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Article

Process Simulation and Techno-Economic Analysis of *Wolffia*-Integrated Recirculating Aquaculture Systems for Nutrient Recovery and CO₂ Utilization

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Abstract

Recirculating aquaculture systems (RAS) enable efficient fish production but generate nutrient-rich effluents requiring sustainable management. Integrating aquatic biomass cultivation offers a circular approach for nutrient recovery, CO₂ utilization, and biomass production. This study presents a process simulation and techno-economic analysis (TEA) of a pilot-scale *Wolffia globosa*-RAS system in Thailand, comparing static and well-mixed suspended aeration cultivation. Within the same system boundary, the suspended configuration required only 5.90 m² for 28.00 m³, whereas static cultivation required 131 m² for 32.80 m³, corresponding to a 19-fold improvement in land-use efficiency (0.21 vs. 4.00 m² m⁻³). Higher annual biomass production was achieved in the suspended system (1056 kg dry weight (DW) yr⁻¹) compared with the static system (690 kg DW yr⁻¹), corresponding to CO₂ fixation of ~1.50 and ~0.98 t CO₂ yr⁻¹, respectively. The static system achieved higher nutrient removal efficiencies (97% N and ~100% P), while the suspended system showed lower removal (64% N and 65.3% P) but higher productivity. Economic analysis confirmed feasibility, with the suspended configuration showing superior performance, achieving higher return on investment (17.56% vs. 12.35%) and a shorter payback period (5.70 vs. 7.76 years). These results demonstrate the potential of RAS-*Wolffia* integration for sustainable aquaculture and resource recovery.

Keywords: *Wolffia* biomass; recirculating aquaculture systems; aquaculture effluent; nutrient recovery; CO₂ capture; techno-economic analysis

1. Introduction

Aquaculture is the fastest-growing food production sector and plays a crucial role in meeting the increasing global demand for sustainable food and bio-based resources [1]. Global fisheries and aquaculture production reached 223.20 million tons in 2022, with aquaculture contributing 130.90 million tons, surpassing capture fisheries for the first time [1]. However, this rapid expansion presents significant sustainability challenges, particularly regarding the discharge of nutrient-rich

effluents and the intensive use of limited fishery resources for feed production [2]. These issues pose risks to aquatic ecosystems and hinder progress toward the United Nations Sustainable Development Goals related to food security, environmental protection, and sustainable production systems [3].

These effluents typically contain elevated nitrogen, phosphorus, carbon, and total suspended solids (TSS). Recirculating aquaculture systems (RAS) improve water-use efficiency by converting toxic nitrogen species such as ammonium and nitrite through biological nitrification [4]. However, nitrification produces nitrate, which remains biologically available and can still contribute to eutrophication, while phosphorus is not removed through nitrification-denitrification pathways and may accumulate unless specifically assimilated or removed [5]. Therefore, additional strategies are required to recover nutrients and reduce environmental discharge from RAS operations.

In this context, integrating photosynthetic organisms such as microalgae into RAS offers an alternative approach by enabling the direct assimilation of nitrogen and phosphorus into biomass, thereby coupling nutrient removal with resource recovery and valorization [6,7]. This approach not only mitigates nutrient discharge but also enhances system sustainability through biological CO₂ capture and concurrent oxygen release [8]. In addition, microalgal biomass represents a valuable source of pigments, proteins, and lipids. However, large-scale outdoor microalgae cultivation in photobioreactors (PBRs) still faces technical and economic barriers, including strain selection, reactor optimization, high harvesting costs, and complex downstream processing [1,9].

Furthermore, CO₂ generated within RAS through fish respiration and nitrifying biofilter activity constitutes a major internal waste source, accounting for approximately 30-60% of the total carbon input from aquafeeds [6]. In addition, incomplete denitrification in conventional biofilters can release nitrous oxide (N₂O), a greenhouse gas with a global warming potential nearly 300 times greater than CO₂, thereby further increasing the environmental footprint of RAS operations [10]. With the continued expansion of aquaculture production, substantial amounts of nitrogen, phosphorus and carbon are discharged into the environment as nitrate, nitrogen gases, and CO₂ without resource recovery, highlighting the urgent need for integrated strategies that enable simultaneous nutrient removal, carbon utilization, and valorization [11].

Among emerging solutions, aquatic plants such as *Wolffia* have attracted growing attention as potential alternatives to microalgae for nutrient (N/P) removal and biological CO₂ capture in integrated aquaculture systems [12–15]. Duckweeds (Lemnaceae) include several genera with distinct morphological characteristics, such as *Lemna*, *Spirodela*, *Landoltia*, *Wolffiella* and *Wolffia*. While commonly known duckweeds form relatively large, leaf-like fronds that float on the water surface, *Wolffia* represents a morphologically distinct group characterized by extremely small, rootless fronds that do not form extended surface mats. This difference in structure leads to fundamentally different cultivation and harvesting behavior compared with larger duckweed species [16]. *Wolffia* can be harvested within 2-3 weeks, a cultivation period comparable to that of microalgae, yet it exhibits substantially higher biomass productivity, reaching 86-159 g wet weight m⁻².day⁻¹ (3.40-10 g dry weight (DW) m⁻² d⁻¹), which is approximately 12-24 times greater than that of conventional aquaponic crops such as spinach or lettuce [17]. Because *Wolffia* grows as small, visible fronds rather than microscopic cells, it can be harvested easily and with low energy input, eliminating the need for costly biomass separation processes. In addition, its dry biomass contains 34-45% protein with a complete essential amino acid profile, highlighting its potential as a high-value nutritional resource [18].

Like microalgae, *Wolffia* assimilates nitrogen and phosphorus, fixes CO₂ through photosynthesis, and releases oxygen, while enabling resource recovery from aquaculture-derived nutrient loads. Similar microalgae and terrestrial plants, *Wolffia* utilizes CO₂ for growth via photosynthesis, underscoring its potential for direct CO₂ capture from both atmospheric and dissolved sources in aquaculture systems [14].

The contribution of *Wolffia* cultivation to aquaculture and the green bioeconomy depends on sustainable production strategies that balance environmental protection, resource efficiency, and economic feasibility. In this context, *Wolffia* cultivation supports circular bioeconomy principles by

enabling nutrient recycling, CO₂ capture, and low-waste biomass generation [19]. Sustainable *Wolffia* cultivation also involves using alternative nutrient sources, such as aquaculture effluents or other wastewater streams, which substantially reduce the need for synthetic fertilizers and lower production costs. Several studies have demonstrated the potential of *Wolffia* species for nutrient recovery from wastewater and aquaculture effluents. Said et al. reported the phytoremediation capacity of *Wolffia arrhiza* cultivated in tilapia aquaculture effluent, achieving efficient nutrient uptake while producing biomass suitable for feed reuse [13]. Similarly, Yongyod and Kamolrat investigated the growth performance and nutrient removal efficiency of *Wolffia globosa* under different nitrogen-to-phosphorus ratios, showing that balanced nutrient conditions promoted higher biomass productivity and specific growth rates, while nitrogen rich conditions enhanced protein accumulation and phosphorus uptake [20]. These findings demonstrate the capacity of *Wolffia* to simultaneously produce valuable biomass and remove nutrients from aquatic systems.

Beyond its application for wastewater treatment and feed production, *Wolffia* has additional high value uses. It is traditionally consumed as food in several Southeast Asian countries [21]. In addition to serving as a nutritious human food rich in protein, vitamins, and essential amino acids, *Wolffia* biomass can also be utilized as a sustainable source of natural chlorophyll [21]. Chlorophyll is a high-value bioactive compound widely used as a natural colorant, antioxidant, and nutraceutical ingredient. In addition to chlorophyll, *Wolffia* sp. is rich in other bioactive metabolites such as carotenoids (lutein, β -carotene, zeaxanthin), flavonoids (quercetin, kaempferol, luteolin, apigenin), phenolic acids (gallic, chlorogenic, and caffeic acids), and vitamin C, all of which contribute to its potent antioxidant and anti-inflammatory activities [16,18,22]. The recovery of these bioactive compounds from *Wolffia* biomass provides multiple valorization pathways that enhance the economic viability of integrated aquaculture systems. Moreover, replacing conventional culture media with nutrient-rich wastewater can decrease the cost of pigment and chlorophyll production by up to 40-60% compared with standard synthetic formulations, making integrated systems both environmentally and economically advantageous [19].

Conventional *Wolffia* cultivation is typically carried out in static systems, where *Wolffia* remains confined to the water surface under quiescent hydrodynamic conditions. While simple and widely practiced, this approach often limits nutrient diffusion, gas exchange, and light distribution, thereby reducing photosynthetic efficiency and biomass productivity [23,24]. It is also associated with operational issues such as inconsistent yields, environmental fluctuations, and hygiene concerns [25]. To address these challenges, a well-mixed, suspended-growth system, where *Wolffia* is maintained in suspension submerged and dispersed throughout the culture medium via continuous aeration, has been proposed to improve the mass transfer of nutrients and gases and enhance light exposure to boost growth rates [18]. Building on this concept, Sucunthowong et al. systematically evaluated well-mixed, suspended-growth cultivation of *Wolffia globosa* using continuous aeration and demonstrated that aerated suspension significantly enhanced biomass productivity (up to ~ 200 mg L⁻¹ d⁻¹) and CO₂ fixation rates (~ 280 mg CO₂ L⁻¹ d⁻¹) compared with static-water systems, primarily due to improved nutrient and gas mass transfer and more uniform light exposure [18].

Comparative assessment of static and suspended-growth *Wolffia* cultivation is therefore essential to identify the most effective approach for integrating *Wolffia* into nutrient recovery and biomass valorization processes within RAS. However, evaluating cultivation performance alone does not provide a complete picture of the feasibility of these systems. To date, the majority of *Wolffia* cultivation studies have been conducted at the laboratory scale, with limited consideration of techno-economic analysis (TEA) or large-scale feasibility [18,26,27]. Therefore, a comprehensive TEA is required to assess the trade-offs between productivity, capital investment, operational costs, and potential revenue.

Previous TEAs have mainly focused on microalgae rather than aquatic macrophytes. Vázquez-Romero et al. evaluated the feasibility of microalgae cultivation in high-rate algal ponds using RAS effluents, demonstrating high nutrient removal but limited economic viability due to harvesting costs [28]. Zappernick et al. evaluated the profitability of a commercial-scale tilapia-lettuce aquaponics

system in the United States using TEA, highlighting the importance of co-product valorization and optimized system design [29]. Valdovinos-García et al. performed a TEA of *Chlorella vulgaris* for CO₂ capture in raceway ponds, underscoring the benefits of coupling algal growth with industrial carbon sources [30]. Ferreira et al. modeled chlorophyll extraction from microalgae using methanol, identifying major cost contributors and efficiency constraints through process simulation in SuperPro Designer [31]. Despite the growing body of experimental studies examining *Wolffia* growth performance, nutrient uptake, and biomass composition under laboratory or pilot-scale conditions [13,18,20,32], the system-level economic feasibility of integrating *Wolffia* cultivation into RAS remains largely unexplored.

The present study develops a process simulation and TEA of an integrated *Wolffia* (*Wolffia globosa*) cultivation with RAS (RAS-*Wolffia*) designed to recover nutrients and utilize CO₂ from aquaculture effluents while producing valuable biomass products. To the best of our knowledge, no previous study has conducted a comprehensive TEA evaluating RAS-*Wolffia* for simultaneous nutrient recovery, carbon utilization, and biomass valorization. This work therefore provides one of the first system-level economic evaluations of a *Wolffia*-based circular aquaculture platform. The analysis investigates the feasibility of producing approximately one ton of dried *Wolffia* biomass annually by comparing two cultivation configurations: conventional static-water systems and aerated suspended-growth cultivation. The two scenarios are evaluated in terms of biomass productivity, resource recovery performance, capital and operating costs, and economic indicators including profitability and investment return. By quantifying both technical and economic performance, this study provides critical insights into the potential of integrating *Wolffia* cultivation within RAS infrastructure to support circular bioeconomy strategies and more sustainable aquaculture production systems.

2. Process Simulation and Techno-Economic Modeling

2.1. System Description and Modeling Framework

Process simulation and TEA were performed in SuperPro Designer v10® to model an integrated bioprocess chain, comprising: (i) a RAS including fish culture tanks for tilapia cultivation and downstream solids removal and nitrification units for effluent conditioning; (ii) nutrient conversion and CO₂ recycling; (iii) *Wolffia* cultivation; and (iv) downstream biomass recovery and processing. The upstream RAS and CO₂ recycling loop were simulated as steady-state continuous operations, while *Wolffia* cultivation and downstream processing were modeled as batch or semi-continuous processes to represent cyclic harvesting. Such hybrid continuous-batch configurations are common in aquaculture-plant integration and nutrient recovery microalgae processes, where continuously generated nutrient streams feed cultivation stages operating on periodic batch harvest cycles [29,33,34].

All unit operations, mass and energy balances, and economic calculations were based on experimental and literature data. The integrated RAS-*Wolffia* process for both scenarios is illustrated in Figure 1.

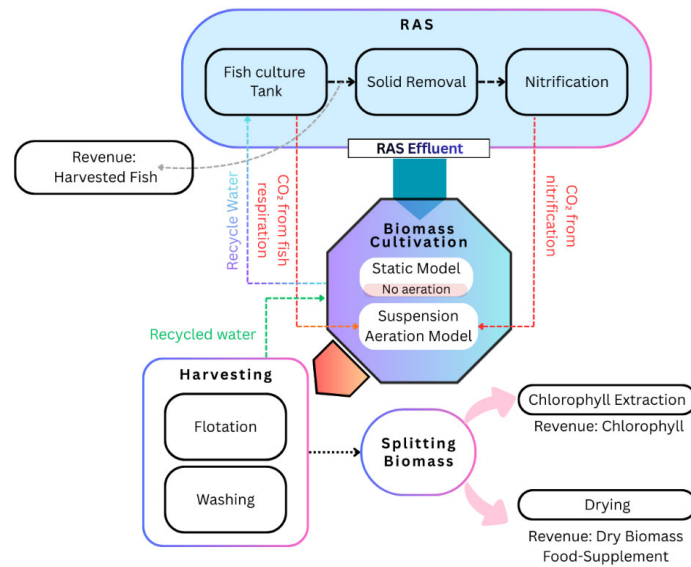


Figure 1. Schematic of the integrated RAS-*Wolffia* system showing fish production, nutrient recovery, CO₂ capture, and co-production of edible *Wolffia* biomass and chlorophyll.

Detailed SuperPro Designer process flow diagrams of the integrated RAS-*Wolffia* system for the static and suspended scenarios are shown in Figures 2 and 3, respectively.

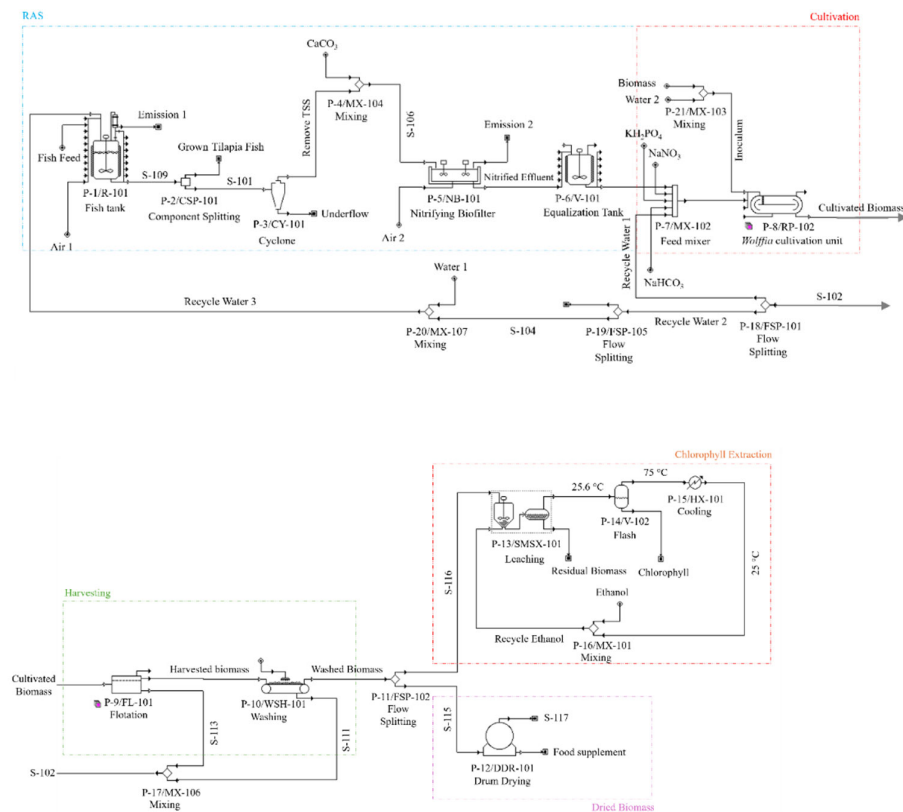


Figure 2. Process flow diagram of the integrated RAS-*Wolffia* system under the static cultivation scenario, developed using SuperPro Designer.

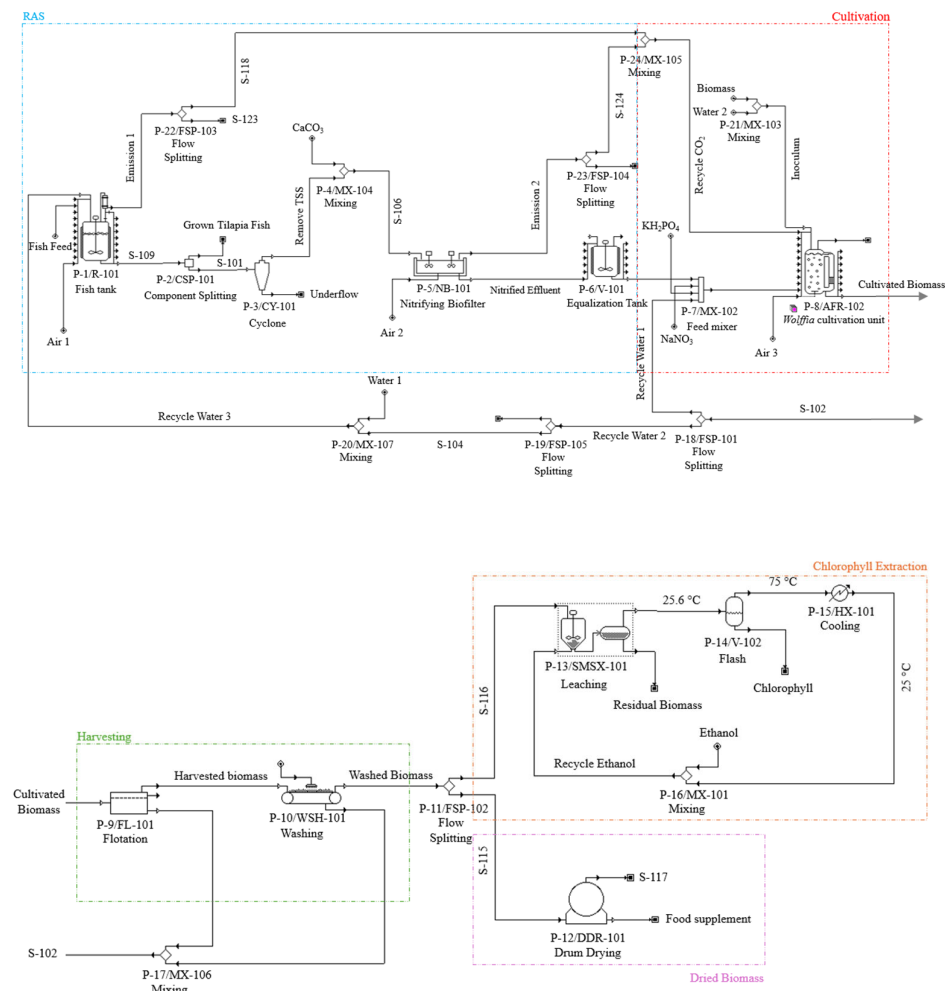


Figure 3. Process flow diagram of the integrated RAS-*Wolffia* system under the suspended cultivation scenario, developed using SuperPro Designer.

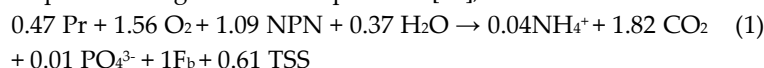
The simulation aimed to evaluate the techno-economic feasibility of co-producing three products, harvested tilapia biomass, edible *Wolffia* biomass for food-supplement applications, and chlorophyll extracts, while simultaneously achieving CO₂ capture and nutrient (N and P) removal from aquaculture effluent. The simulated facility was assumed to operate 330 days y⁻¹, with 35 days allocated for maintenance and cleaning, consistent with TEA practices in bioprocess industries. A 15-year project lifetime was selected to account for depreciation of major equipment (e.g., raceway ponds, PBRs, cyclones, dryers). The modeled production capacity was ~1 ton y⁻¹ of dry *Wolffia* biomass, representing of pilot-to-early commercial-scale systems reported in Thailand. The RAS-*Wolffia* process was assumed to be in Bangkok, Thailand. Key assumptions included constant fish stocking density, steady-state RAS operation, batch *Wolffia* cultivation cycles, and stoichiometric biomass formation based on literature-reported nutrient uptake ratios. These assumptions enable process-level comparison between static and suspended cultivation configurations under consistent upstream RAS conditions.

2.2. Fish Cultivation and RAS

Tilapia (*Oreochromis niloticus*) cultivation, waste conversion, solids removal, and nitrification were modeled based on typical RAS operation. Tilapia were stocked at 20 kg m⁻³ and fed daily at 3% of total body weight using a commercial diet containing ~30% protein [10], consistent with intensive

RAS conditions (20-40 kg m⁻³) [35,36], with a 6-8 month production cycle prior to harvest [29]. The fish-culture tank was simulated in SuperPro Designer as a continuous stirred-tank reactor (CSTR) to represent continuous feeding, respiration, ammonia excretion, CO₂ generation, and effluent discharge, with mixing provided by aeration rather than mechanical agitation [29], and the default CSTR capital cost was replaced with that of an aerated polyethylene aquaculture tank to represent realistic RAS infrastructure.

Dissolved oxygen (DO) was maintained above 5 mg L⁻¹ via continuous aeration. Fish metabolism, oxygen consumption, CO₂ production, ammonia release, and solid-waste generation can be modeled using stoichiometric mass balances based on established tilapia metabolic coefficients and nitrogen–phosphorus budgets for RAS operation [37], as described below:



where Pr represents feed protein nitrogen, NPN digestible carbohydrates and lipids, F_b fish biomass, and TSS total suspended solids. Stoichiometric coefficients are dimensionless and expressed on a mass basis normalized to 1 kg of fish biomass production.

Total Oxygen demand for tilapia metabolism was assumed to be 0.80-1.20 kg O₂ kg⁻¹ feed [37], with a respiratory quotient (RQ) of 0.85 [38]. Protein nitrogen was assumed to represent 16% of protein mass [31]. Approximately 30% of dietary nitrogen was retained in fish biomass, while ~70% was excreted, mainly as ammonia (~50%) via gill excretion and organic nitrogen (~20%) in feces and dissolved forms, consistent with reported nitrogen budgets for tilapia RAS [39].

The feed conversion ratio (FCR) was set to 1.80 kg feed kg⁻¹ fish growth, within the reported range for intensive tilapia RAS (1.40-2.40) [40,41]. Feed phosphorus was assumed to be 1% of feed mass (0.80-1.20% P in commercial aquafeeds) [42], with ~70% excreted as dissolved or particulate waste [35]. TSS, including uneaten feed, fish feces and microbial flocs, were estimated at 20-30% of feed input [37]. Harvested fish were assumed to be sold immediately, and therefore no cold-chain storage was included in the model.

Fish-tank effluent (S-106 in static scenario and S-109 in suspended scenario) was directed to a component splitter (P-2/CSP-101) to separate harvested tilapia biomass, followed by a hydrocyclone (HCY-101) for solids removal. Hydrocyclones are widely used in RASs to separate particulate matter such as uneaten feed, fish feces, and microbial flocs due to their compact design and continuous operation [29,37]. The unit was modeled based on throughput driven sizing, with centrifugal forces concentrating suspended solids in the underflow and clarified liquid existing via the overflow. Dissolved nutrients remained in the overflow and were directed to the nitrification unit, consistent with typical RAS solids-management practices [37,43].

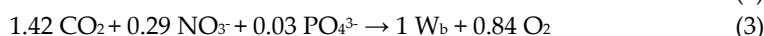
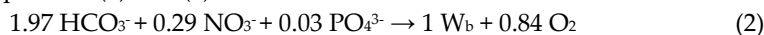
After solids removal, clarified effluent containing ammonium and phosphate was sent to the nitrification unit (NB-101). This reactor was modeled in SuperPro Designer as a well-mixed aerobic bio-oxidation reactor representing a generic nitrifying biofilter. Continuous aeration maintained DO >2 mg L⁻¹, enabling near-complete conversion of ammonium to nitrate under steady-state conditions [37]. Reactor sizing was based on established ammonia oxidation kinetics and typical hydraulic retention times for aquaculture nitrification systems [37,44]. Because nitrification generates acidity, CaCO₃ was added to maintain alkalinity within 100-300 mg L⁻¹ [18]. The unit was simulated using the aerobic bio-oxidation module but costed as a generic RAS nitrifying biofilter. The resulting nitrified effluent, containing mainly nitrate and phosphate with trace ammonium, was routed to the equalization tank and reused as nutrient medium for *Wolffia* cultivation.

2.3. *Wolffia* Cultivation

A continuous-batch interface was implemented using an equalization tank (V-101) receiving steady nitrified RAS effluent. This tank intermittently supplied nutrient medium to batch-operated *Wolffia* cultivation units, enabling continuous RAS operation while decoupling cultivation cycles [45,46]. The stored effluent served as the nutrient medium for two cultivation scenarios: (i) static-water, non-aerated cultivation and (ii) aerated, well-mixed, suspended-growth. The equalization

tank thus enabled the RAS to operate continuously while allowing the *Wolffia* reactors to follow their own batch harvesting and regrowth schedules.

Nitrogen and phosphorus were supplied mainly from RAS effluent. However, stoichiometric analysis based on *Wolffia* biomass composition and the targeted production capacity indicated that the RAS nutrients alone were insufficient; therefore, KH_2PO_4 and NaNO_3 were added to meet nutrient demand, consistent with reported *Wolffia* growth in aquaculture effluents [13]. *Wolffia* growth was modeled using mass-based stoichiometric reactions linking inorganic carbon uptake, nitrate and phosphate consumption, and oxygen evolution to biomass formation. The stoichiometric coefficients were implemented in SuperPro Designer and normalized to 1 kg DW *Wolffia* biomass for clarity and comparability across scenarios, with static (HCO_3^-) and suspended (CO_2 -based) growth described by Equations (2) and (3).



where W_b represents *Wolffia* biomass. In the static scenario, HCO_3^- served as the inorganic carbon source, whereas the suspended system used dissolved CO_2 directly for photosynthetic carbon fixation.

Differences in annual biomass production were determined by cultivation performance such as biomass concentration, batch duration, and number of batches per year, while stoichiometry calculated nutrient uptake and gas exchange per unit biomass. KH_2PO_4 and NaNO_3 were dosed into the cultivation feed mixers (P-7/MX-102) for both scenarios to adjust the influent N and P concentrations to optimal levels for *Wolffia* growth. The mixer received three converging inputs: (i) nitrified RAS effluent from the equalization tank, (ii) recycled process water from the flotation and washing units (total 10%), and (iii) makeup nutrients (KH_2PO_4 and NaNO_3). An inoculum of 10% (v/v) was used in both scenarios, giving an initial biomass concentration of $\sim 500 \text{ mg L}^{-1}$ [18].

In the static scenario, *Wolffia* was cultivated in an outdoor raceway pond (P-8/RP-102) without aeration or mechanical mixing, using nitrified RAS effluent as the nutrient medium. Natural sunlight provided illumination, while NaHCO_3 served as the sole inorganic carbon source since neither gas-phase CO_2 nor recycled CO_2 was introduced. CO_2 fixation was estimated a conversion factor of $1.42 \text{ g CO}_2 \text{ g}^{-1} \text{ biomass}$ [18]. Static cultivation followed a 14-day batch cycle (12 days growth, 2 days harvesting/turnaround).

In the suspended-growth scenario, *Wolffia* was cultivated in a bubble-column PBR, represented in SuperPro Designer by an air-lift fermenter (P-8/AFR-102). White LEDs ($402 \mu\text{mol m}^{-2} \text{ s}^{-1}$, 16:8 photoperiod) provided artificial lighting and the associated electricity demand for LED operation was incorporated into the TEA. Continuous aeration ensured mixing and gas exchange, while recycled CO_2 from fish respiration and nitrification off-gas was supplied through a gas-mixing system, with atmospheric air ($\sim 0.04\% \text{ CO}_2$) as a supplementary carbon source. Each stream was conditioned using flow-splitting units (P-22/FSP-103 and P-23/FSP-104) to regulate gas flow rates, after which the streams were combined in a gas-mixing unit (P-24/MX-105). The blended gas mixture was subsequently sparged into the PBR to supply recycled CO_2 for photosynthetic assimilation. CO_2 capture was estimated by comparing simulated CO_2 input and off-gas streams. Suspended cultivation followed a 12-day batch cycle (10 days growth, 2 days harvest/turnaround) [18].

2.4. Downstream Processing

2.4.1. Harvesting and Washing

At the end of each cultivation cycle, *Wolffia* biomass was recovered and processed. Although laboratory separation can be achieved by nylon cloth filtration for small fronds ($\sim 250\text{-}300 \mu\text{m}$) [18], this approach is unsuitable for large-scale operation. Therefore, flotation unit (FL-101) was selected, exploiting the natural buoyancy of *Wolffia* fronds to achieve rapid surface concentration with low energy input and minimal shear stress.

After flotation, biomass was concentrated to $\sim 80 \text{ g L}^{-1}$ and transferred to a washing unit (WSH-101) for impurity removal. Washing was modeled as a batch process with a 4 h contact time under gentle mixing, the unit was sized for a $2,500 \text{ kg h}^{-1}$ throughput based on standard solid-liquid washing practices [47]. Water from flotation and washing was combined (P-17/MX-106), flow-adjusted (P-18/FSP-101), and recycled as feed water for the next cultivation batch. Additionally, 10% of the recycling stream was diverted (P-19/FSP-105), mixed with fresh water (P-20/MX-108), and returned to the fish culture tank.

2.4.2. Treatment

After washing, the biomass was divided equally for drying (edible biomass) and chlorophyll extraction. This 50:50 allocation was selected because chlorophyll represents $<1\%$ of DB, ensuring meaningful pigment recovery while maintaining edible biomass output. For food applications, *Wolffia* was processed in dried form to avoid cold-chain storage and improve product stability. Drying was performed using a drum dryer (P-12/DDR-101) at $70 \text{ }^\circ\text{C}$ for 6 h with a capacity of $\sim 70 \text{ kg h}^{-1}$, consistent with pilot-scale biorefinery conditions. Drum drying was selected due to its lower energy demand and cost compared with spray or freeze drying [48]. The dried *Wolffia* was suitable for use as a food ingredient or nutritional supplement.

The remaining 50% of wet biomass was used for chlorophyll extraction with ethanol as solvent. Biomass was mixed with food-grade ethanol at a 2:1 (v/v) biomass-to-solvent ratio in a solid mixer-settler extractor (P-13/SMSX-101), homogenized for 5 min, and extracted for 24 h at $4 \text{ }^\circ\text{C}$ to promote pigment diffusion while limiting thermal degradation. The ethanol-chlorophyll phase was then separated and sent to a flash-drum evaporator (P-14/V-102) for solvent recovery at $75 \text{ }^\circ\text{C}$, enabling ethanol recycling and reducing solvent consumption. The flash drum was selected for its simpler configuration and lower capital cost, suitable for pilot-scale pigment recovery [31,49,50].

2.5. Economic Assessment and Assumptions

The economic analysis included all major cost components such as raw materials, utilities, labor, and indirect expenses. Raw material prices were obtained from 2025 supplier data (Alibaba.com), while utility and service charges were taken from the SuperPro Designer database. Labor costs were based on operator wage rates in Thailand. Indirect expenses, including maintenance, supervision, depreciation, and overhead, were estimated by the simulator as standard percentage of equipment purchase cost. Utility, labor, and raw material prices used in the model are summarized in Table S1.

SuperPro Designer calculated key economic indicators including capital investment (CAPEX), operating cost (OPEX), annual revenue, return on investment (ROI), payback time (PBT), and net present value (NPV), based on material flows, equipment costs, and operating conditions. CAPEX included equipment purchase, installation, indirect costs and working capital, while OPEX covered raw materials, utilities, labor, maintenance, and overhead. Annual revenue was derived from tilapia biomass, dried *Wolffia* biomass and chlorophyll extract. Economic performance was evaluated over a 15-year project lifetime, with $\text{ROI} = \text{annual net profit}/\text{CAPEX}$, NPV used a 2% discount rate and PBT defined as the time required to recover the initial capital investment.

2.6. Sensitivity Analysis

A sensitivity analysis was conducted to evaluate the effect of key revenue-related parameters on the economic performance of the integrated RAS-*Wolffia* biorefinery. Two parameters were analyzed: (i) the selling price of dried *Wolffia* and (ii) the allocation ratio between chlorophyll extraction and food-supplement biomass. The *Wolffia* selling price was varied from 115-135 USD kg^{-1} to represent market variability. Allocation sensitivity was evaluated using 30:70, 50:50, and 70:30 chlorophyll: biomass ratios, while all other model inputs were held constant. Changes in NPV, ROI, IRR, gross margin, and PBT were used to identify parameters with the greatest influence on profitability.

3. Results and Discussion

3.1. Integrated RAS Performance: Nutrient Generation and Effluent Characteristics

Tilapia production was held constant across scenarios because the RAS configuration and operating conditions were identical, yielding 420 kg yr⁻¹ of harvested fish from the 10 m³ RAS and generating 2,577 USD yr⁻¹ in fish revenue. This corresponds to an average production rate of ~0.053 kg h⁻¹ (~1.27 kg d⁻¹) over 330 operating days yr⁻¹ and a volumetric productivity of ~42 kg m⁻³ yr⁻¹, consistent with reported values for warm-water RAS of similar scale [29]. Although tilapia is harvested in 6-8 month cycles, fish cultivation was modeled as a continuous unit to represent time-averaged biomass growth, feed input, and nutrient generation. With an FCR of 1.80, the system required ~757.50 kg feed yr⁻¹, consistent with reported values for Nile tilapia [40,41,51,52].

The metabolic release of nitrogen and phosphorus matched expected stoichiometries: fish-tank effluent stabilized at ~0.03 g NH₄⁺-N L⁻¹ and ~0.012 g PO₄³⁻-P L⁻¹, corresponding to TAN and phosphate loadings of ~0.0022 and 0.00062 kg h⁻¹, respectively. On a volumetric basis, this corresponds to average loading rates of ~5.30 g TAN m⁻³ d⁻¹ and ~1.50 g P m⁻³ d⁻¹ for the 10 m³ RAS, equivalent to ~18 kg TAN yr⁻¹ and ~4.90 kg P yr⁻¹ over 330 operating days. Gas emissions from the fish tank were dominated by CO₂, with the aeration off-gas (Emission 1) carrying ~0.08 kg CO₂ h⁻¹ (~666 kg CO₂ yr⁻¹), equivalent to ~1.60 kg CO₂ per kg of harvested tilapia, consistent with the assumed RQ = 0.85.

Suspended solids were removed using a hydrocyclone, producing a concentrated underflow (~1.80 kg h⁻¹) containing fish feces, uneaten feed, and microbial solids (6-10 g L⁻¹). The clarified overflow (~79 kg h⁻¹) contained mainly dissolved nutrients, including NH₄⁺ (~0.03 g L⁻¹), NO₃⁻ (~0.043 g L⁻¹), and PO₄³⁻ (~0.012 g L⁻¹), with minimal particulates.

The overflow was treated in the nitrifying biofilter (NB-101), achieving near-complete conversion of ammonium to nitrate under aerobic conditions. Continuous aeration and CaCO₃ dosing (0.19 kg h⁻¹) maintained adequate DO and alkalinity. The nitrified effluent contained negligible residual ammonium (~0.000016 g L⁻¹), while nitrate accumulated to ~0.11 g L⁻¹, confirming complete conversion of TAN to nitrate under oxygen-sufficient conditions. The nitrifying biofilter received 0.39 kg h⁻¹ of aeration, maintaining adequate DO for nitrifying activity. CO₂ generated as a by-product of nitrification was released at ~0.01 kg h⁻¹ (Emission 2), consistent with expected alkalinity consumption and microbial stoichiometry. The nitrified effluent acted as a consistent nutrient input to both *Wolffia* cultivation modes, ensuring that downstream performance differences were attributed solely to culture configuration.

3.2. The Performance of *Wolffia* Cultivation

Wolffia cultivation performance differed markedly between the static raceway pond and suspended bubble-column PBR due to differences in hydrodynamics, carbon supply, and light conditions, with key parameters summarized in Table S2. In both systems, nitrified RAS effluent served as the main nutrient source, supplying most nitrogen and a substantial portion of phosphorus. Supplemental fertilizers (NaNO₃ ~26 kg batch⁻¹ and KH₂PO₄ ~2.70 kg batch⁻¹) were added only to compensate for remaining nutrient deficits and maintain comparable N and P levels between scenarios.

In the static configuration, each raceway pond operated at 8.20 m³ working volume and 0.25 m depth, reaching a final biomass concentration of ~0.80 g L⁻¹ (~6.60 g DW m⁻² d⁻¹). In contrast, the suspended system achieved ~2.77 g L⁻¹. These values correspond to modeled batch concentration and are consistent with reported experimental ranges for static and aerated *Wolffia* cultivation systems [18].

Reactor geometry strongly influenced land-use requirements. The static system required four raceway ponds providing 32.80 m³ working volume and occupying ~131 m², whereas the suspended system used eight vertical bubble-column PBRs providing 28.80 m³ while occupying only ~10-12 m²,

representing ~12-fold reduction in land area. This advantage arises from the vertical reactor design, which enables higher culture volumes and improved mixing compared with shallow raceways.

Growth kinetics also differed between the systems. Suspended *Wolffia* cultures exhibit shorter lag phases (~1-2 days) than static systems (~6-8 days) [18], enabling 26 batches yr⁻¹ compared with 22 batches yr⁻¹ for the static configuration. Consequently, annual biomass production increased from 690.28 kg DW yr⁻¹ (static) to 1,055.85 kg DW yr⁻¹ (suspended), corresponding to a ~53% increase in biomass output.

Static system operated with minimal energy input, relying on natural sunlight without mixing or aeration, and released CO₂ directly to the atmosphere. In contrast, suspended system required continuous aeration and LED illumination, increasing energy demand but enabling higher biomass density, faster batch turnover, and improved land-use efficiency. The aeration loop also recycled CO₂ from fish respiration and nitrification, allowing partial carbon capture by the *Wolffia* culture.

3.3. Nutrient Removal Performance

Table S3 summarizes nitrogen and phosphorus removal in the two cultivation systems. In the static scenario, nitrogen decreased from ~28.46 kg batch⁻¹ (originating from the combined nitrified RAS effluent and NaNO₃ supplementation) to 0.89 kg batch⁻¹, corresponding to ~97% nitrogen removal and ~606.50 kg yr⁻¹ uptake into biomass. Phosphorus declined from 2.99 kg batch⁻¹ to nearly zero, indicating complete removal, equivalent to ~64.10 kg yr⁻¹ of phosphorus captured in harvested biomass).

In the suspended system, nitrogen decreased from 29.26 to 10.69 kg batch⁻¹, corresponding to 64% removal and an annual nitrogen uptake of ~483 kg yr⁻¹ uptake. Phosphorus decreased from 2.91 to 1.04 kg batch⁻¹ (65.30% removal; ~50.70 kg yr⁻¹ recovery). The lower percentage removals in the suspended system are consistent with its shorter batch cycle and higher volumetric loading, which reduces the time available for nutrient depletion per batch. In contrast, the static system behaves more like a polishing step, achieving near-complete depletion of phosphate and higher overall nutrient removal per batch.

3.4. CO₂ Fixation and Capture

Table S4 summarizes the carbon balance and CO₂ capture of both systems. In the static system, NaHCO₃ served as the sole carbon source, fixing ~0.98 t CO₂ yr⁻¹ through biomass formation. This behavior is characteristic of non-sparged shallow systems, where limited gas-liquid exchange prevents CO₂ stripping and photosynthetic alkalization shifts the carbonate equilibrium toward dissolved HCO₃⁻ and CO₃²⁻ species [53,54]. *Wolffia* can assimilate these DIC forms via HCO₃⁻ uptake and carbon concentrating mechanisms, enabling efficient utilization of supplied inorganic carbon [15]. To sustain this carbon supply, the static scenario required 4,926 kg NaHCO₃ yr⁻¹ (~887 USD yr⁻¹), equivalent to 2.58 t CO₂ yr⁻¹, and the shallow geometry (0.25 m depth, 8.20 m³ working volume) demanded ~131 m² per pond, implying hundreds of ponds per hectare at commercial scale.

The suspended system utilized CO₂ from tilapia respiration and nitrification, supplemented by atmospheric aeration. With ~1,056 kg yr⁻¹ *Wolffia* production, the system fixed ~1.50 t CO₂ yr⁻¹, about 1.50 times higher than the static system. These results align with the established performance of aerated, enclosed cultivation systems, which sustain higher inorganic carbon availability and improved DIC utilization relative to open raceways of comparable footprint [55]. Gas-balance analysis of the PBR further revealed substantial CO₂ capture from the gas phase. Per batch, the reactor received 27.31 kg CO₂ from recycled RAS off-gas and an additional ~14.60 kg CO₂ from atmospheric air, giving a total inflow of ~41.90 kg CO₂ batch⁻¹. The off-gas contained only 14.19 kg CO₂ batch⁻¹, indicating net retention of ~27.70 kg CO₂ batch⁻¹ within the culture and corresponding to a capture efficiency of ~66%. Over 26 batches yr⁻¹, this equates to ~0.72 t CO₂ yr⁻¹ actively removed from the gas phase, of which approximately 65% originates from internal RAS recycling and 35% from atmospheric air.

Beyond carbon performance, the two cultivations differ substantially in spatial requirements, which is a critical consideration for scale-up. The static system required four raceway ponds to

provide 32.80 m³ of working volume, occupying a total footprint of ~131 m². In contrast, the suspended system used eight vertical PBRs to achieve 28.80 m³ of working volume, requiring only ~8-12 m² of floor area even after accounting for realistic spacing, access, and piping. The suspended system required roughly an order of magnitude less land area than the static configuration for similar cultivation capacity. This dramatic spatial advantage highlights the suitability of the suspended PBR scenario for land-limited or high-value production settings, whereas the static raceway scenario remains more appropriate for large open-field installations.

3.5. TEA Results of The Two Cultivation Scenarios

The techno-economic performance of the two *Wolffia* cultivation configurations is summarized in Table 1, including capital investment, operating costs, revenues, and profitability indicators.

Table 1. Techno-economic indicators for integrated RAS-*Wolffia* systems under static and suspended cultivation scenarios.

Parameter	Static Scenario	Suspended Scenario
CAPEX, USD	266,669	392,342
OPEX, USD yr ⁻¹	76,737	94,579
<i>Wolffia</i> revenue (USD yr ⁻¹)	86,286	131,982
Tilapia revenues (USD yr ⁻¹)	2,577	2,577
Chlorophyll revenues (USD yr ⁻¹)	1,193	1,948
Total annual revenues (USD yr ⁻¹)	90,037	136,524
<i>Wolffia</i> production (kg DW yr ⁻¹)	690.30	1055.90
Tilapia production (kg yr ⁻¹)	420	420
Chlorophyll production (kg yr ⁻¹)	5.40	8.90
Unit production cost (USD kg ⁻¹ <i>Wolffia</i>)	112.98	89.58
Unit production revenue (USD kg ⁻¹ <i>Wolffia</i>)	130.44	129.30
GM (%)	14.77%	30.72%
ROI (%)	12.89%	17.56%
PBT (yr)	7.76	5.70
IRR (%)	5.55%	11.02%
NPV (USD, 2% discount rate)	73,657	303,309
Batches (yr ⁻¹)	22	26

Note: Gross margin = GM; Internal rate of return = IRR; Unit production cost = total annual operating cost / annual *Wolffia* biomass production; unit production revenue = total annual revenue / annual *Wolffia* biomass production.

Both scenarios were economically feasible with PBT below 10 years, but the suspended system showed superior performance. Its PBT (5.70 years) was shorter than the static system (7.76 years), while ROI increased from 12.89% to 17.56%. Gross margin (GM) rose from 14.77% to 30.72%, and NPV increased from ~0.07 to ~0.30 million USD, indicating stronger financial performance. Although upstream and downstream processes were identical, differences in cultivation design led to variations in CAPEX and OPEX. Operating costs were 76,737 USD yr⁻¹ (static) and 94,579 USD yr⁻¹

(suspended), while capital investment was 266,669 USD and 392,342 USD, respectively. Thus, the improved economics of the suspended system results from higher biomass productivity and revenue, rather than lower costs.

As presented in Table S5, it was found that for both static and suspended scenarios, as much as 94% and 95% of the CAPEX (static: US\$ 0.25 million, suspended: US\$ 0.37 million) was due to the direct fixed capital cost (DFC). Meanwhile, the DFC was mainly contributed by the costs of equipment purchase (PC), plant construction, engineering, contractor's fee contingency, and equipment installation, process piping, auxiliary facilities, and corresponding contributions to the DFC was shown as pie chart in Figure 4. In both cultivation scenarios, equipment PC and construction dominated the DFC, together contributing the largest shares. PC accounted for ~19.50% of DFC in the static system and ~20.50% in the suspended system, while construction contributed ~19% in both cases, making these two categories the most significant capital cost drivers. This was similar to the study by Chalermthai et al. where the PC also contributed about 19% of the CAPEX for *Spirulina* microalgae cultivation [47]. Also, similar to Hossain et al. where the PC also contributed about 20.50% of the CAPEX for microalgae cultivation [56].

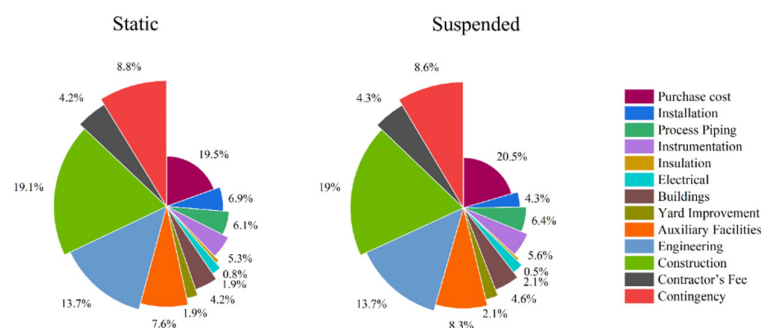


Figure 4. Contribution to the direct fixed capital (DFC) cost in the integrated RAS-*Wolffia* systems under static and suspended scenarios.

The OPEX comprises the costs of raw materials, labor, facility-dependent costs, waste treatment, and laboratory/QC/QA, as summarized in Table S6 and Figures 5a and 5b for the static and suspended scenarios, respectively. In the static system, labor costs dominated OPEX (~55.10%, ~0.04 million USD yr⁻¹), followed by facility-dependent costs (~35.70%, ~0.03 million USD yr⁻¹) (Figure 5a). This distribution reflects the intensive manual effort required to manage large raceway ponds. Because the system relies on natural sunlight, operators must frequently adjust culture depth, remove debris, manually mix the ponds, and perform labor-intensive harvesting.

In contrast, the suspended system was dominated by facility-dependent costs (~71% of OPEX, ~0.07 million USD yr⁻¹), while labor costs decreased to ~16% (~0.02 million USD yr⁻¹) due to the more automated operation of enclosed PBR units (Figure 5b). Utility costs were also higher in the suspended system (~7.20% of OPEX) than in the static system (~2.20%) because of the electricity and chilled-water demand for LED illumination and continuous aeration typical of closed PBRs [49], as also reflected in Table 2. Under a 16 h light/8 h dark photoperiod, LED lighting required ~6,930 kWh yr⁻¹, accounting for ~80% of cultivation electricity use and an energy intensity of ~2.90 kWh kg⁻¹ biomass, consistent with reported values for enclosed column PBR systems, where artificial lighting typically contributes 70-90% of overall electricity consumption [49].

Regarding raw-material inputs, detailed compositions and costs for both scenarios are presented in Tables S7-S8 and Figure 5c. Raw-material costs were lower in the suspended system (1,422 USD yr⁻¹) than in the static system (2,156 USD yr⁻¹) because recycled CO₂ from the RAS replaced NaHCO₃ supplementation. Freshwater demand was also lower due to the enclosed reactor design. However,

ethanol consumption for chlorophyll extraction was higher in the suspended scenario because of its greater biomass production.

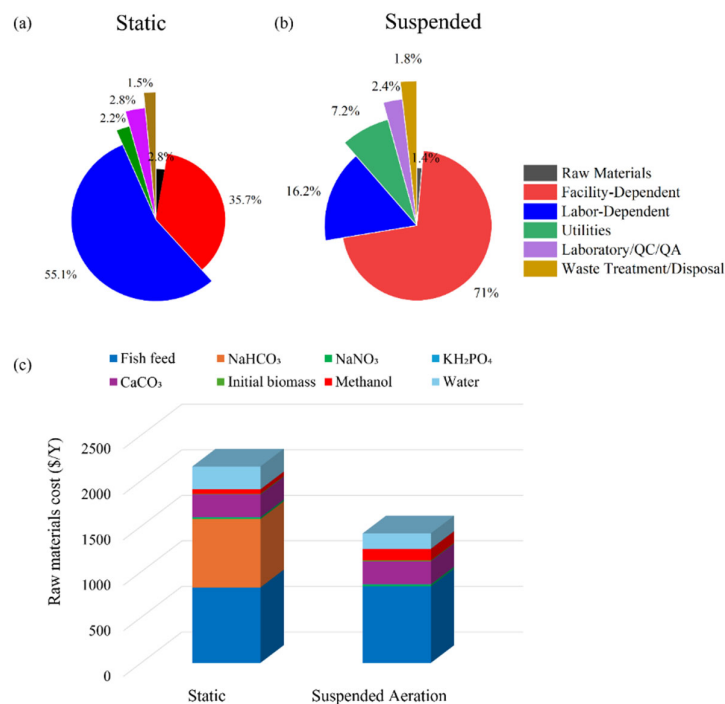


Figure 5. Distribution of operating costs in the integrated RAS-*Wolffia* system for (a) static and (b) suspended scenarios, and (c) raw material distribution for both scenarios.

Table 2. Summary of utility costs for the integrated RAS-*Wolffia* system for both scenarios.

Kind of Services	Unit Cost	Static		Suspended	
		Annual amount	Annual Cost (\$)	Annual amount	Annual Cost (\$)
Electricity (\$/kWh)	0.09	16,107	1,611	9,372	7,084
Chilled water (\$/MT)	0.40	13	5	416	166
Steam (\$/MT)	12	8	101	14	163
Total			1,717		7,413

Figure 6a and b present the distribution of equipment purchase costs across process stages, expressed as their percentage contribution to total CAPEX. As detailed in Table S9, which summarizes the equipment specifications and associated purchase costs, the bubble-column PBR is the dominant capital expense in the suspended scenario, accounting for 66% of total CAPEX (Figure 6b). This concentration of investment reflects

the specialized construction, transparent materials, aeration hardware and illumination requirements of enclosed PBRs, and aligns with previous studies reporting that PBRs commonly contribute 60-90% of overall CAPEX in closed algal cultivation systems [49,57]. In contrast, the static scenario in raceway pond results in a more distributed CAPEX structure, with no single unit approaching the capital intensity of the PBR. Overall, these results indicate that cultivation infrastructure, particularly the PBR, is the primary determinant of total CAPEX in enclosed *Wolffia* production systems.

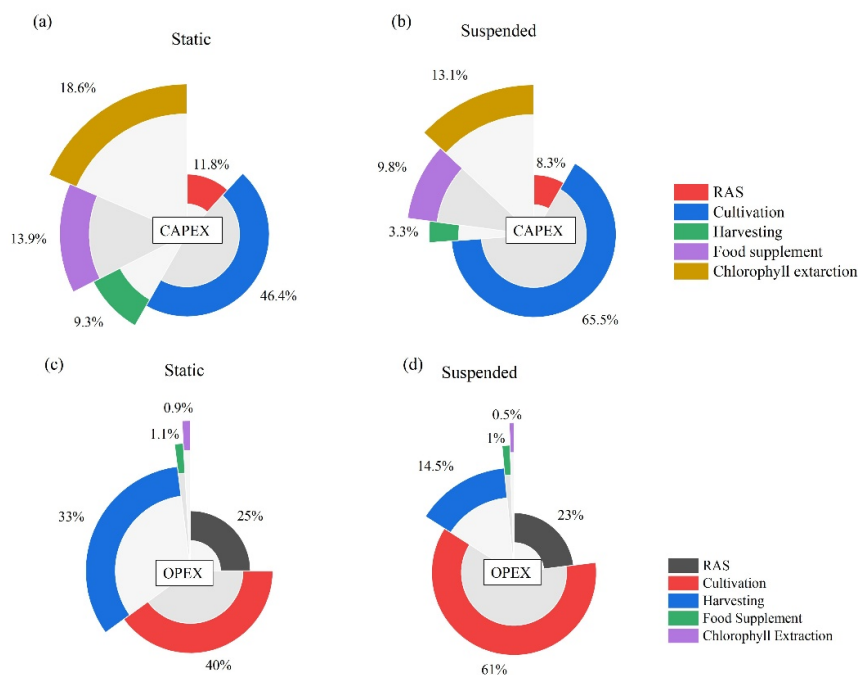


Figure 6. Distribution of equipment costs by process stage in the integrated RAS-*Wolffia* system for (a) static and (b) suspended scenarios (total CAPEX), and energy-use distribution by process stage for the (c) static and (d) suspended systems (total OPEX).

The distribution of energy use across process stages (Figure 6c and d) also reflects the distinct operational characteristics of the two cultivation modes. In the static system, cultivation accounted for ~40% of energy use, followed by harvesting (~33%) and RAS (~25%). The cultivation energy demand arises from hydraulic recirculation and pond flow maintenance, while high harvesting energy reflects the need to wash large volumes of dilute biomass exiting the raceway pond. In the suspended system, cultivation dominated energy consumption (~61%) due to continuous aeration and LED illumination. The RAS contributed a similar share in both systems (~25% static; ~23% suspended), while harvesting energy was lower in the suspended system (~14.50%) due to reduced hydraulic loads. Overall, although the suspended system requires higher capital and energy inputs, its higher biomass productivity, improved spatial efficiency, and greater revenue generation result in superior economic performance compared with the static configuration.

3.6. Product Yields and Economic Performance

In addition to the profitability indicators discussed above, the economic outcomes of the two cultivation scenarios are also influenced by product yields and value recovery. As shown in Table 1, dried *Wolffia* was the dominant product, with annual yields of 690.30 kg yr⁻¹ (static) and 1,055.90 kg yr⁻¹ (suspended), reflecting the higher productivity of the suspended system. The assumed *Wolffia* selling price of 125 USD kg⁻¹ reflects current market estimates for dried duckweed-based food supplements. At this price, *Wolffia* generated 86,286 USD yr⁻¹ in the static scenario and 131,982 USD yr⁻¹ in the suspended scenario, accounting for ~96% of total revenues in both systems and confirming *Wolffia* biomass as the primary economic driver of the integrated RAS-biorefinery.

A portion of the *Wolffia* biomass was allocated to chlorophyll extraction. With a chlorophyll content of 0.80 wt%, annual recoveries were 5.40 kg yr⁻¹ (static) and 8.90 kg yr⁻¹ (suspended). At a selling price of 220 USD kg⁻¹, chlorophyll contributed 1,193 USD yr⁻¹ (static) and 1,948 USD yr⁻¹ (suspended), representing ~1-2% of total revenue. Additional income was generated from tilapia (2,577 USD yr⁻¹) contributed ~2% of total revenue. Consequently, total annual revenues reached 90,037 USD yr⁻¹ (static) and 136,524 USD yr⁻¹ (suspended).

Higher revenues in the suspended system improved economic performance. The IRR increased from 5.55% (static) to 11.02% (suspended), while the PBT decreased from 7.76 (static) to 5.70 (suspended) years. The suspended system also achieved a markedly higher GM (30.72%) compared with the static system (14.77%). These outcomes remain consistent despite the higher capital and operating expenditures of the suspended aeration system (CAPEX 392,342 USD; OPEX 94,579 USD yr⁻¹) compared with the static system (CAPEX 266,669 USD; OPEX 76,737 USD yr⁻¹). Overall, while both systems can recover their investments, the suspended aeration scenario offers stronger economic performance due to its higher *Wolffia* productivity and associated revenue gains.

3.7. Sensitivity Analysis

Sensitivity analysis indicated that economic performance was mainly influenced by (i) the allocation ratio between chlorophyll extraction and food-supplement production and (ii) the selling price of *Wolffia* biomass (Figures 7a-d).

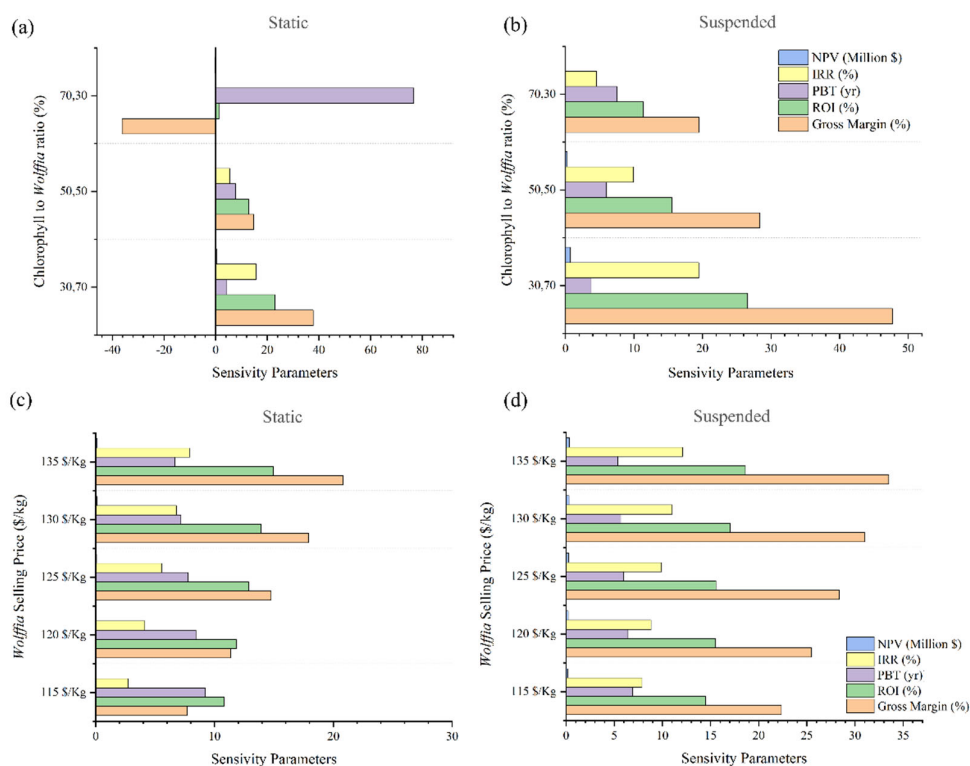


Figure 7. Sensitivity analysis of key economic parameters in the integrated RAS-*Wolffia* system under varying chlorophyll to *Wolffia* allocation ratios (30:70, 50:50, and 70:30) for (a) static and (b) suspended scenarios, and varying *Wolffia* selling price (115-135 USD kg⁻¹) for (c) static and (d) suspended scenarios.

Adjusting chlorophyll to *Wolffia* allocation reveals clear economic trends. In the static scenario, shifting the allocation from 50:50 to 30:70 (chlorophyll: *Wolffia*) improved profitability, increasing the GM from 14.77% to 37.76%, ROI from 12.89% to 22.95%, and reducing PBT from 7.76 to 4.36 years, driven by higher food-biomass revenue (966 kg yr⁻¹), which dominates the total income in the low

chlorophyll configuration. Increasing chlorophyll allocation to 70% resulted in negative economics (NPV = -230,783 USD) because only $\sim 7.50 \text{ kg yr}^{-1}$ chlorophyll is produced with high unit OPEX.

In contrast, the suspended system remained profitable across all allocation ratios due to its higher biomass and chlorophyll productivity. Under the base case (50:50), it achieved 17.56% ROI, 5.70-year PBT, and NPV of 392,342 USD. Reducing chlorophyll allocation to 30:70 further improved performance (ROI 26.56%, PBT 3.77 years, NPV 700,367 USD) due to higher biomass production output ($1,478 \text{ kg yr}^{-1}$). Even at 70:30, the suspended scenario maintained 11.36% ROI and a positive NPV, indicating strong economic robustness. Since both *Wolffia* biomass and chlorophyll represent important revenue streams, a 50:50 allocation was adopted as the base scenario.

Changes in the *Wolffia* selling price (115-135 USD kg^{-1}) also affected profitability. In the static scenario, a 5 USD kg^{-1} price increase raised NPV by ~ 0.03 million USD and reduced PBT from 9.24 to 6.68 years. However, even at 135 USD kg^{-1} (NPV = 132,196 USD), the static system remained less profitable than the suspended system. In the suspended scenario, increasing the price from 115 to 135 USD kg^{-1} raised NPV from 193,083 to 362,874 USD and reduced PBT from 6.90 to 5.30 years, while remaining profitable even at the lowest price.

The base case (125 USD kg^{-1} and a 50:50 allocation) was selected as a realistic balance between food-biomass revenue and chlorophyll recovery. Although dried *Wolffia* prices range from 115-135 USD kg^{-1} , the upper value typically reflects premium-grade products requiring additional processing and serving niche markets; therefore, 125 USD kg^{-1} represents a more practical mid-range price for large-scale production. Similarly, allocating only 30% of biomass to chlorophyll extraction underutilizes this high-value co-product (chlorophyll $\sim 0.80\%$ of dry biomass), whereas allocating 70% reduces food-supplement output and weakens overall profitability. A 50:50 allocation therefore provides a practical compromise, maintaining food-biomass revenue while enabling meaningful chlorophyll recovery and overall economic performance.

4. Conclusions

Integrating *Wolffia* cultivation with RAS offers a promising strategy for nutrient recovery, CO_2 utilization, and biomass production in resource-efficient aquaculture systems. This study developed a process-based TEA of a pilot-scale RAS-*Wolffia* system under tropical conditions, comparing static-water cultivation and aerated suspended-growth cultivation. Economic performance was mainly influenced by biomass productivity, *Wolffia* selling price, and biomass allocation between food production and chlorophyll extraction. The suspended system consistently outperformed the static configuration, achieving higher biomass productivity, improved CO_2 utilization, greater land-use efficiency, and reduced labor demand, resulting in higher ROI and shorter PBT. Although both systems were economically feasible, the suspended configuration showed stronger performance.

Overall, the results highlight the potential of RAS-*Wolffia* integration to convert aquaculture-derived nutrients and carbon into valuable biomass products, supporting circular aquaculture and resource recovery. The TEA framework developed in this study also provides a transferable approach for evaluating integrated aquaculture-biomass production systems. However, because the model relies on literature-based parameters, the results represent process-level feasibility rather than experimental validation.

Future research should investigate the environmental implications of RAS-*Wolffia* integration at the system level. In particular, the effects of carbon recycling, organic load reduction, and water quality improvement, such as changes in CO_2 emissions, biological oxygen demand (BOD), and nutrient discharge, should be assessed. Life cycle assessment (LCA) could provide a comprehensive framework to quantify energy use, greenhouse gas emissions, and resource consumption across the entire production chain. Integrating TEA with LCA would enable a more holistic evaluation of the sustainability potential of RAS-*Wolffia* systems and support their development as scalable circular aquaculture technologies.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1. Utility, labor, and raw material costs in integrated RAS-*Wolffia* systems used in the techno-economic model. Table S2. Comparison of cultivation performance in integrated RAS-*Wolffia* systems under static and suspended cultivation systems. Table S3. Nitrogen and phosphorus removal performance in integrated RAS-*Wolffia* systems under static and suspended cultivation systems. Table S4. Carbon balance and CO₂ fixation in integrated RAS-*Wolffia* systems under static and suspended cultivation scenarios. Table S5. CAPEX calculation and results in the integrated RAS-*Wolffia* system for both scenarios. Table S6. Contributions to OPEX in the integrated RAS-*Wolffia* system for both scenarios. Table S7. Summary of raw material costs in the integrated RAS-*Wolffia* system for static scenario. Table S8. Summary of raw material costs in the integrated RAS-*Wolffia* system for suspended scenario. Table S9. Equipment costs and specifications in the integrated RAS-*Wolffia* system for both scenarios.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “Conceptualization, K.N.; methodology, S.R.M. and K.N.; software, S.R.M. and B.C.; formal analysis, S.R.M. and R.K.; investigation, S.R.M.; data curation, S.R.M.; writing—original draft preparation, S.R.M.; writing—review and editing, B.C., R.K., M.E., C.Y.C., K.N.; supervision, K.N. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

TSS	Total suspended solids
RAS	Recirculating aquaculture system
NH ₄ ⁺	Ammonium
NO ₂ ⁻	Nitrite
NO ₃ ⁻	Nitrate
PBRs	Photobioreactors
N ₂ O	Nitrous oxide
TEA	Techno-economic evaluation
CSTR	Continuous stirred-tank reactor
RQ	Respiratory quotient
FCR	Feed conversion ratio
DW	Dried weight
CAPEX	Capital investment
OPEX	Operating cost
ROI	Return on investment
PBT	Payback time
NPV	Net present value
GM	Gross margin
IRR	Internal rate of return
DFC	Direct fixed capital
DO	Dissolved oxygen
NaHCO ₃	Sodium bicarbonate
CaCO ₃	Calcium carbonate
PC	Purchases cost

BOD	Biological oxygen demand
LCA	Life-cycle assessment

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