	1,766.59	3,179.14	457.80	613.72	1,104.44	159.04
70	1,738.17	3,128.00	450.43	613.72	1,104.44	159.04
71	1,746.29	3,142.61	452.54	613.72	1,104.44	159.04
72	1,761.52	3,170.01	456.48	613.72	1,104.44	159.04
73	1,762.87	3,172.44	456.83	613.72	1,104.44	159.04
74	1,737.83	3,127.39	450.34	613.72	1,104.44	159.04
75	1,688.77	3,039.10	437.63	613.72	1,104.44	159.04
76	1,621.62	2,918.24	420.23	613.72	1,104.44	159.04
77	1,572.56	2,829.96	407.51	613.72	1,104.44	159.04
78	1,515.04	2,726.45	392.61	613.72	1,104.44	159.04
79	1,460.06	2,627.51	378.36	613.72	1,104.44	159.04
80	1,424.71	2,563.89	369.20	613.72	1,104.44	159.04
81	1,404.74	2,527.96	364.03	613.72	1,104.44	159.04
82	1,360.59	2,448.51	352.59	613.72	1,104.44	159.04
83	1,333.36	2,399.49	345.53	613.72	1,104.44	159.04
84	1,321.34	2,377.88	342.41	613.72	1,104.44	159.04
85	1,318.13	2,372.09	341.58	613.72	1,104.44	159.04
86	1,304.43	2,347.44	338.03	613.72	1,104.44	159.04
87	1,293.26	2,327.34	335.14	613.72	1,104.44	159.04
88	1,312.72	2,362.35	340.18	613.72	1,104.44	159.04
89	1,307.64	2,353.22	338.86	613.72	1,104.44	159.04
90	1,265.18	2,276.81	327.86	613.72	1,104.44	159.04
91	875.59	1,575.70	226.90	620.83	1,117.23	160.88
92	790.84	1,423.18	204.94	620.83	1,117.23	160.88
93	1,573.57	2,831.79	407.78	620.83	1,117.23	160.88
94	1,693.34	3,047.32	438.81	620.83	1,117.23	160.88
95	1,637.69	2,947.16	424.39	620.83	1,117.23	160.88
96	1,621.62	2,918.24	420.23	620.83	1,117.23	160.88
97	1,603.68	2,885.97	415.58	620.83	1,117.23	160.88
98	1,585.08	2,852.49	410.76	620.83	1,117.23	160.88
99	1,565.11	2,816.56	405.59	620.83	1,117.23	160.88
100	1,580.00	2,843.35	409.44	620.83	1,117.23	160.88
101	1,622.12	2,919.16	420.36	620.83	1,117.23	160.88
102	1,601.32	2,881.71	414.97	620.83	1,117.23	160.88
103	1,542.61	2,776.07	399.75	620.83	1,117.23	160.88
104	1,485.10	2,672.57	384.85	620.83	1,117.23	160.88
105	1,425.38	2,565.10	369.37	620.83	1,117.23	160.88
106	1,375.65	2,475.60	356.49	620.83	1,117.23	160.88
107	1,339.95	2,411.37	347.24	620.83	1,117.23	160.88
108	1,323.21	2,381.23	342.90	620.83	1,117.23	160.88
109	1,327.94	2,389.75	344.12	620.83	1,117.23	160.88
110	1,308.15	2,354.13	339.00	620.83	1,117.23	160.88
111	1,292.76	2,326.43	335.01	620.83	1,117.23	160.88
112	1,274.15	2,292.94	330.18	620.83	1,117.23	160.88
113	1,275.50	2,295.38	330.53	620.83	1,117.23	160.88
114	1,274.15	2,292.94	330.18	620.83	1,117.23	160.88
115	1,001.28	1,801.90	259.47	620.83	1,117.23	160.88
116	705.07	1,268.84	182.71	631.99	1,137.32	163.77
117	1,481.04	2,665.26	383.80	631.99	1,137.32	163.77
118	1,689.45	3,040.32	437.81	631.99	1,137.32	163.77
119	1,712.80	3,082.33	443.86	631.99	1,137.32	163.77
120	1,720.41	3,096.03	445.83	631.99	1,137.32	163.77
121	1,709.58	3,076.55	443.02	631.99	1,137.32	163.77
122	1,713.81	3,084.16	444.12	631.99	1,137.32	163.77
123	1,727.18	3,108.21	447.58	631.99	1,137.32	163.77
124	1,701.80	3,062.54	441.01	631.99	1,137.32	163.77
125	1,641.07	2,953.25	425.27	631.99	1,137.32	163.77
126	1,578.14	2,840.01	408.96	631.99	1,137.32	163.77
127	1,530.94	2,755.07	396.73	631.99	1,137.32	163.77
128	1,489.33	2,680.18	385.95	631.99	1,137.32	163.77

Cultivating time (Hour)	Microalgal cultivation inlet carbon dioxide			Microalgal cultivation outlet carbon dioxide		
	CCO _{2F1} (ppm)	CCO _{2F1} (mg/m ³)	HCO _{2F1} (mg)	CCO _{2Fo} (ppm)	CCO _{2Fo} (mg/m ³)	HCO _{2Fo} (mg)
129	1,464.97	2,636.34	379.63	631.99	1,137.32	163.77
130	1,444.16	2,598.90	374.24	631.99	1,137.32	163.77
131	1,419.80	2,555.06	367.93	631.99	1,137.32	163.77
132	1,394.26	2,509.09	361.31	631.99	1,137.32	163.77
133	1,388.33	2,498.43	359.77	631.99	1,137.32	163.77
134	1,372.60	2,470.12	355.70	631.99	1,137.32	163.77
135	1,351.46	2,432.07	350.22	631.99	1,137.32	163.77
136	1,345.20	2,420.80	348.60	631.99	1,137.32	163.77
137	1,369.05	2,463.73	354.78	631.99	1,137.32	163.77
138	1,347.40	2,424.76	349.17	631.99	1,137.32	163.77
139	1,338.26	2,408.32	346.80	631.99	1,137.32	163.77
140	1,074.02	1,932.80	278.32	631.99	1,137.32	163.77
141	893.86	1,608.58	231.64	692.89	1,246.92	179.56
142	1,801.27	3,241.55	466.78	692.89	1,246.92	179.56
143	1,790.27	3,221.76	463.93	692.89	1,246.92	179.56
144	1,773.87	3,192.23	459.68	692.89	1,246.92	179.56
145	1,806.01	3,250.07	468.01	692.89	1,246.92	179.56
146	1,860.82	3,348.71	482.21	692.89	1,246.92	179.56
147	1,981.77	3,566.38	513.56	692.89	1,246.92	179.56
148	2,054.68	3,697.59	532.45	692.89	1,246.92	179.56
149	2,068.55	3,722.55	536.05	692.89	1,246.92	179.56
150	2,138.42	3,848.28	554.15	692.89	1,246.92	179.56
151	2,171.75	3,908.25	562.79	692.89	1,246.92	179.56
152	2,129.79	3,832.75	551.92	692.89	1,246.92	179.56
153	2,061.79	3,710.37	534.29	692.89	1,246.92	179.56
154	2,001.56	3,602.00	518.69	692.89	1,246.92	179.56
155	1,980.59	3,564.25	513.25	692.89	1,246.92	179.56
156	1,931.36	3,475.66	500.49	692.89	1,246.92	179.56
157	1,925.44	3,465.00	498.96	692.89	1,246.92	179.56
158	1,916.47	3,448.87	496.64	692.89	1,246.92	179.56
159	1,890.25	3,401.68	489.84	692.89	1,246.92	179.56
160	1,913.94	3,444.30	495.98	692.89	1,246.92	179.56
161	1,906.83	3,431.51	494.14	692.89	1,246.92	179.56
162	1,903.95	3,426.34	493.39	692.89	1,246.92	179.56
163	1,725.15	3,104.56	447.06	692.89	1,246.92	179.56
164	1,518.09	2,731.93	393.40	692.89	1,246.92	179.56
165	821.63	1,478.59	212.92	609.66	1,097.14	157.99
166	635.88	1,144.33	164.78	609.66	1,097.14	157.99
167	894.54	1,609.80	231.81	609.66	1,097.14	157.99
168	2,525.81	4,545.43	654.54	609.66	1,097.14	157.99
169	2,693.29	4,846.81	697.94	609.66	1,097.14	157.99
170	2,688.38	4,837.98	696.67	609.66	1,097.14	157.99
171	2,640.68	4,752.13	684.31	609.66	1,097.14	157.99
172	2,632.56	4,737.52	682.20	609.66	1,097.14	157.99
173	2,609.72	4,696.42	676.29	609.66	1,097.14	157.99
174	2,587.73	4,656.85	670.59	609.66	1,097.14	157.99
175	2,516.17	4,528.07	652.04	609.66	1,097.14	157.99
176	2,463.05	4,432.48	638.28	609.66	1,097.14	157.99
177	2,389.46	4,300.06	619.21	609.66	1,097.14	157.99
178	2,295.91	4,131.70	594.97	609.66	1,097.14	157.99
179	2,240.93	4,032.76	580.72	609.66	1,097.14	157.99
180	2,205.07	3,968.23	571.42	609.66	1,097.14	157.99
181	2,187.65	3,936.87	566.91	609.66	1,097.14	157.99
182	2,140.45	3,851.93	554.68	609.66	1,097.14	157.99
183	2,113.38	3,803.22	547.66	609.66	1,097.14	157.99
184	2,091.22	3,763.34	541.92	609.66	1,097.14	157.99
185	2,091.05	3,763.04	541.88	609.66	1,097.14	157.99
		Sum	77,581		Sum	29,478

Appendix G

G1. Result from High-Performance Liquid Chromatography analysis

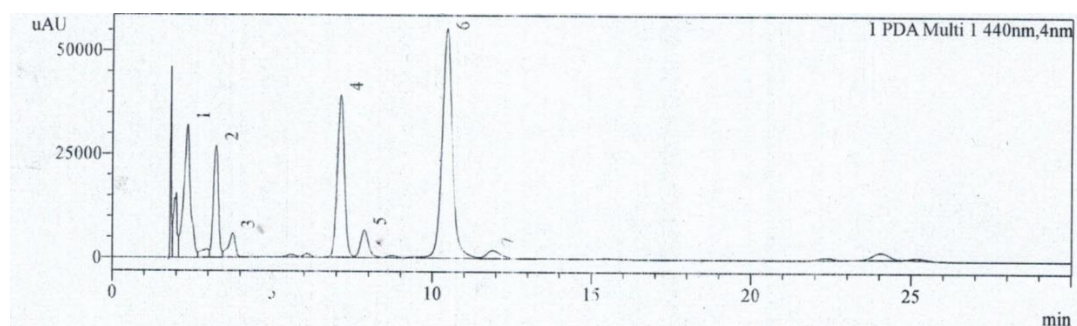


Fig G1 Chromatogram of extracted pigments solution from *Scenedesmus armatus*

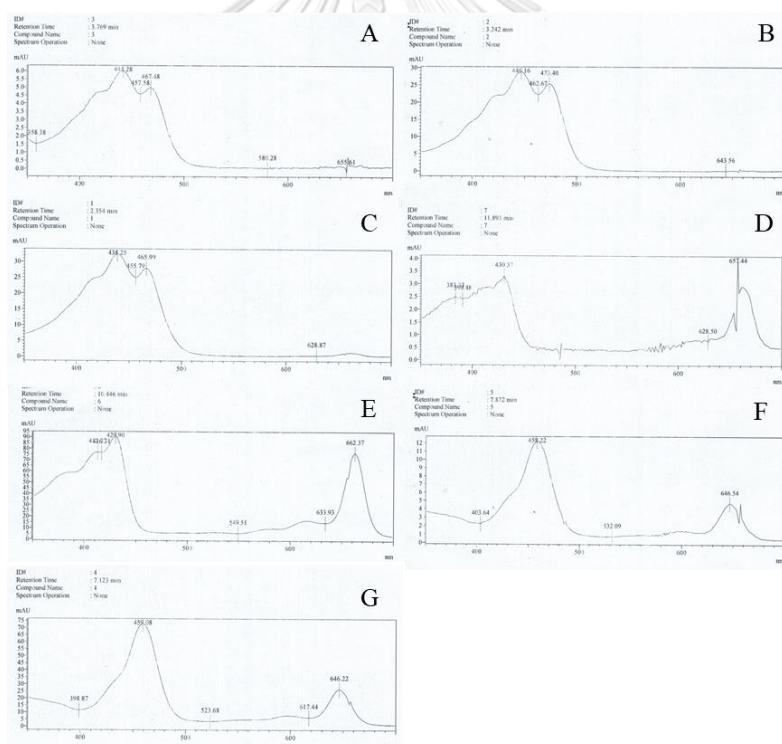


Fig G2 (A) Spectrum of the 1st peak in chromatogram [Unidentified carotenoid 1], (B) Spectrum of the 2nd peak in chromatogram [Lutein], (C) Spectrum of the 3rd peak in chromatogram [Unidentified carotenoid 2], (D, E) Spectrum of the 4th and 5th peaks in chromatogram [Chlorophyll-b] and (F, G) Spectrum of the 6th and 7th peaks in chromatogram [Chlorophyll-a]; These peak's spectrums were identified by comparing with the pigment spectrums as references (Mantoura and Wrigh, 1997).

Table G1 Pigment analysis from BG-11 treatment by High-Performance Liquid Chromatography (Shimadzu Model SPD-M20A) (Sample: solvent =3:1)

Peak no.	Retention time (min)	Peak area			Pigment
		Rep 1	Rep 2	Rep 3	
1	2.375	882,420	688,075	407,963	Unidentified carotenoid 1
2	3.289	816,128	436,553	432,535	Lutein
3	3.844	139,590	127,146	60,282	Unidentified carotenoid 2
4	7.283	1,141,598	894,021	433,011	Chlorophyll-b
5	8.047	247,127	165,774	67,571	Chlorophyll-b
6	10.711	2,584,279	1,819,933	899,306	Chlorophyll-a
7	12.186	162,545	86,757	54,577	Chlorophyll-a

Table G2 Pigment analysis from Effluent treatment by High-Performance Liquid Chromatography (Shimadzu Model SPD-M20A) (Sample: solvent =3:1)

Peak no.	Retention time (min)	Peak area			Pigment
		Rep 1	Rep 2	Rep 3	
1	2.400	526,573	457,978	59,282	Unidentified carotenoid 1
2	3.288	572,396	487,853	109,335	Lutein
3	3.834	133,584	113,784	108,860	Unidentified carotenoid 2
4	7.290	626,151	482,282	62,886	Chlorophyll-b
5	8.060	140,273	105,947	0	Chlorophyll-b
6	10.749	1,134,007	852,738	96,513	Chlorophyll-a
7	12.244	102,920	86,355	0	Chlorophyll-a

Table G3 Pigment analysis from C treatment by High-Performance Liquid Chromatography (Shimadzu Model SPD-M20A) (Sample: solvent =1:2)

Peak no.	Retention time (min)	Peak area			Pigment
		Rep 1	Rep 2	Rep 3	
1	2.410	18,239	52,207	36,277	Unidentified carotenoid 1
2	3.374	30,758	82,232	58,515	Lutein
3	7.648	27,694	53,814	36,409	Chlorophyll-b
4	11.398	39,251	90,867	64,490	Chlorophyll-a

Table G4 Pigment analysis from T1 treatment by High-Performance Liquid Chromatography (Shimadzu Model SPD-M20A) (Sample: solvent =1:2)

Peak no.	Retention time (min)	Peak area			Pigment
		Rep 1	Rep 2	Rep 3	
1	2.410	76,924	37,918	32,399	Unidentified carotenoid 1
2	3.373	64,146	45,166	34,016	Lutein
3	7.630	63,468	46,319	43,071	Chlorophyll-b
4	11.364	86,047	63,962	59,838	Chlorophyll-a

Table G5 Pigment analysis from T2 treatment by High-Performance Liquid Chromatography (Shimadzu Model SPD-M20A) (Sample: solvent =1:2)

Peak no.	Retention time (min)	Peak area			Pigment
		Rep 1	Rep 2	Rep 3	
1	2.400	0	30,627	58,533	Unidentified carotenoid 1
2	3.358	62,565	32,582	49,157	Lutein
3	7.607	51,586	25,264	74,763	Chlorophyll-b
4	11.325	67,147	32,502	99,590	Chlorophyll-a

Table G6 Pigment analysis from T3 treatment by High-Performance Liquid Chromatography (Shimadzu Model SPD-M20A) (Sample: solvent =1:2)

Peak no.	Retention time (min)	Peak area			Pigment
		Rep 1	Rep 2	Rep 3	
1	2.401	131,877	82,857	64,603	Unidentified carotenoid 1
2	3.345	78,816	74,895	60,932	Lutein
3	7.565	114,978	92,514	58,833	Chlorophyll-b
4	11.266	166,383	105,698	66,472	Chlorophyll-a



G2. Result from CHN analysis

RESULT FROM CHN ELEMENTAL ANALYSIS

Sender kittikoon sucunthowong Date 25/10/2019

NO.	Sample code	Analysis required			Remark
		%C	%H	%N	
1	NI	49.90	7.72	6.76	
2	OR	29.73	4.24	3.14	
3	FD	41.68	6.33	2.97	
4	SC	47.87	7.53	8.11	

OPERATER ID: NATTHAPAT
Perkin-Elmer 2400 Series CHNS/O Analyser

Where NI = nile tilapia biomass
OR = Suspended organic solids
FD = Approximately 15% protein fish feed
SC = *Scenedesmus armatus* biomass which obtained from experiments

G3. Result from GC

ภาควิชาเคมีเทคนิค คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	เอกสาร: ScFM-CT-06-002-A	FM โทร 0-2218-7523-5 โทรสาร 0-2255-5831
	แบบรายงานผลการวิเคราะห์และทดสอบ	ลำดับการแก้ไข 0 หน้าที่ 1

วันที่ 22 ตุลาคม 2562

รายงานผลการวิเคราะห์และทดสอบ

เรื่อง รายงานผลการวิเคราะห์ Gas Chromatography (GC)
 ผู้ส่งตัวอย่าง : นายกิตติคุณ สุคันโธวงศ์
 ใบเสนอราคาเลขที่: CT005/63
 หน่วยงาน : คณะวิศวกรรมศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
 ชนิดตัวอย่าง : อากาศจากระบบเพาะเลี้ยงสัตว์น้ำ
 วันที่รับตัวอย่าง : 21 ตุลาคม 2562
 เครื่องมือวิเคราะห์/ทดสอบ : Gas Chromatography (GC) Shimadzu GC-2014
 สภาวะการวิเคราะห์ : Injection Temp: 150°C
 Column: Unibeads C, Column flow (He): 30 mL/min, Equilibration: 3.00 min
 Column Temp: 150°C hold 10.00 min
 Detector: TCD, Detector Temp: 200°C, Current: 120 mA
 มาตรฐานอ้างอิงสำหรับการทดสอบ : -
 ผลการวิเคราะห์และทดสอบ

ลำดับ	ชื่อตัวอย่าง	ผลการวิเคราะห์
1	Outlet MCU (Amb)	ตามเอกสารแนบ
2	Outlet MCU (CO ₂)	ตามเอกสารแนบ
3	Outlet SSU	ตามเอกสารแนบ
4	Outlet AU	ตามเอกสารแนบ
5	Ambient Air	ตามเอกสารแนบ
6	CO ₂ Pure	ตามเอกสารแนบ
7	CO ₂ 1%	ตามเอกสารแนบ
8	CO ₂ 1%	ตามเอกสารแนบ
9	CO ₂ 1%	ตามเอกสารแนบ
10	CO ₂ 1%	ตามเอกสารแนบ
11	CO ₂ 1%	ตามเอกสารแนบ

รับรองผลการวิเคราะห์ถูกต้อง

Amiya
 (นางสาวอภิญญา ลาญายติ)
 นักวิทยาศาสตร์
 วันที่ 22 ตุลาคม 2562

Ujan
 (รองศาสตราจารย์ ดร.ชวลิต งามจรัสศรีวิชัย)
 หัวหน้าห้องปฏิบัติการเครื่องมือวิเคราะห์
 วันที่ 22 ตุลาคม 2562

หมายเหตุ : 1. ผลการวิเคราะห์ในรายงานฉบับนี้ใช้อ้างอิงสำหรับตัวอย่างที่ส่งมาเท่านั้น
 2. ห้ามทำสำเนารายงานฉบับนี้เพียงบางส่วนโดยไม่ได้รับอนุญาตอย่างเป็นทางการ

เอกสาร	ผู้จัดเก็บ	วิธีการจัดเก็บ	สถานที่เก็บ/แพคเกจเก็บ	ระยะเวลาที่เก็บ	ผู้อนุมัติให้ทำลาย	วิธีการทำลาย	ผู้มีหน้าที่ทำลาย
ScFM-CT-06-002-A	เจ้าหน้าที่วิเคราะห์	เข้าแฟ้ม	แฟ้มบริการวิชาการรอง เจ้าหน้าที่วิเคราะห์	1 ปี	QMR	ทิ้ง	เจ้าหน้าที่วิเคราะห์

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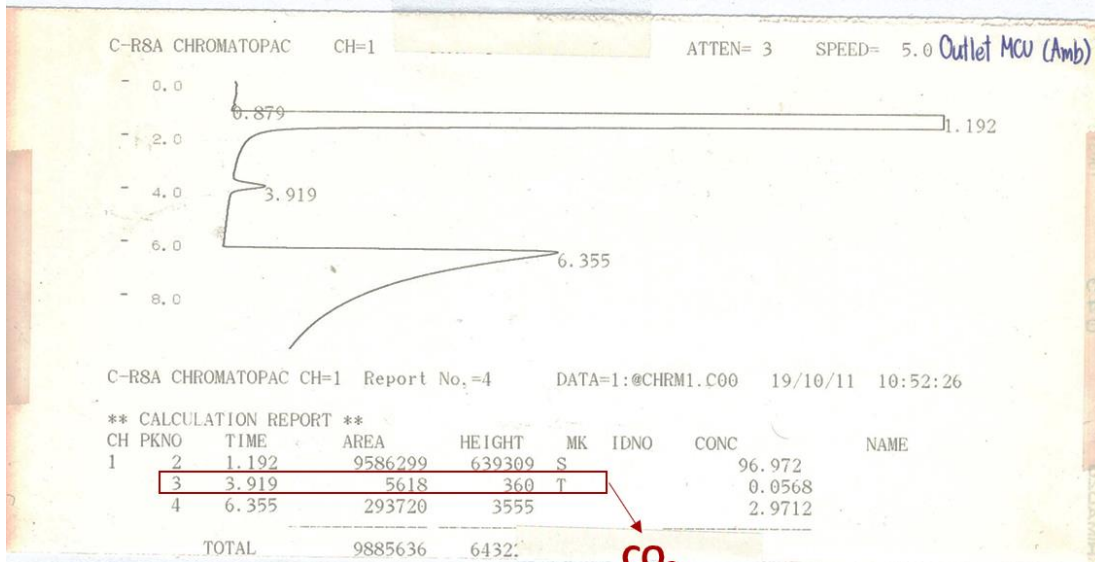
INTEGRATION PARAMETERS

WIDTH (sec)	5	SLOPE (uV/min)	100
DRIFT (uV/min)	0	T.DBL (min)	1000
STOP.TM (min)	10	ATTEN (2 ^X mV)	3
SPEED (mm/min)	5		

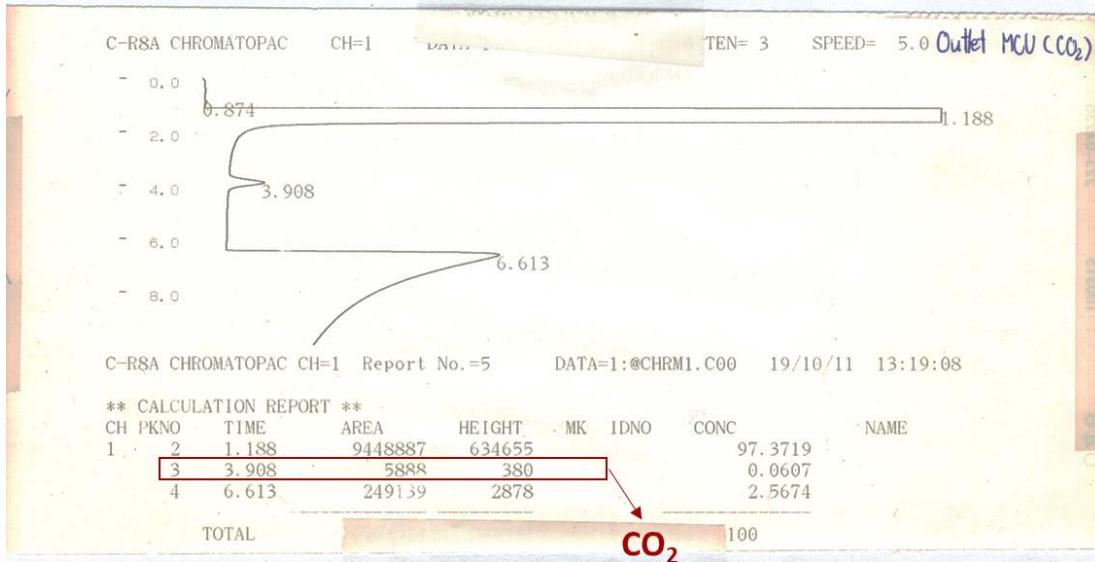
QUANTITATION PARAMETERS

METHOD (0 - 8)	1	CURVE (CALIB FIT TYPE)	0
CAL.LEVL (0 - 15)	1	MIN.AREA (Count)	100
WIN.BAND (0:WIN 1:BAND)0		WINDOW (%)	5
SPL.WT	100	IS.WT	1
DILFACT	1		

Outlet air from microalgal cultivation (Control-10)

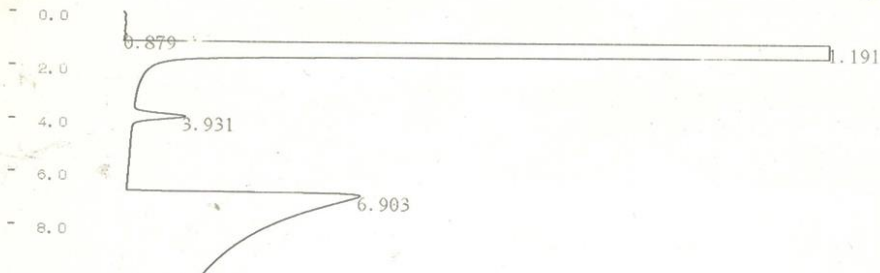


Outlet air from microalgal cultivation (CO2-10)



Outlet air from solid separating unit at fish density 10 kg/m³

C-RSA CHROMATOPAC CH=1 TEN= 3 SPEED= 5.0 Outlet ssu



C-RSA CHROMATOPAC CH=1 Report No.=6 DATA=1:@CHRM1.C00 19/10/11 13:37:44

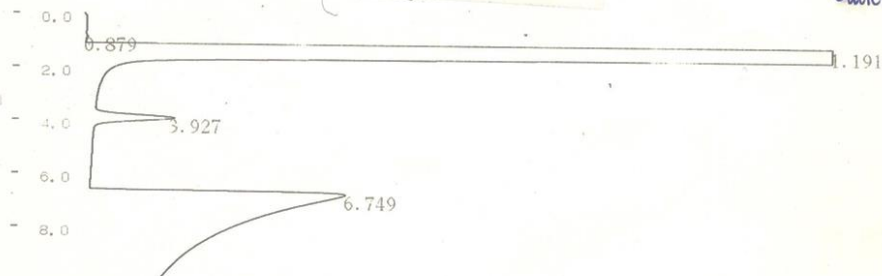
** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	2	1.191	9702697	642944			97.8515	
	3	3.931	8836	560			0.0891	
	4	6.903	204203	27			2.0594	
TOTAL			9915736	645				

CO₂

Outlet air from fish tank at fish density 10 kg/m³

C-RSA CHROMATOPAC CH=1 EN 3 SPEED= 5.0 Outlet AU



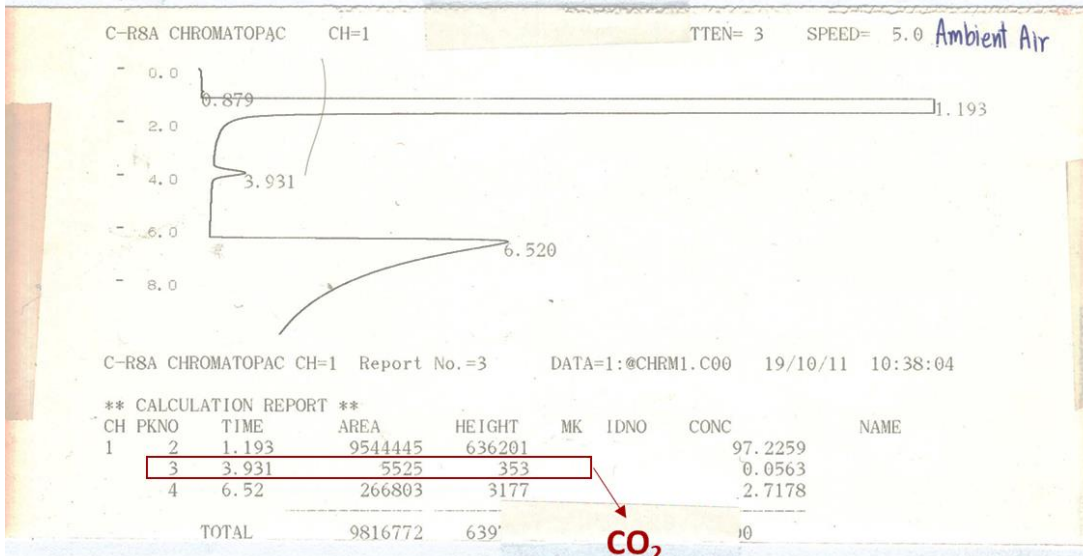
C-RSA CHROMATOPAC CH=1 Report No.=7 DATA=1:@CHRM1.C00 19/10/11 14:00:58

** CALCULATION REPORT **

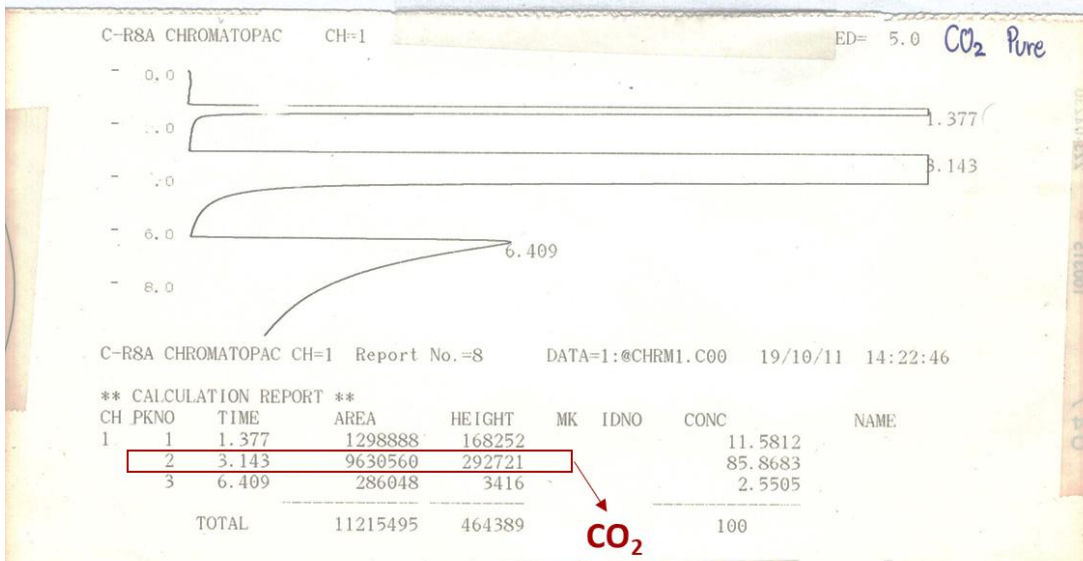
CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	2	1.191	9612502	639499			97.5771	
	3	3.927	13606	860			0.1381	
	4	6.749	225080	2702			2.2848	
TOTAL			9851188	64306				

CO₂

Inlet air to solid separating unit and fish tank (ambient air)



Pure CO₂



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INTEGRATION PARAMETERS

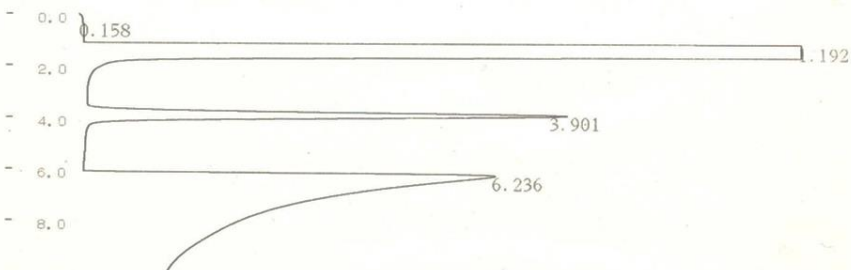
WIDTH (sec)	5	SLOPE (uV/min)	100
DRIFT (uV/min)	0	T.DBL (min)	1000
STOP.TM (min)	10	ATTEN (2^X mV)	3
SPEED (mm/min)	5		

QUANTITATION PARAMETERS

METHOD (0 - 8)	1	CURVE (CALIB FIT TYPE)	0
CAL.LEVL (0 - 15)	1	MIN.AREA (Count)	100
WIN.BAND (0:WIN 1:BAND)	0	WINDOW (%)	5
SPL.WT	100	IS.WT	1
DILFACT	1		

CO₂ 9901 ppm

C-R8A CHROMATOPAC CH=1 DATA=1:@CHRM1.C00 ATTEN= 3 SPEED= 5.0 CO₂ 1+



C-R8A CHROMATOPAC CH=1 Report No.=1 DATA=1:@CHRM1.C00 19/10/17 08:44:06

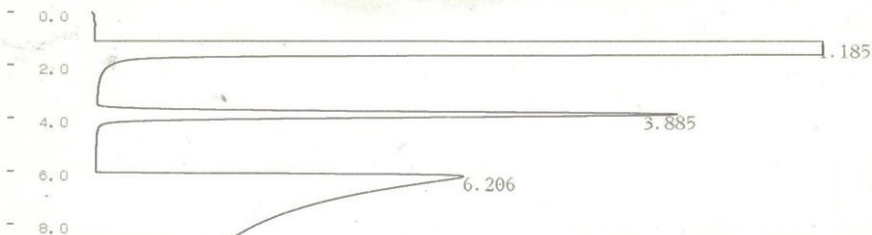
** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	1	0.158	165	14			0.0016	
	2	1.192	9566568	644760			95.4911	
	3	3.901	83899	5323			0.8375	
	4	6.236	367654	4528			3.6698	
TOTAL			1001828				100	

CO₂

CO₂ 9901 ppm

C-RSA CHROMATOPAC CH=1 DATA=1:@CHRM1.C00 ATTEN= 3 SPEED= 5.0 CO₂ 1%



C-RSA CHROMATOPAC CH=1 Report No.=2 DATA=1:@CHRM1.C00 19/10/17 09:13:56

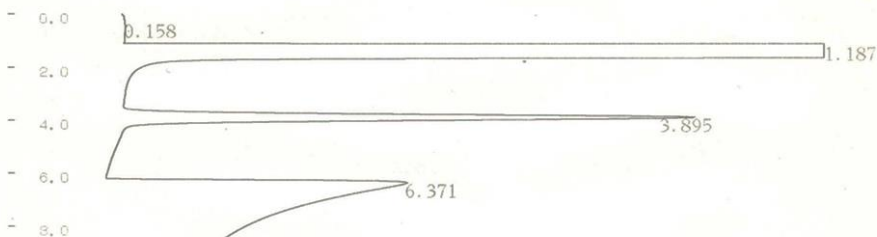
** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	1	1.185	9360414	638009			95.4378	
	2	3.885	98454	6274			1.0038	
	3	6.206	349005	3929			3.5584	
TOTAL			9807872	648212			100	

CO₂

CO₂ 9901 ppm

C-RSA CHROMATOPAC CH=1 DATA=1:@CHRM1.C00 ATTEN= 3 SPEED= 5.0 CO₂ 1%



C-RSA CHROMATOPAC CH=1 Report No.=3 DATA=1:@CHRM1.C00 19/10/17 09:28:48

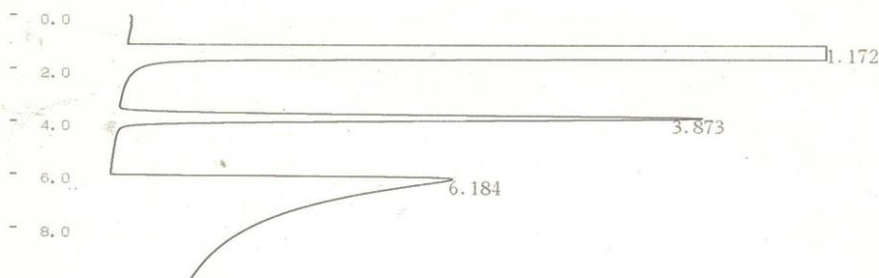
** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	1	0.158	152	13			0.0015	
	2	1.187	9505452	643235			96.1808	
	3	3.895	100564	6200			1.0176	
	4	6.371	276735	3210			2.8001	
TOTAL			9882903	652658			100	

CO₂

CO₂ 9901 ppm

C-RSA CHROMATOPAC CH=1 DATA=1:@CHRM1.C00 ATTEN= 3 SPEED= 5.0 CO₂ 1%



C-RSA CHROMATOPAC CH=1 Report No.=4 DATA=1:@CHRM1.C00 19/10/17 09:44:26

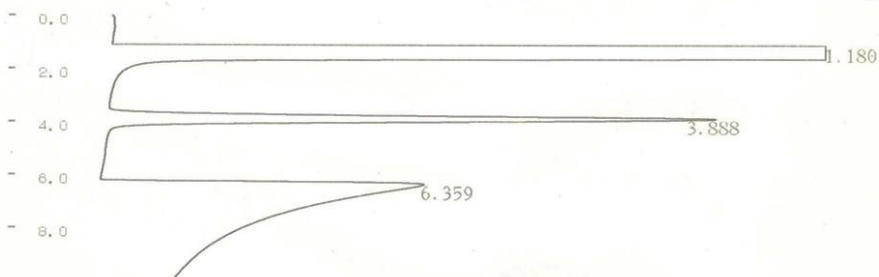
**** CALCULATION REPORT ****

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	1	1.172	9407958	640145			95.7927	
	2	3.873	99054	6306			1.0086	
	3	6.184	314152	3654			3.1987	
TOTAL			9821163	650105			100	

CO₂

CO₂ 9901 ppm

C-RSA CHROMATOPAC CH=1 DATA=1:@CHRM1.C00 ATTEN= 3 SPEED= 5.0 CO₂ 1%



C-RSA CHROMATOPAC CH=1 Report No.=5 DATA=1:@CHRM1.C00 19/10/17 10:01:14

**** CALCULATION REPORT ****

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	1	1.18	9477430	642347			95.9703	
	2	3.888	103763	6558			1.0507	
	3	6.359	294185	3452			2.979	
TOTAL			9875377	652356			100	

CO₂

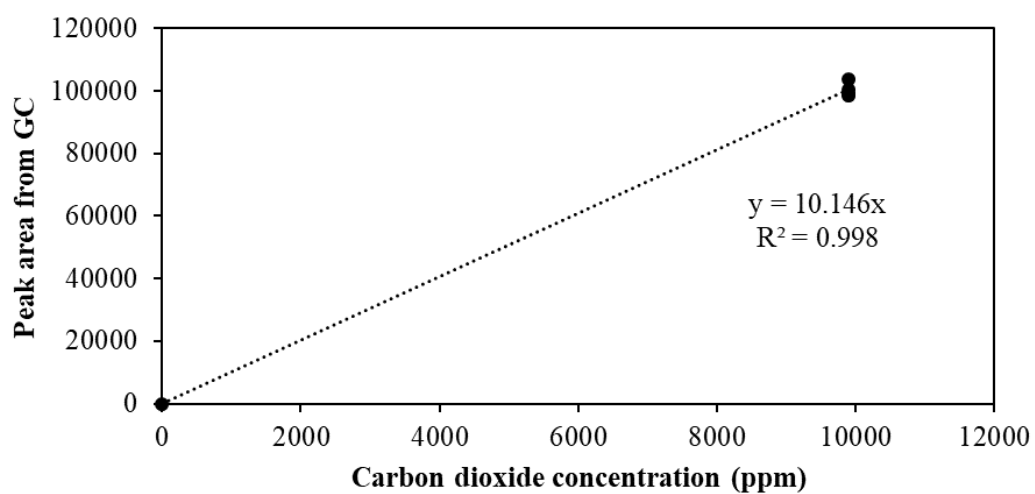


Fig F1 Relative graph between carbon dioxide concentrations and CO₂ peak area from packed column gas chromatography analysis



Appendix G

G1. Statistical analysis in “Utilization of nitrifying biofilter effluent from commercial aquaculture to cultivate microalgae”

Specific growth rate

T-TEST GROUPS=Group(1 2)
 /MISSING=ANALYSIS
 /VARIABLES=SGR
 /CRITERIA=CI(.95).



Group	N	Mean	Std. Deviation	Std. Error Mean
SGR 1.00	3	.8866	.09427	.05443
2.00	3	1.5481	.11857	.06845

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
SGR	Equal variances assumed	.183	.691	-7.564	4
	Equal variances not assumed			-7.564	3.807

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference
					Lower
SGR	Equal variances assumed	.002	-.66150	.08745	-.90431
	Equal variances not assumed	.002	-.66150	.08745	-.90926

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Upper	
SGR	Equal variances assumed	-.41869	
	Equal variances not assumed	-.41374	

Maximum cells number density

```

T-TEST GROUPS=Group(1 2)
/MISSING=ANALYSIS
/VARIABLES=Maxcells
/CRITERIA=CI(.95).

```

T-Test**Group Statistics**

Group	N	Mean	Std. Deviation	Std. Error Mean
Maxcells 1.00	3	603.6111	111.69258	64.48574
2.00	3	648.8889	72.99321	42.14265

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Maxcells	Equal variances assumed	.269	.632	-.588	4
	Equal variances not assumed			-.588	3.445

Independent Samples Test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
Maxcells	Equal variances assumed	.588	-45.27778	77.03515
	Equal variances not assumed	.593	-45.27778	77.03515

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Maxcells	Equal variances assumed	-259.16163	168.60607
	Equal variances not assumed	-273.46696	182.91141

Chlorophyll content

```
T-TEST GROUPS=Group(1 2)
/MISSING=ANALYSIS
/VARIABLES=Chlo_a
/CRITERIA=CI(.95).
```

T-Test**Group Statistics**

Group	N	Mean	Std. Deviation	Std. Error Mean
Chlo_a 1.00	3	16.7183	7.39964	4.27218
2.00	3	10.5180	6.05551	3.49615

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Chlo_a	Equal variances assumed	.212	.669	1.123	4
	Equal variances not assumed			1.123	3.849

Independent Samples Test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
Chlo_a	Equal variances assumed	.324	6.20036	5.52038
	Equal variances not assumed	.326	6.20036	5.52038

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Chlo_a	Equal variances assumed	-9.12667	21.52739
	Equal variances not assumed	-9.36614	21.76686

Carotenoid content

T-TEST GROUPS=Group (1 2)
 /MISSING=ANALYSIS
 /VARIABLES=Caro
 /CRITERIA=CI (.95) .

T-Test**Group Statistics**

Group	N	Mean	Std. Deviation	Std. Error Mean
Caro 1.00	3	3.9569	.99829	.57637
2.00	3	3.0941	1.99724	1.15311

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Caro	Equal variances assumed	1.709	.261	.669	4
	Equal variances not assumed			.669	2.941

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference
					Lower
Caro	Equal variances assumed	.540	.86281	1.28913	-2.71639
	Equal variances not assumed	.552	.86281	1.28913	-3.28703

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Upper	
Caro	Equal variances assumed	4.44200	
	Equal variances not assumed	5.01264	

Lutein content

```
T-TEST GROUPS=Group(1 2)
/MISSING=ANALYSIS
/VARIABLES=Lutien
/CRITERIA=CI(.95).
```

T-Test**Group Statistics**

Group	N	Mean	Std. Deviation	Std. Error Mean
Lutien 1.00	3	1.5851	.62170	.35894
2.00	3	1.1185	.70748	.40847

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Lutien	Equal variances assumed	.090	.779	.858	4
	Equal variances not assumed			.858	3.935

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference
					Lower
Lutien	Equal variances assumed	.439	.46661	.54377	-1.04312
	Equal variances not assumed	.440	.46661	.54377	-1.05301

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Upper	
Lutien	Equal variances assumed	1.97635	
	Equal variances not assumed	1.98624	

G2. Statistical analysis in “Utilization of carbon dioxide from aquaculture for microalgal cultivation”

Carbon dioxide concentration

```
T-TEST GROUPS=group(1 2)
  /MISSING=ANALYSIS
  /VARIABLES=co2
  /CRITERIA=CI(.95).
```

T-Test

Group Statistics

group	N	Mean	Std. Deviation	Std. Error Mean
co2 1.00	372	566.3730	13.94107	.72281
2.00	372	935.0075	93.43166	4.84421

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
co2	Equal variances assumed	634.344	.000	-75.265	742
	Equal variances not assumed			-75.265	387.512

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference
					Lower
co2	Equal variances assumed	.000	-368.63449	4.89784	-378.24975
	Equal variances not assumed	.000	-368.63449	4.89784	-378.26415

independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Upper	
co2	Equal variances assumed	-359.01922	
	Equal variances not assumed	-359.00483	

Specific growth rate

ONEWAY SGR BY Group
 /MISSING ANALYSIS
 /POSTHOC=TUKEY ALPHA (0.05) .

Oneway

ANOVA

SGR

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.048	3	.016	2.230	.162
Within Groups	.058	8	.007		
Total	.106	11			

Post Hoc Tests



Multiple Comparisons

Dependent Variable: SGR

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	1.00	-.05333	.06944	.867	-.2757	.1690
	2.00	.12000	.06944	.371	-.1024	.3424
	3.00	.00000	.06944	1.000	-.2224	.2224
1.00	.00	.05333	.06944	.867	-.1690	.2757
	2.00	.17333	.06944	.135	-.0490	.3957
	3.00	.05333	.06944	.867	-.1690	.2757
2.00	.00	-.12000	.06944	.371	-.3424	.1024
	1.00	-.17333	.06944	.135	-.3957	.0490
	3.00	-.12000	.06944	.371	-.3424	.1024
3.00	.00	.00000	.06944	1.000	-.2224	.2224
	1.00	-.05333	.06944	.867	-.2757	.1690
	2.00	.12000	.06944	.371	-.1024	.3424

Homogeneous Subsets

SGR

Tukey HSD^a

Group	N	Subset for alpha = 0.05
		1
2.00	3	.5200
.00	3	.6400
3.00	3	.6400
1.00	3	.6933
Sig.		.135

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Maximum cell number density

ONEWAY Maxcell BY Group

/MISSING ANALYSIS

/POSTHOC=TUKEY ALPHA(0.05) .

Oneway

ANOVA

Maxcell

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1059814.363	3	353271.454	5.501	.024
Within Groups	513764.313	8	64220.539		
Total	1573578.677	11			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Maxcell

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	1.00	-418.03333	206.91470	.257	-1080.6470	244.5804
	2.00	369.00000	206.91470	.347	-293.6137	1031.6137
	3.00	-256.23333	206.91470	.622	-918.8470	406.3804
1.00	.00	418.03333	206.91470	.257	-244.5804	1080.6470
	2.00	787.03333*	206.91470	.022	124.4196	1449.6470
	3.00	161.80000	206.91470	.861	-500.8137	824.4137
2.00	.00	-369.00000	206.91470	.347	-1031.6137	293.6137
	1.00	-787.03333*	206.91470	.022	-1449.6470	-124.4196
	3.00	-625.23333	206.91470	.065	-1287.8470	37.3804
3.00	.00	256.23333	206.91470	.622	-406.3804	918.8470
	1.00	-161.80000	206.91470	.861	-824.4137	500.8137
	2.00	625.23333	206.91470	.065	-37.3804	1287.8470

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Maxcell

Tukey HSD^a

Group	N	Subset for alpha = 0.05	
		1	2
2.00	3	414.3667	
.00	3	783.3667	783.3667
3.00	3	1039.6000	1039.6000
1.00	3		1201.4000
Sig.		.065	.257

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Biomass productivity

ONEWAY Product BY Group

/MISSING ANALYSIS

/POSTHOC=TUKEY ALPHA(0.05) .

Oneway

ANOVA

Product

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2343.048	3	781.016	2.128	.175
Within Groups	2936.000	8	367.000		
Total	5279.048	11			

Post Hoc Tests



Multiple Comparisons

Dependent Variable: Product

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	1.00	-19.76000	15.64183	.608	-69.8506	30.3306
	2.00	14.24333	15.64183	.800	-35.8473	64.3340
	3.00	-18.08000	15.64183	.668	-68.1706	32.0106
1.00	.00	19.76000	15.64183	.608	-30.3306	69.8506
	2.00	34.00333	15.64183	.210	-16.0873	84.0940
	3.00	1.68000	15.64183	1.000	-48.4106	51.7706
2.00	.00	-14.24333	15.64183	.800	-64.3340	35.8473
	1.00	-34.00333	15.64183	.210	-84.0940	16.0873
	3.00	-32.32333	15.64183	.242	-82.4140	17.7673
3.00	.00	18.08000	15.64183	.668	-32.0106	68.1706
	1.00	-1.68000	15.64183	1.000	-51.7706	48.4106
	2.00	32.32333	15.64183	.242	-17.7673	82.4140

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Homogeneous Subsets

Product

Tukey HSD^a

Group	N	Subset for alpha =
		0.05
		1
2.00	3	75.9200
.00	3	90.1633
3.00	3	108.2433
1.00	3	109.9233
Sig.		.210

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Maximum biomass

ONEWAY Maxbiomass BY Group
 /MISSING ANALYSIS
 /POSTHOC=TUKEY ALPHA(0.05) .

Oneway

ANOVA

Maxbiomass

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	152153.794	3	50717.931	2.359	.148
Within Groups	172019.426	8	21502.428		
Total	324173.220	11			

Post Hoc Tests



Multiple Comparisons

Dependent Variable: Maxbiomass

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	1.00	-165.55667	119.72866	.542	-548.9700	217.8566
	2.00	119.99667	119.72866	.753	-263.4166	503.4100
	3.00	-128.33333	119.72866	.715	-511.7466	255.0800
1.00	.00	165.55667	119.72866	.542	-217.8566	548.9700
	2.00	285.55333	119.72866	.158	-97.8600	668.9666
	3.00	37.22333	119.72866	.989	-346.1900	420.6366
2.00	.00	-119.99667	119.72866	.753	-503.4100	263.4166
	1.00	-285.55333	119.72866	.158	-668.9666	97.8600
	3.00	-248.33000	119.72866	.240	-631.7433	135.0833
3.00	.00	128.33333	119.72866	.715	-255.0800	511.7466
	1.00	-37.22333	119.72866	.989	-420.6366	346.1900
	2.00	248.33000	119.72866	.240	-135.0833	631.7433

Homogeneous Subsets

Maxbiomass

Tukey HSD^a

Group	N	Subset for alpha =
		0.05
		1
2.00	3	630.0033
.00	3	750.0000
3.00	3	878.3333
1.00	3	915.5567
Sig.		.158

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Chlorophyll content

ONEWAY chlo_a BY Group
 /MISSING ANALYSIS
 /POSTHOC=TUKEY ALPHA(0.05) .

Oneway

ANOVA

chlo_a

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	25.082	3	8.361	1.485	.291
Within Groups	45.051	8	5.631		
Total	70.133	11			

Post Hoc Tests



Multiple Comparisons

Dependent Variable: chlo_a

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	1.00	.68391	1.93758	.984	-5.5209	6.8887
	2.00	-1.79377	1.93758	.792	-7.9986	4.4110
	3.00	-2.97405	1.93758	.462	-9.1789	3.2308
1.00	.00	-.68391	1.93758	.984	-6.8887	5.5209
	2.00	-2.47768	1.93758	.600	-8.6825	3.7271
	3.00	-3.65796	1.93758	.305	-9.8628	2.5469
2.00	.00	1.79377	1.93758	.792	-4.4110	7.9986
	1.00	2.47768	1.93758	.600	-3.7271	8.6825
	3.00	-1.18028	1.93758	.926	-7.3851	5.0245
3.00	.00	2.97405	1.93758	.462	-3.2308	9.1789
	1.00	3.65796	1.93758	.305	-2.5469	9.8628
	2.00	1.18028	1.93758	.926	-5.0245	7.3851

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Homogeneous Subsets

chlo_a

Tukey HSD^a

Group	N	Subset for alpha =
		0.05
		1
1.00	3	3.5696
.00	3	4.2535
2.00	3	6.0473
3.00	3	7.2276
Sig.		.305

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Total carotenoid content

ONEWAY caro BY Group
 /MISSING ANALYSIS
 /POSTHOC=TUKEY ALPHA(0.05) .

Oneway

ANOVA

caro

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.007	3	.336	1.798	.225
Within Groups	1.493	8	.187		
Total	2.500	11			

Post Hoc Tests



Multiple Comparisons

Dependent Variable: caro

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	1.00	.00839	.35274	1.000	-1.1212	1.1380
	2.00	.17021	.35274	.961	-.9594	1.2998
	3.00	-.59081	.35274	.394	-1.7204	.5388
1.00	.00	-.00839	.35274	1.000	-1.1380	1.1212
	2.00	.16182	.35274	.966	-.9678	1.2914
	3.00	-.59920	.35274	.384	-1.7288	.5304
2.00	.00	-.17021	.35274	.961	-1.2998	.9594
	1.00	-.16182	.35274	.966	-1.2914	.9678
	3.00	-.76102	.35274	.215	-1.8906	.3686
3.00	.00	.59081	.35274	.394	-.5388	1.7204
	1.00	.59920	.35274	.384	-.5304	1.7288
	2.00	.76102	.35274	.215	-.3686	1.8906

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Homogeneous Subsets

caro

Tukey HSD^a

Group	N	Subset for alpha =
		0.05
		1
2.00	3	1.4057
1.00	3	1.5675
.00	3	1.5759
3.00	3	2.1667
Sig.		.215

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Lutein content

ONEWAY lutein BY Group
 /MISSING ANALYSIS
 /POSTHOC=TUKEY ALPHA(0.05) .

Oneway

ANOVA

lutein

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.156	3	.052	3.138	.087
Within Groups	.133	8	.017		
Total	.289	11			

Post Hoc Tests



Multiple Comparisons

Dependent Variable: lutein

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	1.00	.15000	.10525	.519	-.1871	.4871
	2.00	.14333	.10525	.554	-.1937	.4804
	3.00	-.12667	.10525	.642	-.4637	.2104
1.00	.00	-.15000	.10525	.519	-.4871	.1871
	2.00	-.00667	.10525	1.000	-.3437	.3304
	3.00	-.27667	.10525	.112	-.6137	.0604
2.00	.00	-.14333	.10525	.554	-.4804	.1937
	1.00	.00667	.10525	1.000	-.3304	.3437
	3.00	-.27000	.10525	.123	-.6071	.0671
3.00	.00	.12667	.10525	.642	-.2104	.4637
	1.00	.27667	.10525	.112	-.0604	.6137
	2.00	.27000	.10525	.123	-.0671	.6071

Homogeneous Subsets

lutein

Tukey HSD^a

Group	N	Subset for alpha =
		0.05
		1
1.00	3	.6633
2.00	3	.6700
.00	3	.8133
3.00	3	.9400
Sig.		.112

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

G3. Statistical analysis in “Effect of fish density on carbon dioxide production and utilization of carbon dioxide and nitrate from recirculating aquaculture system by microalgae”

Carbon dioxide concentration of fish tank and solid separating inlet air

ONEWAY CO22 BY G2
 /MISSING ANALYSIS
 /POSTHOC=TUKEY ALPHA(0.05) .

Oneway



CO22

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7416.780	2	3708.390	3.288	.057
Within Groups	23681.775	21	1127.704		
Total	31098.556	23			



Carbon dioxide concentration of fish tank outlet air

ONEWAY outletFU BY group
 /MISSING ANALYSIS
 /POSTHOC=TUKEY ALPHA(0.05) .

Oneway

ANOVA

outletFU

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	58355227.276	2	29177613.638	431.212	.000
Within Groups	37621261.563	556	67664.140		
Total	95976488.839	558			

Post Hoc Tests



Multiple Comparisons

Dependent Variable: outletFU

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
3.00	5.00	-107.66205 [*]	26.93822	.000	-170.9670	-44.3571
	10.00	-732.71961 [*]	26.90170	.000	-795.9387	-669.5005
5.00	3.00	107.66205 [*]	26.93822	.000	44.3571	170.9670
	10.00	-625.05756 [*]	27.00996	.000	-688.5311	-561.5840
10.00	3.00	732.71961 [*]	26.90170	.000	669.5005	795.9387
	5.00	625.05756 [*]	27.00996	.000	561.5840	688.5311

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

outletFU

Tukey HSD^{a,b}

group	N	Subset for alpha = 0.05		
		1	2	3
3.00	188	880.7997		
5.00	185		988.4617	
10.00	186			1613.5193
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 186.325.

b. The group sizes are unequal. The harmonic mean of the group sizes is used.

Type I error levels are not guaranteed.

Carbon dioxide concentration of Solid separating outlet air

ONEWAY CO2SU BY G2
 /MISSING ANALYSIS
 /POSTHOC=TUKEY ALPHA(0.05) .

Oneway

ANOVA

CO2SU

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	157604.122	2	78802.061	19.844	.000
Within Groups	83392.820	21	3971.087		
Total	240996.941	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: CO2SU

Tukey HSD

(I) G2	(J) G2	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
3.00	5.00	-101.48500 [*]	31.50828	.011	-180.9038	-22.0662
	10.00	-198.48000 [*]	31.50828	.000	-277.8988	-119.0612
5.00	3.00	101.48500 [*]	31.50828	.011	22.0662	180.9038
	10.00	-96.99500 [*]	31.50828	.015	-176.4138	-17.5762
10.00	3.00	198.48000 [*]	31.50828	.000	119.0612	277.8988
	5.00	96.99500 [*]	31.50828	.015	17.5762	176.4138

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

CO2SU

Tukey HSD^a

G2	N	Subset for alpha = 0.05		
		1	2	3
3.00	8	710.0988		
5.00	8		811.5838	
10.00	8			908.5788
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

Rate of CO₂ production from fish tank

```

ONEWAY rCO2 BY group
  /MISSING ANALYSIS
  /POSTHOC=TUKEY ALPHA(0.05) .

```

Oneway

ANOVA

rCO2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9368.243	2	4684.122	.360	.702
Within Groups	272891.239	21	12994.821		
Total	282259.482	23			



Specific growth rate of *S. armatus* at fish density 3 kg/m³

T-TEST GROUPS=Group (0 1)
 /MISSING=ANALYSIS
 /VARIABLES=SGR
 /CRITERIA=CI (.95) .

T-Test

Group Statistics

Group	N	Mean	Std. Deviation	Std. Error Mean
SGR .00	3	1.1400	.16371	.09452
1.00	3	.8900	.13077	.07550

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
SGR	Equal variances assumed	.164	.706	2.067	4
	Equal variances not assumed			2.067	3.814

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference
					Lower
SGR	Equal variances assumed	.108	.25000	.12097	-.08586
	Equal variances not assumed	.111	.25000	.12097	-.09243

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Upper	
SGR	Equal variances assumed	.58586	
	Equal variances not assumed	.59243	

Specific growth rate of *S. armatus* at fish density 5 kg/m³

T-TEST GROUPS=Group(0 1)
 /MISSING=ANALYSIS
 /VARIABLES=SGR
 /CRITERIA=CI(.95).

T-Test

Group Statistics

Group	N	Mean	Std. Deviation	Std. Error Mean
SGR .00	3	1.3600	.07000	.04041
SGR 1.00	3	1.3333	.13650	.07881

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
SGR	Equal variances assumed	1.203	.334	.301	4
	Equal variances not assumed			.301	2.984

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference
					Lower
SGR	Equal variances assumed	.778	.02667	.08857	-.21924
	Equal variances not assumed	.783	.02667	.08857	-.25606

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Upper	
SGR	Equal variances assumed	.27257	
	Equal variances not assumed	.30940	

Specific growth rate of *S. armatus* at fish density 10 kg/m³

```
T-TEST GROUPS=Group(0 1)
/MISSING=ANALYSIS
/VARIABLES=SGR
/CRITERIA=CI(.95).
```

T-Test

Group Statistics

Group	N	Mean	Std. Deviation	Std. Error Mean
SGR .00	3	1.6600	.14107	.08145
SGR 1.00	3	1.8300	.12166	.07024

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means	
	F	Sig.	t	df
SGR Equal variances assumed	.020	.894	-1.581	4
SGR Equal variances not assumed			-1.581	3.915

Independent Samples Test

	t-test for Equality of Means			
	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference
				Lower
SGR Equal variances assumed	.189	-.17000	.10755	-.46860
SGR Equal variances not assumed	.191	-.17000	.10755	-.47116

Independent Samples Test

	t-test for Equality of Means	
	95% Confidence Interval of the Difference	
	Upper	
SGR Equal variances assumed	.12860	
SGR Equal variances not assumed	.13116	

Maximum cell number density of *S. armatus* at fish density 3 kg/m³

```
T-TEST GROUPS=Group(0 1)
/MISSING=ANALYSIS
/VARIABLES=Maxcell
/CRITERIA=CI(.95).
```

T-Test

Group Statistics

Group	N	Mean	Std. Deviation	Std. Error Mean
Maxcell .00	3	231.9467	34.70561	20.03730
1.00	3	235.5533	102.24617	59.03185

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Maxcell	Equal variances assumed	4.355	.105	-.058	4
	Equal variances not assumed			-.058	2.455

Independent Samples Test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
Maxcell	Equal variances assumed	.957	-3.60667	62.33982
	Equal variances not assumed	.958	-3.60667	62.33982

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Maxcell	Equal variances assumed	-176.68975	169.47642
	Equal variances not assumed	-229.41202	222.19869

Maximum cell number density of *S. armatus* at fish density 5 kg/m³

T-TEST GROUPS=Group (0 1)
 /MISSING=ANALYSIS
 /VARIABLES=Maxcell
 /CRITERIA=CI (.95) .

T-Test

Group Statistics

Group	N	Mean	Std. Deviation	Std. Error Mean
Maxcell .00	3	371.7600	114.69909	66.22155
1.00	3	441.6667	115.22905	66.52752

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Maxcell	Equal variances assumed	.000	.993	-.745	4
	Equal variances not assumed			-.745	4.000

Independent Samples Test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
Maxcell	Equal variances assumed	.498	-69.90667	93.86802
	Equal variances not assumed	.498	-69.90667	93.86802

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Maxcell	Equal variances assumed	-330.52607	190.71273
	Equal variances not assumed	-330.52825	190.71492

Maximum cell number density of *S. armatus* at fish density 10 kg/m³

T-TEST GROUPS=Group(0 1)

/MISSING=ANALYSIS

/VARIABLES=Maxcell

/CRITERIA=CI(.95).

T-Test

Group Statistics

Group	N	Mean	Std. Deviation	Std. Error Mean
Maxcell .00	3	272.2233	47.46281	27.40267
1.00	3	506.9433	81.50080	47.05451

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Maxcell	Equal variances assumed	1.550	.281	-4.311	4
	Equal variances not assumed			-4.311	3.217

Independent Samples Test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
Maxcell	Equal variances assumed	.013	-234.72000	54.45211
	Equal variances not assumed	.020	-234.72000	54.45211

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Maxcell	Equal variances assumed	-385.90331	-83.53669
	Equal variances not assumed	-401.59190	-67.84810

Maximum biomass of *S. armatus* at fish density 3 kg/m³

T-TEST GROUPS=Group(0 1)
 /MISSING=ANALYSIS
 /VARIABLES=Maxbiomass
 /CRITERIA=CI(.95).

T-Test

Group Statistics

Group	N	Mean	Std. Deviation	Std. Error Mean
Maxbiomass .00	3	219.2567	27.60812	15.93955
1.00	3	237.7800	13.35726	7.71182

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Maxbiomass	Equal variances assumed	3.165	.150	-1.046	4
	Equal variances not assumed			-1.046	2.888

Independent Samples Test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
Maxbiomass	Equal variances assumed	.355	-18.52333	17.70710
	Equal variances not assumed	.375	-18.52333	17.70710

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Maxbiomass	Equal variances assumed	-67.68613	30.63947
	Equal variances not assumed	-76.13582	39.08915

Maximum biomass of *S. armatus* at fish density 5 kg/m³

```
T-TEST GROUPS=Group(0 1)
/MISSING=ANALYSIS
/VARIABLES=Maxbiomass
/CRITERIA=CI(.95).
```

T-Test

Group Statistics

Group	N	Mean	Std. Deviation	Std. Error Mean
Maxbiomass .00	3	349.4467	41.70947	24.08097
1.00	3	353.8900	83.68630	48.31631

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Maxbiomass	Equal variances assumed	1.759	.255	-.082	4
	Equal variances not assumed			-.082	2.936

Independent Samples Test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
Maxbiomass	Equal variances assumed	.938	-4.44333	53.98480
	Equal variances not assumed	.940	-4.44333	53.98480

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Maxbiomass	Equal variances assumed	-154.32917	145.44250
	Equal variances not assumed	-178.38966	169.50299

Maximum biomass of *S. armatus* at fish density 10 kg/m³

```
T-TEST GROUPS=Group(0 1)
/MISSING=ANALYSIS
/VARIABLES=Maxbiomass
/CRITERIA=CI(.95).
```

T-Test

Group Statistics

Group	N	Mean	Std. Deviation	Std. Error Mean
Maxbiomass .00	3	234.0733	54.62974	31.54049
1.00	3	409.6300	22.25694	12.85005

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Maxbiomass	Equal variances assumed	4.396	.104	-5.155	4
	Equal variances not assumed			-5.155	2.646

Independent Samples Test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
Maxbiomass	Equal variances assumed	.007	-175.55667	34.05769
	Equal variances not assumed	.019	-175.55667	34.05769

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Maxbiomass	Equal variances assumed	-270.11598	-80.99735
	Equal variances not assumed	-292.62279	-58.49055

Productivity of *S. armatus* at fish density 3 kg/m³

T-TEST GROUPS=Group (0 1)
 /MISSING=ANALYSIS
 /VARIABLES=Product
 /CRITERIA=CI (.95) .

T-Test

Group Statistics

Group	N	Mean	Std. Deviation	Std. Error Mean
Product .00	3	38.9233	9.22162	5.32410
1.00	3	43.8900	7.89270	4.55685

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Product	Equal variances assumed	.144	.723	-.709	4
	Equal variances not assumed			-.709	3.907

Independent Samples Test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
Product	Equal variances assumed	.518	-4.96667	7.00792
	Equal variances not assumed	.518	-4.96667	7.00792

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Product	Equal variances assumed	-24.42378	14.49045
	Equal variances not assumed	-24.60801	14.67467

Productivity of *S. armatus* at fish density 5 kg/m³

T-TEST GROUPS=Group (0 1)

/MISSING=ANALYSIS

/VARIABLES=Product

/CRITERIA=CI (.95) .

T-Test

Group Statistics

Group	N	Mean	Std. Deviation	Std. Error Mean
Product .00	3	41.2833	7.26133	4.19233
1.00	3	40.6833	11.50168	6.64050

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Product	Equal variances assumed	1.256	.325	.076	4
	Equal variances not assumed			.076	3.376

Independent Samples Test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
Product	Equal variances assumed	.943	.60000	7.85314
	Equal variances not assumed	.943	.60000	7.85314

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Product	Equal variances assumed	-21.20382	22.40382
	Equal variances not assumed	-22.88985	24.08985

Productivity of *S. armatus* at fish density 10 kg/m³

T-TEST GROUPS=Group (0 1)
 /MISSING=ANALYSIS
 /VARIABLES=Product
 /CRITERIA=CI (.95) .

T-Test

Group Statistics

Group	N	Mean	Std. Deviation	Std. Error Mean
Product .00	3	46.3933	16.70219	9.64302
1.00	3	95.4000	7.91260	4.56834

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Product	Equal variances assumed	2.095	.221	-4.593	4
	Equal variances not assumed			-4.593	2.855

Independent Samples Test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
Product	Equal variances assumed	.010	-49.00667	10.67040
	Equal variances not assumed	.022	-49.00667	10.67040

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Product	Equal variances assumed	-78.63245	-19.38088
	Equal variances not assumed	-83.96378	-14.04956

Amount of removed nitrate in *S. armatus* at fish density 3 kg/m³

T-TEST GROUPS=Group (0 1)
 /MISSING=ANALYSIS
 /VARIABLES=RemoveNO3
 /CRITERIA=CI (.95) .

T-Test

Group Statistics

Group	N	Mean	Std. Deviation	Std. Error Mean
RemoveNO3 .00	3	13.4267	4.83026	2.78875
1.00	3	19.0167	3.18500	1.83886



Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
RemoveNO3	Equal variances assumed	.702	.449	-1.673	4
	Equal variances not assumed			-1.673	3.463

Independent Samples Test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
RemoveNO3	Equal variances assumed	.170	-5.59000	3.34044
	Equal variances not assumed	.181	-5.59000	3.34044

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
RemoveNO3	Equal variances assumed	-14.86456	3.68456
	Equal variances not assumed	-15.46070	4.28070

Amount of removed nitrate in *S. armatus* at fish density 5 kg/m³

T-TEST GROUPS=Group (0 1)
 /MISSING=ANALYSIS
 /VARIABLES=RemoveNO3
 /CRITERIA=CI (.95) .

T-Test

Group Statistics

Group	N	Mean	Std. Deviation	Std. Error Mean
RemoveNO3 .00	3	30.0933	.93039	.53716
1.00	3	28.0233	2.34413	1.35338

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
RemoveNO3	Equal variances assumed	5.020	.089	1.422	4
	Equal variances not assumed			1.422	2.615

Independent Samples Test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
RemoveNO3	Equal variances assumed	.228	2.07000	1.45609
	Equal variances not assumed	.263	2.07000	1.45609

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
RemoveNO3	Equal variances assumed	-1.97275	6.11275
	Equal variances not assumed	-2.97500	7.11500

Amount of removed nitrate in *S. armatus* at fish density 10 kg/m³

T-TEST GROUPS=Group (0 1)
 /MISSING=ANALYSIS
 /VARIABLES=RemoveNO3
 /CRITERIA=CI (.95) .

T-Test

Group Statistics

Group	N	Mean	Std. Deviation	Std. Error Mean
RemoveNO3 .00	3	17.3033	7.48086	4.31908
1.00	3	34.4400	1.82748	1.05510

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
RemoveNO3	Equal variances assumed	8.426	.044	-3.854	4
	Equal variances not assumed			-3.854	2.238

Independent Samples Test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
RemoveNO3	Equal variances assumed	.018	-17.13667	4.44609
	Equal variances not assumed	.051	-17.13667	4.44609

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
RemoveNO3	Equal variances assumed	-29.48098	-4.79235
	Equal variances not assumed	-34.44436	.17102

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