Optimizing Hydrodynamics for Enhanced Co-Cultivation in Bubble Column Reactors

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Abstract**—** Hydrodynamics significantly influences algal growth rates and biomass productivity in bubble column reactors, particularly when different column diameters are used during cultivation. This study investigates the effects of hydrodynamics on the growth of *Scenedesmus sp.* and *Lemna sp.* (Duckweed) co-cultivated in sewage wastewater, focusing on fluid flow patterns and nutrient distribution, as well as how co-cultivation supports novel cultivation and attached harvesting methods. A synthetic mixture of Bold’s Basal Medium (BBM) and pre-treated sewage wastewater ratio was used as the cultivation medium in an automatic triple bubble column, illuminated with blue LED light, operated under 18-hour lighting conditions, with an aeration rate of 5 L/hr and cyclic CO_2 injection (5 %). The growth medium varied between BBM-to-wastewater ratios, with pH levels ranging from 7.5 to 8.5, and a gas supply consisting of 50% air and 50% nitrogen at 5 L/hr, along with 5% CO_2 sparging. Results indicate that wider diameter columns improve gas exchange and mixing efficiency, leading to more uniform nutrient distribution. However, the widest column (15 cm) also created dead zones due to dissipated mixing efficiency, resulting in decreased nutrient gradients. Under optimised conditions, the highest algal growth rate of 0,74/day was achieved using a 10 cm diameter column, compared to 0,52/day in a 5 cm column and 0,68/day in a 15 cm column.

Additionally, co-culturing *Scenedesmus sp.* with Duckweed led to an 11,45 % increase in growth rates and almost 53 % boost in biomass productivity, demonstrating multiple benefits including enhanced nutrient uptake, improved gas exchange, higher photosynthetic efficiency, sustainable bioremediation, and increased lipid and protein content. Despite the limitations of reduced turbulence in wider columns, efficient mixing minimized the negative impact of such gradients. The findings of this study conclude that optimizing the diameter of bubble columns and enhancing hydrodynamic conditions can significantly improve algal biomass productivity, offering a scalable and sustainable solution for biofuel production and wastewater treatment. Furthermore, this research provides valuable insights into reactor design, contributing to the advancement of large-scale cultivation of microalgae for sustainable bioenergy production.

Keywords**—** Attached Cultivation, Bubble Columns, Co-Cultivation, Hydrodynamics, *Lemna*, *Scenedesmus Sp*.

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I. Introduction

Besides population growth, industrialization and fossil fuel depletion, carbon emissions are rising at an unprecedented rate with an expected increase of 30 % by 2030 if no intervention is developed (Elena, 2024). This phenomenon and its effects are visible in the drastic weather patterns, natural disasters and climate changes over the last decade. To keep global warming below 1.5 °C, the Intergovernmental Panel on Climate Change (IPCC) stresses that CO_2 emissions must decrease by approximately 45 % compared to 2010 levels by 2030 and reach net zero by 2050 (IPCC, 2022). Reducing the carbon footprint and emissions (CO_2) should be prioritized to counteract the repercussions of human actions. Furthermore, CO_2 capture for the utilization of biomass valorisation and energy production will combat environmental concerns. Microalgae appear as the most attractive resource to utilize this CO_2 in the form of carbon capture for the generation of clean energy, as they utilize approximately 50 times more than terrestrial plants. Microalgae, the most propitious feedstock for biofuel production, with high lipid yield, fatty acid content and ability to cultivate on non-arable land, is highly advantageous compared to other biofuel producing food crops (Dalrymple *et al*, 2013). However, most large-scale studies utilize outdoor raceway ponds, occupying space instead of photobioreactors (PBR). Although outdoor ponds are said to be economical and durable, evaporation, bacterial/protozoan contamination and land occupation are of severe concern. Unlike normal PBRs, bubble columns (BCPBRs) are cylindrical vessels of liquid in which gas is “bubbled” through. These reactors are utilized as one of the most applicable multiphase liquid-gas reactors used in various applications (biochemical, chemical, petrochemical and metallurgical). These reactors have various uses in wastewater treatment, cell culturing, biochemical processing, photo-bioreaction, hydrogenation, effluent treatment and oxidation; hence remain a very attractive and promising tool (Gemello *et al*, 2018).

The challenge of scaling up remains a daunting and ongoing challenge for several years. Some of the advantages that make bubble columns attractive include their easy and simple design, simplicity in construction, good mass transfer, less occupation of land space, low operational costs and no mechanically moving parts. BCPBR are more feasible, offer more control over the cultivation process and are prone to less contamination, with efficient CO_2 utilization compared to open pond systems. Although BCPBR gained significant attention as a benchmark reactor for the last 20 years, much interest remains in improving its performance and efficiency. Furthermore, the synergistic relationships between microalgae and other organisms cannot be ignored (Rawat *et al*, 2012). Bubble columns are preferred for their slow operation processes, hence the selection of this reactor for the growth of microalgae *Scenedesmus sp*. and *Lemna sp*. The optimization of bubble column reactors for algal biomass production has been widely studied, with research focusing on the effects of hydrodynamics, light availability, gas exchange, and nutrient distribution. These factors play a crucial role in achieving maximum biomass yield and enhancing reactor efficiency. While previous studies have extensively investigated the influence of hydrodynamics, gas exchange, and flow regimes on biomass productivity, this study introduces a novel approach by incorporating the synergistic relationships of co-cultivation strategies in a controlled bubble column reactor system. Unlike earlier research, which primarily focused on single-species cultivation, this study evaluates the synergistic effects of co-culturing *Scenedesmus sp*. and *Lemna sp*., demonstrating enhanced growth rates and biomass productivity.

This study provides a more detailed and systematic comparison of varying column diameters (5 cm, 10 cm, and 15 cm), identifying an optimal balance between mixing efficiency, gas exchange, and light penetration. Prior studies often explored single-diameter setups or focused solely on computational modeling, whereas this study integrates empirical data with real-time monitoring of pH, dissolved oxygen, and growth rates. Furthermore, this research advances the field by demonstrating how optimized hydrodynamic conditions mitigate dead zones in larger-diameter columns, a challenge previously noted but not extensively quantified in experimental setups. The use of automated pH and dissolved oxygen control provides a higher level of precision in evaluating the dynamic interactions within the reactor. This study contributes new insights into reactor optimization for scalable biofuel production and wastewater treatment, setting a foundation for future large-scale implementations.

Beyond the actual cultivation process, conventional microalgae harvesting methods such as flocculation, sedimentation, flotation, and centrifugation are associated with high costs. In response to these challenges, recent studies have explored an alternative harvesting approach known as attached cultivation. This method utilizes vertical supporting materials (e.g., plastic, glass, cotton cloth, sponge, or filter paper) to facilitate the formation of an algal biofilm, thereby enhancing surface area properties, improving light exposure, and simplifying the harvesting process (Liu *et al*., 2012). Attached cultivation is highly adaptable and compatible with most microalgal strains. Compared to traditional pond-based cultivation systems, attached cultivation presents several advantages, including reduced water consumption, more efficient harvesting, enhanced contamination control, and improved scalability. Additionally, since the microalgae are pre-attached to the supporting material, the harvesting process is streamlined, leading to higher biomass recovery than in conventional suspended monoculture systems.

Therefore, this study’s integration of co-cultivation of *Scenedesmus sp*. and *Lemna sp*. as an attached technique is a promising technique for the viability of biofuel production from microalgae, breaking the bottleneck of expensive harvesting costs and low algal biomass production yields. By integrating these insights, this research builds upon existing knowledge and contributes to the ongoing optimization of bubble column reactors for biofuel production.

II. Materials And Methods

Scenedesmus sp. and *Lemna sp*. (Duckweed) were collected from Nelson Mandela University (NMU) and a localized freshwater pond in Durban, South Africa, respectively. The cultivation medium utilized was in a ratio of 3 parts sewage wastewater and 1 part Bolds Basal Medium (BBM) as previously attained, bench scale and 30 L optimized experimental results.

A. Experimental set-up of bubble column

Experiments were conducted simultaneously in the three 1 m stainless steel and glass columns (C1, C2, and C3) with various column diameters of 5 cm, 10 cm and 15 cm, as seen in Figure 1a. Inserting gas (50 % nitrogen and 50 % air) with CO_2 sparging (5 % volume) was done at the bottom of the column, through perforated sparges and mixed with the culture medium in the plenum section. Each sparger varied hole size and quantity, such as C1 (19 holes of 0,45 mm), C2 (37 holes of 0,35 mm) and C3 (56 holes of 0,25 mm), ensuring a homogenous supply of gas. The pH was automatically controlled and recorded by the bubble column programmable logic controller (PLC) unit using pre-loaded solution chambers of sodium bicarbonate and hydrochloric acid (Figure 1b). The pH and DO data were automatically recorded via probes at every 1-hour interval onto a portable microSD memory card. Daily growth rates were taken by removing a small culture sample from the drain valve and inserting it into the spectrophotometer (HACH DR 300) with absorbance measured at 650 nm.

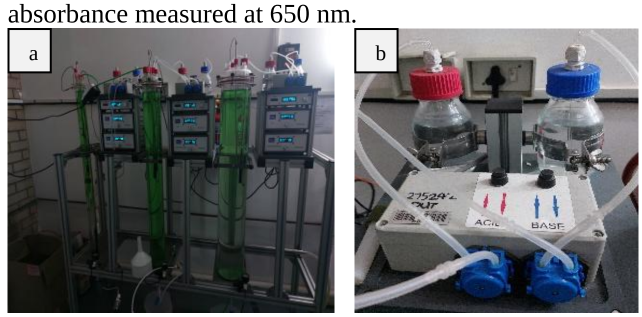

Fig. 1: (a) automated triple bubble column cultivation apparatus; (b) automatic pH control mechanism

III. Results

A. Daily results achieved during the cultivation period in columns C1, C2 and C3

Average daily growth monitoring accompanied by pH and DO management displayed growth rates peaking at the exponential growth phase and decreasing during the stationary and decline phases. During the peak photosynthetic and respiration activity period, the pH was largely influenced by the presence of carbon dioxide. During photosynthesis, the co-culture absorbs CO_2 present in the growth medium and converts it to organic carbon, hence decreasing the concentration of dissolved CO_2 to an equilibrium level of carbonic acid (H_2CO_3). As a result, H_2CO_3 breaks down into H_2O and CO_2, decreasing the concentration of free hydrogen (H+) ions in the cultivation medium. This results in an increase in pH concentration. This pH spike is noticeable during all light periods and photosynthetic activity, with more drastic fluctuations during the exponential growth period. However, the automated bubble column was able to stabilise the pH within the optimum pH range 8,3. During the operation of the bubble column, CO_2 is injected to aid with growth and to stabilise pH by buffering changes, as CO_2 replenishes carbonic acid in the system.

The daily analysis of the growth of *Scenedesmus sp*. and *Lemna sp*. within the bubble column reactors followed distinct phases influenced by variations in pH, dissolved oxygen (DO), CO_2 availability, and reactor hydrodynamics. During the lag phase (Days 0 – 2), minimal metabolic activity was observed as the microalgae and duckweed adapted to the new environment. CO_2 uptake was low, leading to stable pH levels, while DO remain at its lowest due to limited photosynthetic activity. Differences in reactor diameter did not significantly impact growth during this phase, but the smaller column (C1: 5 cm) exhibited higher turbulence, which likely hindered early-stage CO_2 retention, whereas the larger columns (C2: 10 cm and C3: 15 cm) facilitated more gradual CO_2 diffusion. As the culture entered the exponential growth phase (Days 3 – 7), a sharp increase in biomass productivity was observed, driven by high CO_2 uptake and oxygen production from photosynthesis. pH fluctuations became more pronounced, particularly in C1, where rapid CO_2 depletion caused erratic shifts in alkalinity, whereas C2 maintained a more stable pH profile due to balanced CO_2 dissolution and gas-liquid mass transfer efficiency. DO levels peaked during this phase, reflecting the highest photosynthetic activity, with C2 achieving the most efficient oxygen retention, supporting its superior growth rate of 0,74/day, compared to 0,68/day in C3 and 0,52/day in C1. In the absence of light, photosynthetic activity stops, and the co-culture undergoes a respiration process. During this process, CO_2 is given off into the growth medium, increasing the concentration of the dissolved carbon and carbonic acid. This further leads to an excessive increase in H+ ions and a decrease in the overall pH of the cultivating medium.

The stationary phase (Days 8 – 12) marked stability in growth as CO_2 and nutrient limitations began to set in. The pH remained elevated due to ongoing photosynthesis but stabilized as respiration rates increased. DO levels declined slightly, though C2 maintained better oxygen retention, preventing premature growth inhibition. Conversely, C1 exhibited more rapid pH drops, suggesting CO_2 depletion, while C3 experienced localized dead zones that impeded uniform nutrient and gas distribution. The decline phase (Days 13 – 18) was characterized by a significant reduction in growth rates as nutrient depletion and CO_2 imbalances led to increased respiration and oxygen stress. The pH gradually decreased as CO_2 accumulation shifted the system toward acidity, while DO levels dropped sharply, particularly in C1, where poor oxygen retention led to localized anaerobic conditions. C3 also experienced mixing inefficiencies that contributed to cellular stress and biomass loss, whereas C2 maintained the most stable decline, allowing for prolonged productivity. Overall, column C2 emerged as the optimal system, providing the best balance between CO_2 availability, oxygen retention, and mixing efficiency, leading to enhanced biomass productivity and a more controlled pH and DO environment. These findings reinforce the critical role of hydrodynamic optimization in reactor design, ensuring stable conditions for large-scale biofuel production and wastewater treatment applications.

B. Hourly results of bubble column operation and trends observed during the cultivation period in columns C1, C2 and C3

The hourly variations in pH and dissolved oxygen reveal the dynamic interactions between photosynthesis, respiration, gas-liquid exchange, and hydrodynamic efficiency within the bubble column reactors of varying diameters. These fluctuations provide critical insight into the stability of the cultivation environment, the efficiency of CO_2 utilization, and the reactor’s ability to maintain optimal conditions for microalgal growth. The timing and magnitude of pH and DO shifts directly correlate with light exposure, mixing patterns, and gas exchange efficiency, all of which impact biomass productivity and reactor performance.

1) pH and dissolved oxygen fluctuations

During the first 1 – 11 hours of each day, pH was observed to rise, indicating photosynthesis activity taking place and consuming CO_2. After 12 hours, pH decreased at a slow rate until the light illumination was switched off (dark period), causing pH to drop more drastically. Furthermore, it is seen that a drastic spike in pH was observed at the start and end of the exponential growth phase and thereafter stabilizing during the exponential phase with no drastic changes in between. This explains the active photosynthetic activity taking place during these days. However, during the decline growth phase, the pH was constantly high, possibly due to an over-efficient supply of CO_2 facilitated through the gas exchange period within the column.

Comparing the data trends between the smallest column against the largest column, faster pH fluctuations were present in the smaller column (C1) due to the limited gas-liquid interface area and possibly less CO_2 retention, contributing to the sub-optimal growth of microalgae. C2 and C3 provided a larger interface for gas-liquid transfer, hence enhancing CO_2 dissolution within the column and maintaining a more stable pH environment. C1 displayed a greater pH spike as compared to C2 and C3, illustrating that the diameter of the column and mixing efficiency have a direct impact on pH fluctuations.

Likewise, the DO levels shared a similar trend to the growth rate as the DO levels increased during photosynthetic periods and periods of high light illumination. C1, C2 and C3 indicate DO levels increasing through the exponential and stationary phases, with a gradual decrease in the decline phase. DO was observed to decrease during dark phases or in poorly mixed systems (C1), leading to anaerobic zones that inhibit growth. Column C1 experienced much lower DO levels (2 – 5,7 mg/L) due to faster saturation of DO cause by limited volume and mixing; hence leading to higher oxygen stress. The wider columns (10 cm and 15 cm) supported better gas exchange and mixing, hence higher DO levels (2,6 – 7,3 mg/L) and (2,9 – 8,4 mg/L) were observed in each of the systems, supporting the optimal growth range.

2) Hydrodynamics and mixing patterns

The column diameter and optimal hydrodynamics directly influence the efficient mass transfer of gases (CO_2 and O_2), preventing dead zones; hence, the proper column diameter, aeration rates and the balance of shear stress with nutrient distribution is crucial. C1 experienced larger bubbles, higher turbulence, higher shear stress and more intense mixing, causing damage to fragile cells and negatively affecting growth rates (maximum μ = 0,52). The smaller columns lead to higher gas hold ups, resulting in smaller residence times and reduced gas efficiencies. Larger columns (10 cm and 15 cm) experienced lower shear forces and a more uniform mixing regime, promoting even light distribution and minimizing the shear stress on the cells. The gas hold up present in the larger columns was lower, enhancing CO_2 distribution due to prolonged residence time.

3) Lighting efficiency and active photosynthetic activity

The 18-hour light and 6-hour dark period directly affected photosynthetic activity, which affected CO_2 concentrations and inturn, the pH fluctuations. Column C1 experienced more drastic pH fluctuations as compared to the larger columns C2 and C3, due to the narrower columns experiencing higher light penetration per unit volume, compared to the larger columns facing limited light exposure towards the middle of the culture volume and depth culture density, creating a self-shading effect (optical path is longer). This further motivates the slower, gradual pH fluctuations experienced in the 15 cm column. However, decreased light intensity in the wider column C3 can easily be mitigated to sustain microalgal growth by efficient mixing and proper aeration patterns.

4) Mass transfer and gas exchange

Mass transfer and gas exchange share a direct relationship with the hydrodynamics and mixing patterns present in the column. It was evident that the smaller column C1 has a lesser surface area for gas exchange, limiting CO_2 availability and nutrient distribution; hence, the lower growth rate was achieved. Columns C2 and C3 have larger surface area to volume ratios, increasing the gas exchange efficiencies. However, it is necessary to adjust the air flow rate as CO_2 depletion in higher deeper regions is prone to occur. This is seen as the pH and DO levels were much lower during the exponential phases of column C1, as compared to C3. The wider column was able to provide sufficient DO for optimum growth, but dead zones were present.

The hourly pH and DO data confirm that optimal reactor design is crucial for maintaining stable growth conditions in bubble column reactors. While C1 suffered from rapid pH and DO shifts due to limited gas retention and excessive turbulence, and C3 faced mixing inefficiencies and oxygen stratification, C2 provided the best balance of gas exchange, pH stability, and DO retention, making it the most efficient system for large-scale biofuel production and wastewater treatment. These findings emphasize the importance of reactor hydrodynamics to prevent growth-inhibitory conditions, ensuring stable and sustainable biomass cultivation.

C. Monoculture versus Co-culturing

Tables 1 and 2 provide a comparative analysis of mono-culturing vs. co-culturing and the optimized bubble column configurations, offering insights into the impact of reactor scale-up, hydrodynamics, and co-cultivation strategies on biomass productivity and growth rates. These results highlight the critical role of reactor diameter, sparger design, and aeration efficiency in enhancing microalgal cultivation.

Fig. 2: (a) Illustration of co-cultivation attached surface methodology; (b) Harvested biomass

D. Impact of Reactor Scaling and Co-Culturing

Table 1 illustrates the effect of reactor upscaling on biomass productivity and growth rates under both monoculture (*Scenedesmus sp*.) and co-culture (*Scenedesmus sp*. with duckweed) conditions. The results demonstrate a clear enhancement in growth performance with co-cultivation, suggesting that duckweed contributes to improved nutrient utilization and enhanced gas exchange. Across all reactor sizes, co-cultivation resulted in higher growth rates and cumulative biomass production, supporting the hypothesis that synergistic interactions between microalgae and duckweed enhance overall productivity.

The 30 L reactor exhibited the highest mono-culture growth rate (0,752/day), likely due to smaller working volumes, efficient gas exchange, and uniform light exposure. However, when scaled up to the bubble column reactors, the growth rates declined, emphasizing the challenges associated with larger-scale cultivation, such as mixing inefficiencies and light limitations. Among the three bubble column reactors, C2 (10 cm) exhibited the highest co-culture growth rate (0,74/day), surpassing both C1 (0,65/day) and C3 (0,68/day). The superior performance of C2 is attributed to its optimal balance between mixing, gas retention, and light penetration, which facilitated higher biomass accumulation compared to the narrower C1 or the larger C3, where dead zones likely inhibited uniform growth. Despite C3 having a larger gas-liquid interface and better CO_2 retention, it exhibited slightly lower productivity than C2. This could be due to mixing inefficiencies and light attenuation in deeper zones, which limited photosynthetic activity and biomass accumulation. C1 showed the lowest mono-culture growth rate (0,585/day), likely due to higher turbulence and rapid CO_2 stripping, preventing sustained carbon availability for microalgal uptake. However, co-cultivation improved growth rates (0,65/day), reinforcing the idea that *Lemna sp*. helps mitigate carbon limitations and enhances nutrient assimilation.

These findings emphasize that reactor design and cultivation strategies must be optimized in tandem to maximize productivity. While larger reactors offer potential for upscaling, their hydrodynamic limitations (e.g., dead zones, light attenuation) must be carefully managed to prevent productivity loss. Furthermore, co-cultivation served as an attached cultivation methodology in the study. The integration of attached cultivation within the bubble column reactors significantly improved biomass yield by enhancing surface area utilization, nutrient uptake efficiency, and ease of harvesting. Compared to traditional suspended cultivation, where microalgae remain dispersed in the liquid phase, co-cultivation provides a natural attached cultivation method, with a stable growth environment, reducing shear stress and cell loss due to turbulence. This method also minimized cell sedimentation and washout, which are common challenges in large-scale photobioreactors.

The co-cultivation strategy further amplified biomass productivity, as *Lemna sp*. provided a natural matrix for *Scenedesmus sp*. attachment, facilitating enhanced gas exchange and CO_2 capture. The floating Lemna layer likely contributed to micro-environmental stability, reducing pH fluctuations and localized oxygen depletion, thereby sustaining higher photosynthetic efficiency. Additionally, biofilm formation on the reactor walls and Lemna surfaces allowed for better light penetration, preventing self-shading effects observed in deeper regions of the reactor. The dual nature of attached and suspended growth ensured continuous biomass accumulation while maintaining optimal nutrient cycling. The harvested biofilm exhibited dense algal attachment, highlighting the effectiveness of surface immobilization in improving microalgal retention and reducing harvesting costs. The combination of co-culture/attached and suspended growth modes resulted in a cumulative biomass yield of 700.46 mg, compared to 371.25 mg per 7.5 L during monoculture, demonstrating a nearly 53 % increase in biomass productivity. The reduction in harvesting costs due to pre-attached biomass is a crucial advantage for large-scale biofuel production, as it eliminates the need for energy intensive dewatering processes such as centrifugation and filtration. The findings emphasize the potential of attached cultivation in enhancing biofuel production efficiency by addressing key limitations of traditional photobioreactors. This approach offers a scalable and economically viable solution for sustainable biomass generation, wastewater treatment, and CO_2 sequestration, reinforcing the benefits of integrating co-cultivation strategies with innovative reactor designs.

TABLE I
THE EFFECT OF UPSCALING REACTOR CONFIGURATIONS DURING MONO-CULTURING AND CO-CULTURING PERIODS

	30 L reactor	C1	C2	C3
Column diameter (cm)	30	5	10	15
Column height (cm)	50	100	100	100
No. of sparger holes	50 x 2 spargers	18	30	45
Size of holes (mm)	0,1	0,45	0,35	0,25
Maximum monoculture growth	0,752	0,585	0,664	0,602
Maximum cumulative co-culture growth	0,829	0,65	0,74	0,68

E. Optimized growth performance in the 10 cm bubble column

Mono-culture cultivation (*Scenedesmus sp*.) achieved a growth rate of 0,664/day, with biomass productivity of 49,50 mg/L/day, accumulating 371.25 mg/7.5 L over 18 days. Co-cultivation resulted in a higher growth rate (0,75/day), with increased biomass productivity of 700.46 mg, leading to an almost 53 % higher biomass than the mono-culture system (Table 2).

The increase in biomass productivity under co-culture conditions can be attributed to multiple factors, including enhanced nutrient recycling by *Lemna sp*., increased CO_2 sequestration, and improved gas exchange dynamics. The floating duckweed layer likely contributed to better oxygenation and reduced photo-inhibition, leading to more efficient photosynthetic performance and higher lipid accumulation potential for biofuel applications.

The optimized reactor configuration in C2 allowed for efficient gas-liquid mass transfer, minimizing shear stress while maintaining sufficient turbulence to prevent sedimentation. Additionally, the moderate sparger hole size (0,35 mm) and sparger distribution ensured uniform bubble dispersion, preventing localized CO_2 depletion or oxygen saturation stress, which were issues observed in C1 (high turbulence) and C3 (dead zones). These results validate the study's hypothesis that reactor optimization, coupled with strategic co-cultivation, enhances microalgal biomass productivity and growth efficiency. The 10 cm diameter column emerged as the best-performing system, achieving the highest biomass yield and most stable hydrodynamic conditions, making it the optimal configuration for large scale biofuel production and wastewater treatment applications. The 10 cm bubble column reactor (C2) proved to be the most effective configuration, balancing light penetration, hydrodynamic efficiency, and gas retention, which led to the highest biomass productivity.

TABLE II
FINAL OPTIMISED BUBBLE COLUMN C2 DATA WITH MAXIMUM GROWTH RATE AND BIOMASS PRODUCTIVITY

	Monoculture	*Co-culture*
Growth rate (per day)	0,664	0,74
Biomass Produced (mg/L/day)	49,50	93, 395
Cumulative biomass (mg)	371,25	700,46

IV. Discussion

This study demonstrated the significant impact of hydrodynamic conditions on biomass productivity in bubble column reactors, particularly concerning column diameter, gas exchange, and flow regimes. The observed trends in pH fluctuations, dissolved oxygen levels, and mixing efficiency aligned with several prior studies, reinforcing the importance of optimizing reactor design for enhanced microalgal growth. Several studies support and align with these findings. Nauha and Alopaeus (2013) emphasize the role of light intensity in biomass production, noting that while increased light enhances growth, it is limited at the center of wider columns. Their research further cautions against using maximum light intensity, as it may lead to inaccurate results. Instead, maintaining an optimal range and employing co-culturing strategies help in self-shading and preventing excessive illumination, similar to what was observed in this study.

Dudukovic *et al*. (2019) provide insights into the interplay between bubble column hydrodynamics and mass transfer, reinforcing the significance of flow regimes, which were also evident in this study. Wild *et al*. (2003) investigated hydrodynamics, computational fluid dynamics (CFD) modelling, and airlift reactors, confirming that bubble column operation is highly dependent on internal flow regimes, a conclusion that aligns with the results of this research. Esmaeilli *et al*. (2016) reported that gas hold-up and increased pressure significantly impact mass transfer, mirroring this study's results. Wu and Merchilli (2002) demonstrated that biomass yield in a 13 L bubble column decreases as column diameter increases, reinforcing the importance of identifying an optimal column size to maximize productivity, a critical consideration in this study's reactor setup. Gemelolo *et al*. (2018) further explored column diameter, sparger types, and bubble size effects, validating the mechanisms of bubble coalescence and breakage. Their findings also support the understanding of inhibitory flow behaviors, which were evident in this study. Alhamed, Edris, and Gadelhek (2014) evaluated the impact of CO_2 sequestration in a bubble column using Chlorella vulgaris, with their findings confirming that CO_2 levels significantly affect pH balance. Depra *et al*. (2020) analyzed large-scale challenges in photobioreactor setups, emphasizing the necessity of optimization for economic and environmental sustainability. This aligns with the current study's findings on reactor efficiency and operational feasibility. Stephenson *et al*. (2010) reported that raceway pond cultivation is more environmentally sustainable than open ponds, with 80 % lower global warming potential. Uyar *et al*. (2024) compared different cultivation systems and concluded that bubble columns exhibit the highest volumetric mass transfer and growth efficiency.

V. Conclusion

This study's results highlight that optimizing reactor design, particularly column diameter and gas exchange efficiency, can significantly enhance biomass yield. Although the 5 cm column experienced greater turbulence, larger bubbles and higher light intensities, challenges such as frequent pH fluctuations, inadequate DO retention and oxygen stress were evident. The rapid decline in DO during the dark phase (reaching as low as 2.1 – 2.6 mg/L) and unstable pH limited overall growth in this column. In contrast, the wider columns demonstrated better mixing, agitation and homogenous air supply, leading to more stable pH and DO levels. The 10 cm column exhibited the most consistent pH (~ 8.3 – 8.5) and DO levels (~ 5.2 – 5.5) during the stationary phase, preventing inhibitory effects observed in the smaller column. Light distribution was initially a limiting factor; it was effectively mitigated with adjusted aeration and CO_2 supply, ensuring sustained photosynthetic activity. The 10 cm diameter column emerged as the most effective, maintaining stable hydrodynamic conditions and striking a balance between light penetration, mixing efficiency, and gas-liquid mass transfer, ensuring sufficient oxygenation and CO_2 availability throughout the cultivation period. This supports the argument that reactor design is essential in improving biofuel production efficiency. The study reaffirms the viability of co-cultivation in enhancing biomass productivity, by the increased growth rates in co-culture conditions. Future research should explore additional reactor configurations and aeration techniques to further optimize large-scale production.

VI. Conflict of Interest

The authors declare no conflict of interest.

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