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Optimization of hydrodynamics for enhanced co-cultivation in bubble column reactors: Investigating flow dynamics and biomass productivity

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ABSTRACT

Hydrodynamic effects on algal cultivation have been studied, but limited work has addressed how bubble column diameter influences the co-cultivation of microalgae and duckweed in sewage wastewater systems and biofuel production. This is the first empirical study that fills the gap by experimentally evaluating the co-cultivation of *Scenedesmus sp.* and *Lemna sp.* (Duckweed) in wastewater fed reactors. This study evaluated how bubble column diameter and co-cultivation with Lemna sp. influence the biomass productivity and hydrodynamics of Scenedesmus-Lemna regimes. Three bubble columns with internal diameters of 5, 10, and 15 cm were operated under identical aeration (3 – 9 L/hr) and illumination conditions in both monoculture and co-culture configurations. The 10 cm column (C2) achieved the highest performance in co-culture, with a specific growth rate of 0,74 ± 0,05 mg d^{-1} and biomass productivity of 93,85 ± 4,12 mg L^{-1} d^{-1}, representing almost 90% increase compared with monoculture. Additionally, co-culturing *Scenedesmus sp.* with Duckweed led to an 11 % increase in growth rates. Increased biomass suggests potential for increased biomolecules, including lipids and hydrocarbons. Co-cultivation also stabilized pH and dissolved oxygen and promoted attached growth on Lemna roots, offering a low-cost and novel attached harvesting pathway. These results highlight that reactor geometry and co-cultivation strategies are critical determinants of algal productivity and can substantially influence productivity and harvesting efficiency in large scale algal phycoremediation systems and enhance 3stability in wastewater treatment systems.

1. Introduction

The urgent need to reduce carbon emissions, mitigate climate change and address fuel depletion has created the need to develop a renewable and sustainable energy source. Carbon emissions are rising at an unprecedented rate, with an expected increase of 30 % by 2030 if no intervention is developed [1]. 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) compares 2010 levels and stresses that human caused CO_2 emissions must decrease by approximately 45 % by 2030 and reach net zero by 2050 [2]. 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 utilise CO_2 in the form of carbon capture for the generation of clean energy. They can utilise approximately 50 times more than terrestrial plants [3]. Microalgae are particularly attractive as a feedstock for biofuel production due to their high lipid yield, fatty acid content, ability to cultivate on non-arable land and their ability to utilize wastewater. Microalgae are highly advantageous compared to other biofuel producing food crops [4]. However, large scale cultivation faces significant challenges such as contamination, evaporation, high land demand and excessive harvesting costs [5]. The challenge of scaling up remains a daunting and ongoing challenge for several years. The majority of previous studies of large-scale setups utilise outdoor raceway ponds, occupying space instead of photobioreactors (PBR). Unlike normal PBRs, bubble columns photobioreactors (BCPBR's) are cylindrical vessels of liquid in which gas

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is "bubbled" through. These have emerged as alternatives to open ponds, with a simple design, high mass transfer, reduced contamination risks, less occupation of land space, low operational costs, no mechanically moving parts and efficient CO_2 utilisation. Although BCPBR gained significant attention as a benchmark reactor for the last 20 years, much interest remains in improving its performance and efficiency. Most hydrodynamic studies on bubble columns have focused on single species cultivation or computational modelling. There is a lack of experimental evidence on the actual hydrodynamic effects, conditions (particularly reactor diameter) and co-cultivation strategies that may improve productivity and harvesting [6,7]. The synergistic relationships between microalgae and other organisms cannot be ignored ([8] and 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*.

Another challenge in utilizing microalgae for biodiesel production involves the harvesting process and its associated cost. Lipid-accumulating microalgae are unicellular, low in density, and suspended in liquid, therefore complicating separation [9]. Large-scale lipid extraction remains technically complex and is still under development, with ongoing research to mitigate these limitations [5]. Incorporating Duckweed offers synergistic benefits when combined with microalgae, including nutrient recycling, surface attachment for biofilm growth and extended surface areas, and natural attached methods for harvesting.

Previous studies on bubble column reactors have largely focused on hydrodynamic optimization under synthetic media or monoculture conditions, often neglecting the combined role of reactor geometry and interspecies co-cultivation. In contrast, this study addresses these research gaps by integrating two critical but underexplored dimensions: (i) experimentally evaluating bubble column diameters under controlled wastewater based cultivation conditions, (ii) assessing the hydrodynamic effects on dissolved oxygen (DO), pH and mixing patterns and (iii) evaluating the novel use of Lemna sp. to stabilize hydrodynamics and enable attached-growth harvesting. Additional novelty lies in utilising state of the art novel equipment for the integration of hydrodynamic optimisation with attached cultivation to provide scalable solutions for bioenergy production and wastewater treatment. By coupling reactor-scale hydrodynamic optimization with a biologically-assisted co-cultivation strategy, this work extends beyond traditional hydrodynamic studies and provides a scalable, low-cost pathway for wastewater treatment and biomass recovery.

2. Industrial wastewater phycoremediation

Industrialisation, globalisation and population growth lead to an increase in the volumes of wastewater produced. Wastewater is generated in large volumes from industries (dairy, brewery, sugar, sewage and refinery), municipalities and households; and dissipates into the environment without adequate treatment, causing severe damage to ecosystems, human health and the environment. However, these large volumes are not solely considered as waste, but rather a nutrient-rich source and excess CO_2. Microalgae have been widely recognised and proven effective in treating wastewater with high concentrations of nitrogen (N_2), phosphorus (P), heavy metals and toxic organic chemicals. The use of microalgae in the bio-treatment of wastewater is an attractive method to convert solar energy to valuable biomass, while utilising nutrients, heavy metals and pollutants present in the wastewater. Several advantages associated with the biotransformation of pollutants by microalgae have been proven to be an effective method in industrial wastewater treatment [5].

Microalgae photosynthesis allows CO_2 fixation from both atmospheric and industrial emissions while generating O_2 to support and enable microbial degradation of organic matter in wastewater [4,10]. The nutrient mechanism used by microalgae is clearly understood by researchers to optimise the efficiency of the algae growth and performance when utilised in wastewater treatment processes. Algae mainly utilise essential nutrients for effective growth, namely carbon, nitrogen, phosphorus and sulphur; as well as several other trace elements such as potassium, sodium, iron, calcium and magnesium. This enables their adaptability and growth in diverse effluent streams. Recent studies confirm that phycoremediation simultaneously achieves nutrient removal and biomass generation, positioning algae as central to sustainable wastewater management (Takahashi *et al.*, 2024).

Recent advances in reactor engineering highlighted the importance of reactor design and hydrodynamics in enhancing algal biomass productivity. BCPBRs in particular offer high volumetric mass transfer, low contamination risks, and efficient CO_2 utilisation compared to airlift or stirred-tank designs [11]. In this study, specific diameters (5 cm, 10 cm and 15 cm) were selected to evaluate and optimise the influence of increasing reactor scale on mixing, bubble distribution and light penetration, as these parameters are known to strongly influence growth kinetics [12,13]. A 50:50 air/N_2 mix was chosen to balance carbon availability with controlled oxygen levels, thereby simulating industrial aeration conditions while reducing oxidative stress on cultures. These choices were made to ensure that the experimental setup not only generated comparable results with the literature but also reflected realistic operational conditions.

A recent review noted that pairing microalgae with complementary organisms significantly enhances biomass accumulation and wastewater treatment performance compared to monocultures [14]. For example, co-utilization of microalgae with heterotrophic microorganisms has achieved over 90 % phosphate and 80 % organic carbon removal efficiencies without the need for energy-intensive aeration (Takahashi *et al.*, 2024). These findings highlight the potential of mixed systems to lower operational costs while improving treatment outcomes. Our study contributes to this growing field by experimentally co-cultivating *Scenedesmus sp.* with *Lemna sp.* under controlled hydrodynamic conditions in bubble columns. *Lemna sp.* has recently gained attention for its advantages in wastewater applications, including rapid growth and high protein yield. Besides Duckweed being the fastest growing plant on Earth, its large-scale reviews demonstrate the potential of duckweed-based bioreactors for cost-effective wastewater remediation and biomass production, especially in controlled indoor systems [15]. Unlike microalgae alone, Duckweed provides a natural platform for attached cultivation, facilitating biofilm formation, enhancing gas exchange, and simplifying harvesting. These properties directly address one of the main bottlenecks in algal biofuel production, the high cost and complexity of harvesting suspended biomass [16,17].

Beyond the actual cultivation process, conventional microalgae harvesting methods such as flocculation, sedimentation, flotation and centrifugation are associated with high costs (de Assis *et al.*, 2019). 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 [18]. Attached cultivation is highly adaptable and compatible with most microalgal strains. Zhao *et al.* [19] further demonstrated that the physicochemical properties of the supporting substrate significantly influence microalgal attachment. Their study identified the highest attached biomass of *Scenedesmus sp.* as 414.74 mg/L when cultivated in swine wastewater, with notable improvements in pollutant removal efficiency as well as protein and lipid accumulation. 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

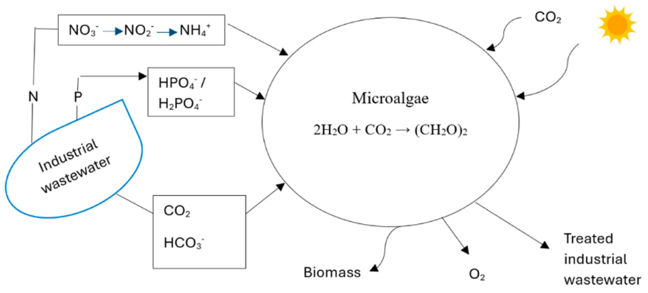

Fig. 1. Microalgal mechanism followed during photosynthesis.

pre-attached to the supporting material, the harvesting process is streamlined, leading to higher biomass recovery than in conventional suspended monoculture systems. Therefore, the synergistic effects of co-culturing *Scenedesmus sp.* and *Lemna sp.* not only improve hydrodynamic balance and biomass yield but also provide a practical pathway to overcoming cost barriers in algal harvesting.

By bridging the gap between theoretical modeling and practical application, this study contributes new insights into reactor optimization for scalable biofuel production and wastewater treatment, setting a foundation for future large-scale implementations. Integration of co-cultivation of *Scenedesmus sp.* and *Lemna sp.* is a promising technique for the viability of biofuel production from microalgae. By combining phycoremediation with innovative reactor engineering and co-cultivation strategies, this study contributes to the ongoing optimization of scalable microalgal cultivation systems for both wastewater treatment and biofuel production. This research builds upon existing knowledge and contributes to novel optimization of bubble column reactors for biofuel production.

Although the cultivation of microalgae is generally a simple and effective process, several limiting parameters that affect growth are present, such as the cultivation period, stock culture, pH, light, DO, aeration, flow rate and temperature. These factors are crucial and have tolerable value ranges necessary for optimal algal growth and biomass generation

- pH: The general pH range for microalgae cultivation ranges between 7 – 9, with optimum values between 8,2 – 8,7 [20]. pH fluctuations during cultivation can lead to a full collapse of the cultivation process. Khalil et al [21] and Han et al. [22] stated that the pH also affects the content of constituents present in the microalgae, such as proteins, pigments and lipids, among others.
- Light illumination: Biological processes occurring in wastewater require the presence of sunlight and CO2. Light as an energy source in the form of light intensity and illumination period has a significant role in microalgal growth. However, light intensity as a primary source also depends on the depth, volume, and density of the cultivation environment. Sfroza et al. [16] further proved that artificial lighting (fluorescent tubes) is sufficient for growing microalgae, although overheating and excessive lighting (photoinhibition) remain a challenge ([23,24] and Dosadas et al. 2014, [25])
- Temperature: The optimum temperature range for cultivating microalgae lies between 20°C – 30°C, with tolerable ranges between 16°C – 27°C. Temperatures below 16°C cause a decrease in microalgae growth rates, whereas temperatures over 35°C have a damaging effect on microbial cells (Anouch et al, 2019). Furthermore, Converti et al. [26] stated that temperature also has a significant effect on the ability of lipid accumulation in the microalgal species.
- Carbon dioxide (CO2): Besides the numerous other nutrients, carbon in the form of CO2 is a necessity for microalgal growth; usually provided in the form of bicarbonates, carbonates, or organic carbon compounds that are supplied to the reactor. Although carbon is the primary necessity, it is vital to have efficient mixing and proper aeration regimes present for optimum mass transfer and nutrient distribution.

All these single factors directly affect the microalgal cultivation growth period. However, their interdependent interactions also greatly complicate and significantly modulate microalgal growth dynamics. The combinatory and/or antagonistic interactions in effect between these parameters affect productivity, cellular composition and ultimately the biomass yield. Therefore, understanding these relations in detail is important to optimize growth conditions. It is necessary to set and maintain such optimal ranges before proceeding to the analysis of the hydrodynamic components of the cultivation environment. Characterization and control of target ranges are a prerequisite to hydrodynamic studies of the cultivation environment due to the interdependence of microalgae physiological responses with fluid dynamics and mass transfer efficiency.

3. Materials and methods

3.1. Strains and media

Scenedesmus sp. and *Lemna sp* were collected from Nelson Mandela University (NMU) and a localized freshwater pond in Durban, South

Fig. 2. Experimental setup of bubble column apparatus (C1, C2, C3), (b) automatic pH controller, and (c) flowmeter.

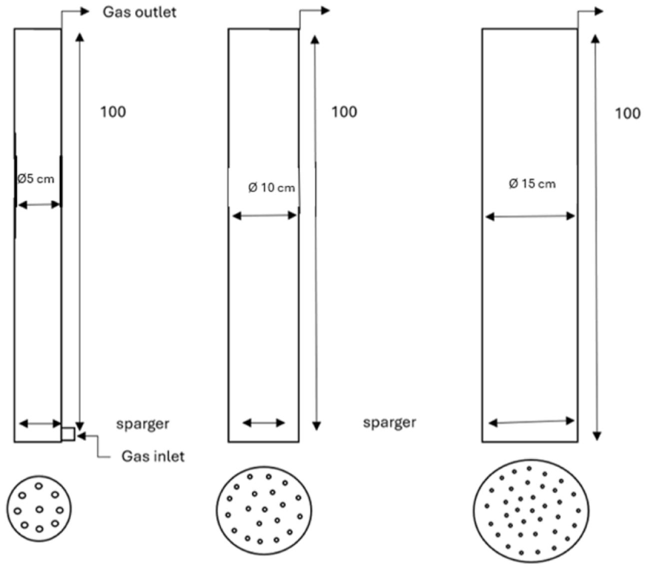

Fig. 3. Axial section (cm) of individual bubble columns C1, C2 and C3 with its associated perforated spargers type.

Africa. The cultures were grown in a 3:1 growth medium ratio of sewage wastewater and Bold's Basal Medium (BBM), pretreated with ultraviolet (UV) radiation treatment. This ratio and nutrients were selected to support optimal growth based on prior bench-scale and 30 L PBR optimization studies. Bold's Basal Medium had a stock composition of 25.0 g/L $NaNO_3$, 2.5 g/L $CaCl_2•2H_2O$, 7.5 g/L $MgSO_4•7H_2O$, 7.5 g/L K_2HPO_4, 17.5 g/L KH_2PO_4, 2.5 g/L NaCl, 50.0 g/L EDTA, 31.0 g/L KOH, 4.98 g/L $FeSO_4•7H_2O$, 1.0 mL/L H_2SO_4, 11.42 g/L H_3BO_3, 8.82 g/L $ZnSO_4•7H_2O$, 1.44 g/L $MnCl_2•4H_2O$, 0.71 g/L MoO_3, 1.57 g/L $CuSO_4•5H_2O$, 0.49 g/L $Co(NO_3)_2•6H_2O$ (Bischoff and Bold, 1963).

3.2. Experimental set-up of a bubble column

Fig. 1.

Three glass bubble column reactors (height: 1 m with 95 % working volume; internal diameters: 5 cm, 10 cm and 15 cm) were operated simultaneously, exposed to 18-h light: 6-h dark lighting (Fig. 2a). Gas supply with a composition of 50 % N_2 and 50 % air, with periodic CO_2 sparging (5 % volume), was done at the bottom of the column (Fig. 2c) through perforated sparges and mixed with the culture medium in the plenum section. Each sparger has a varying number of holes and diameters (Fig. 3), 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 consistent bubble supply and a homogenous supply of gas. The pH was automatically controlled at 8,3 concentration and recorded hourly by the bubble column programmable logic controller (PLC) unit using pre-loaded solution chambers of sodium bicarbonate and hydrochloric acid (Fig. 2b). The pH and DO data were automatically recorded via probes onto a portable microSD memory card. Daily growth rates were taken by temporarily removing a small sample of culture solution from the drain valve and inserting it into the spectrophotometer (HACH DR 300). To measure its optical density at 650 nm (OD_{650}). A calibration curve was prepared by relating OD_{650} to dry weight (mg/L) using serial dilutions of known biomass: Dry weight (mg/L) = a × OD_{650} + b (R^2= 0.99). Biomass productivity was calculated from dry weight changes over time (Eq. 3). All cultivation experiments were designed using Design of Experiments (Design Expert, version 13) and Box Bhenken design, which incorporated and performed experiments in biological triplicate (n = 3). Data are reported as mean ± standard deviation (SD). Statistical analyses were performed using Analysis of variance (ANOVA), where a significance threshold of $p < 0.0001$ was achieved.

Illustration of experimental setup

3.3. Growth rate and biomass productivity analysis

The kinetic growth of *Scenedesmus sp.* was assessed by determining the maximum growth rate (day^{-1}) using Eqs. (1) and (2) for division per day (Dd) and doubling time (td), respectively. The exponential phase of the growth curve was analysed to obtain the maximum specific growth rate (μ_{max}), following the methodology outlined by Zwietering *et al.* [27] and Lin *et al.* [28]. This parameter was estimated following the approach described by Gani *et al.* [29].

$$\boldsymbol{Dd} = \frac{\boldsymbol{\mu_{max}}}{\boldsymbol{ln}2} \tag{1}$$

$$\boldsymbol{td} = \frac{1}{\boldsymbol{Dd}} \tag{2}$$

Biomass productivity was calculated based on kinetic parameters using Eq. (3) within the phycoremediation experiment. The calculation follows the methodology detailed by Gani *et al.* [29] and Arbib *et al.* [30]:

$$\boldsymbol{Biomass\ productivity} = \frac{\boldsymbol{\mu_{max}}\ (0,9\ \boldsymbol{X_m} - 1,1\ \boldsymbol{X}_0)}{9\ (\ \boldsymbol{X_m} - 1,1\ \boldsymbol{X}_0)1,1\ \boldsymbol{X}_0} \tag{3}$$

The derived growth kinetic parameters provided a basis for estimating biomass productivity under the experimental conditions.

4. Results

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

The relationship between reactor hydrodynamics, including column diameter, pH, DO, and microalgal growth, was evaluated across the three bubble columns with varying diameters (Figs. 4,5,6). These figures illustrate how each parameter varied daily at set time intervals and during the distinct phases of microalgal growth: lag, exponential (log), stationary, and decline (death). During this cultivation process of *Scenedesmus sp.* and *Lemna sp.*, the vital parameters were able to be automatically controlled, preventing deviations that could occur due to photosynthetic and biological activity. All results are presented as mean ± SD of triplicate experiments (n = 3).

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

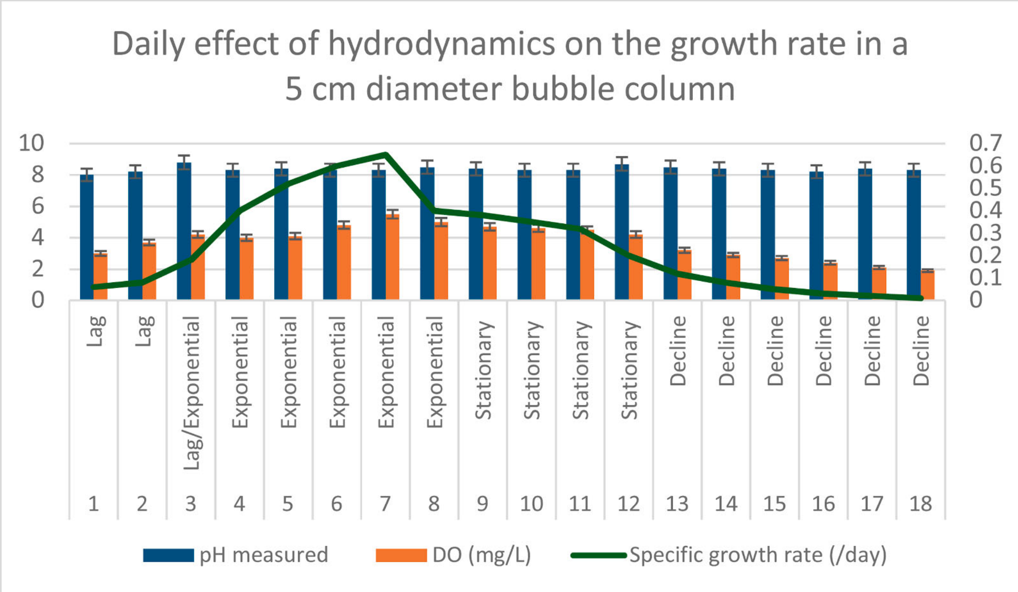

Fig. 4. Illustration of the effect of hydrodynamics on the co-cultivation period in column C1 (5 cm) daily.

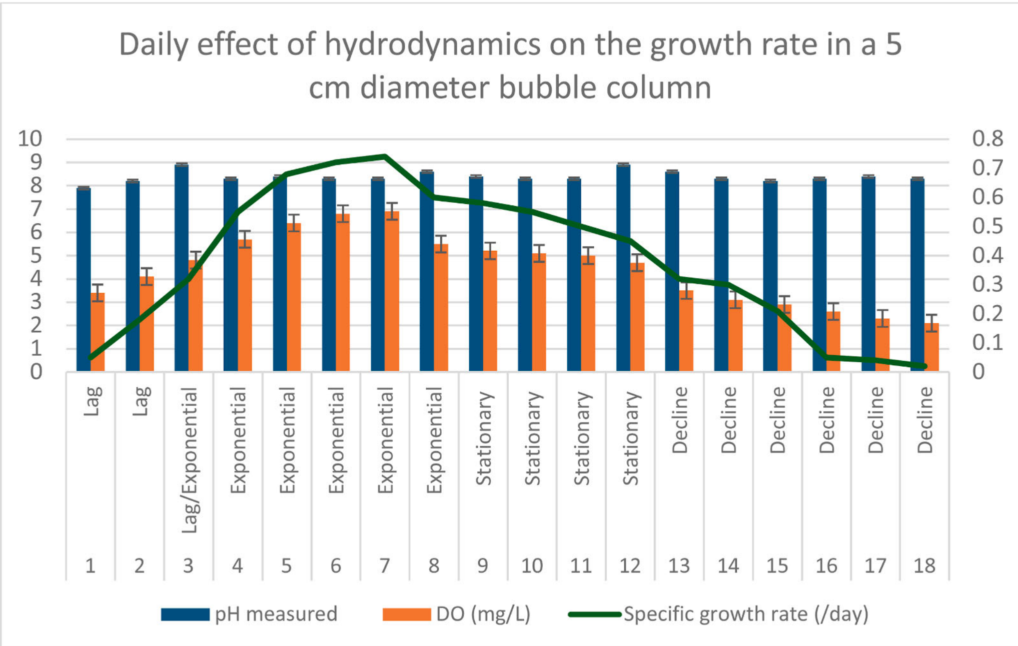

Fig. 5. Illustration of the effect of hydrodynamics on the co-cultivation period in column C2 (10 cm) daily.

result, 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 the 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, 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

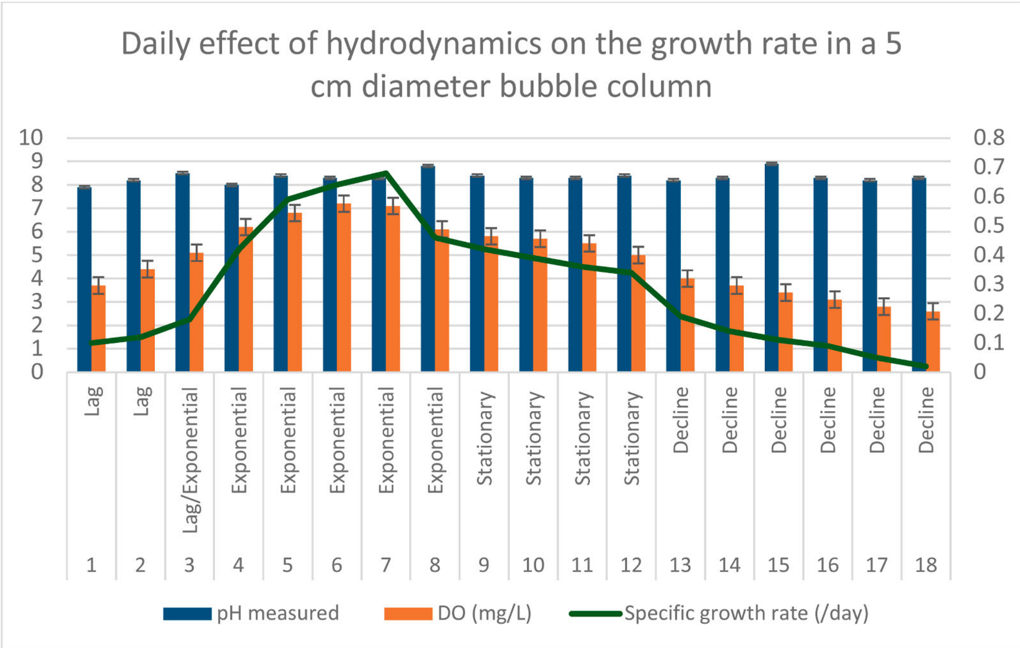

Fig. 6. Illustration of the effect of hydrodynamics on the co-cultivation period in column C3 (15 cm) daily.

retention, supporting its superior growth rate of 0,74 ± 0,05 mg d[-1], compared to 0,68 ± 0,03 mg d[-1] in C3 and 0,65 ± 0,08 mg d[-1] 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, the 10 cm diameter 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

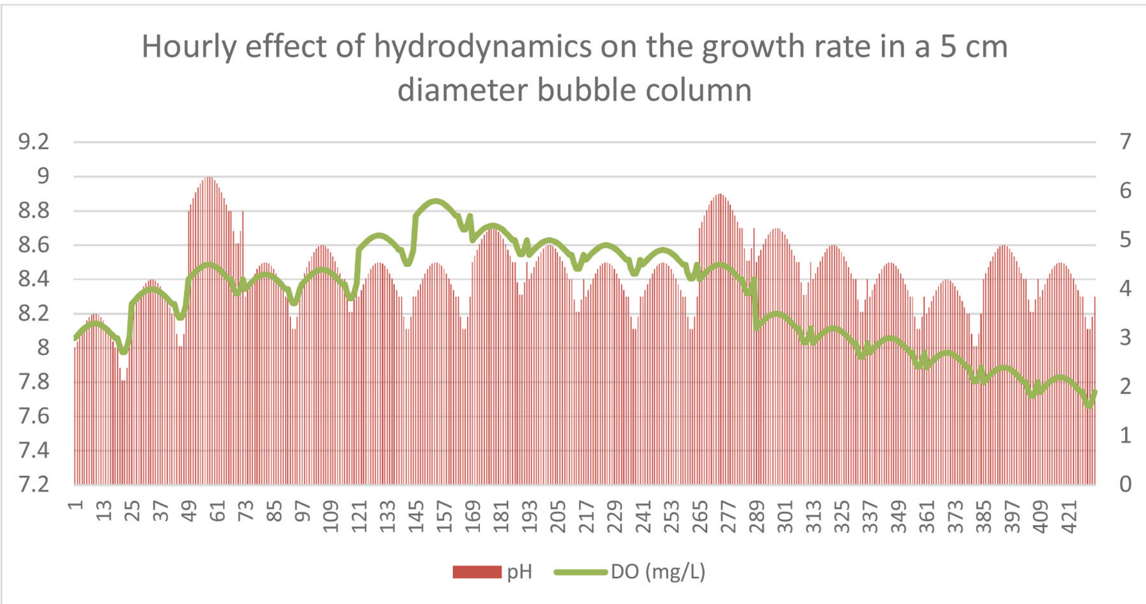

Fig. 7. Hourly deviations of pH and DO in column C1 (5 cm).

biofuel production and wastewater treatment applications.

4.2. 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 DO reveal the dynamic interactions between photosynthetic activity, respiration, gas-liquid exchange, and hydrodynamic efficiency within the 5 cm, 10 cm and 15 cm bubble column reactors (Figs. 7,8,9). 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.

- pH and DO fluctuations/variations and their impact on growth rates

Figs. 7,8 and 9 indicate the rise of pH levels during the light hours of each day, indicating CO_2 availability and uptake during the photosynthetic period. During the first 1 – 11 h of each day, pH was observed to rise, indicating photosynthesis was actively taking place and consuming CO_2. After 12 h, the pH decreased at a slow rate until the light illumination was switched off (dark period), causing the 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 (5 cm diameter) against the largest column (15 cm diameter), 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. The larger columns (10 cm and 15 cm) 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.

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. The 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 caused 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.

- 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 sheer stress with nutrient distribution is crucial. The smaller diameter column C1 (5 cm) experienced larger bubbles, higher turbulence, higher shear stress and more intense mixing causing damage to fragile cells and negatively affecting growth rates (maximum $\mu = 0{,}65$). 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 sheer forces and a more uniform mixing regime, promoting even light distribution and minimising 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.

- Lighting efficiency and active photosynthetic activity

Light intensity during the 18 h light and 6 h dark period directly affected photosynthetic activity, which affected the CO_2 concentrations and in turn the pH fluctuations. Column C1 experienced more drastic pH

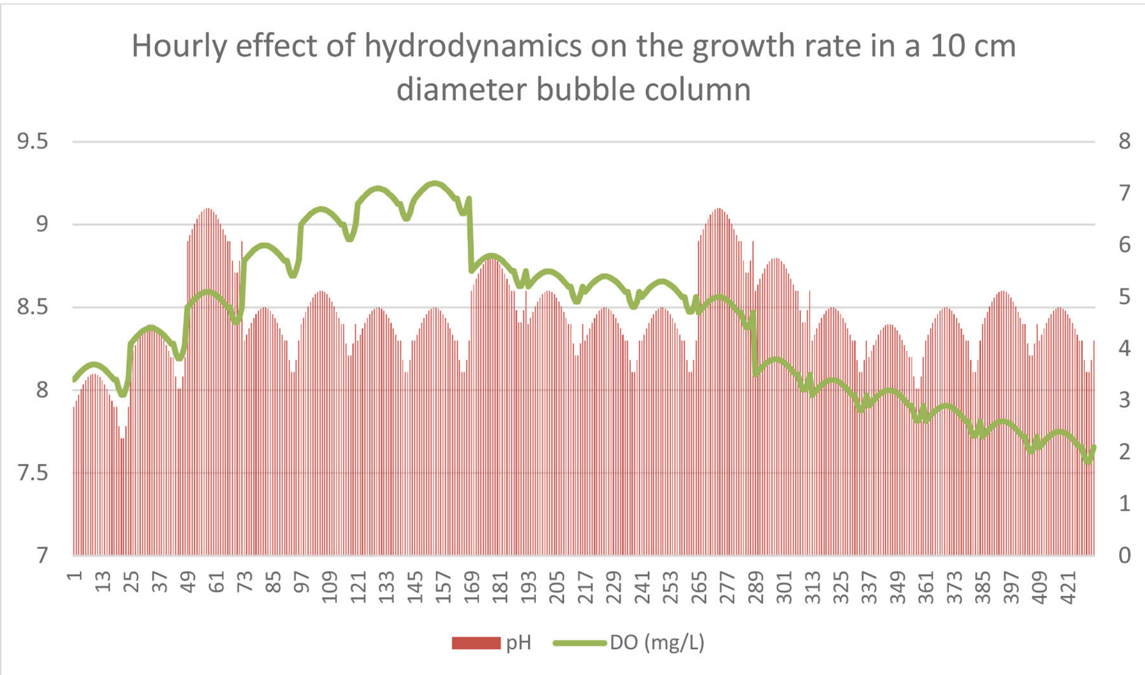

Fig. 8. Hourly deviations of pH and DO in column C2 (10 cm).

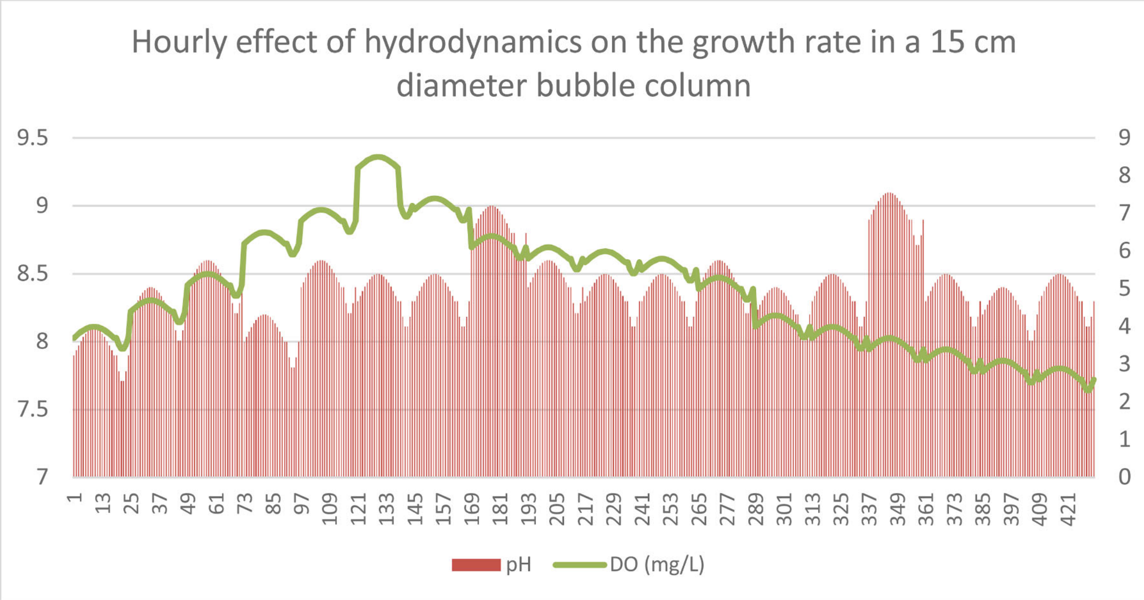

Fig. 9. Hourly deviations of pH and DO in column C1 (15 cm).

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 column 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.

- 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 growth rates achieved. The wider columns C2 and C3 have larger surface area to volume ratios, increasing the gas exchange efficiencies. However, it may be 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 the 5 cm column as compared to the 15 cm column. The wider column was able to provide sufficient DO for optimum growth, but dead zones were visually observed in larger sized columns.

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 controllable and efficient system for large-scale biofuel production and wastewater treatment applications. These findings emphasize the necessity of fine-tuning reactor hydrodynamics to prevent growth-inhibitory conditions, ensuring stable and sustainable biomass cultivation.

4.3. Monoculture versus Co-culturing

Co-cultivation of microalgae with complementary species offers synergistic advantages such as enhanced nutrient removal and metabolite exchange; however, it also introduces challenges related to interspecies competition, differential growth kinetics, and sensitivity to shear or light limitation. These interactions can destabilize culture performance if not properly controlled. The co-cultivation of Scenedesmus sp. and Lemna sp. was selected based on their complementary physiological traits and suitability to bubble column operation. Scenedesmus sp. is a robust microalga with rapid growth kinetics, high lipid accumulation capacity, and tolerance to fluctuating pH and DO conditions, making it well-suited for hydrodynamically dynamic systems such as bubble columns. Its relatively small, non-motile colonies also promote stable suspension under aerated conditions without excessive sedimentation. Lemna sp has low oxygen demand and provides a floating matrix that stabilizes the reactor environment. Its roots enhance nutrient assimilation and act as natural surfaces for attached microalgal growth, reducing washout in bubble-driven mixing regimes. Importantly, the contrasting gas exchange requirements of the two organisms establish a mutually beneficial balance. Scenedesmus sp. releases oxygen during photosynthesis, which supports Lemna respiration, while Lemna roots recycle nutrients and may release exudates that improve algal carbon uptake.

This physiological complementing relationship is specifically advantageous in bubble columns, where mixing intensity and gas transfer efficiency dictate growth performance. Scenedesmus sp. thrives under elevated CO_2 transfer rates promoted by sparging, whereas Lemna sp. mitigates turbulence stress by providing biofilm attachment zones. Together, their growth kinetics and gas-exchange interactions stabilize pH and DO fluctuations, enhance biomass productivity, and reduce the energetic burden of harvesting through natural biofilm formation. Such strategies help sustain balanced co-culture growth and improve overall reactor productivity.

Table 1.

Tables 2 and 3 below provide a comparative analysis of monoculturing vs. co-culturing and the optimized bubble column configurations, respectively, 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 for biofuel production.

4.3.1. Impact of reactor scaling and Co-culturing

Table 2 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-

Table 1
Advantages of using microalgae as a phycoremediation as opposed to the conventional wastewater treatment process.

Phycoremediation	Conventional method
Simple design, setup and operational cost	Large start-up investment and high operational costs
Utilise natural microalgal species and their capabilities, characteristics and adaptability	Solely depends on physical and chemical operation methods
Easily adaptable to changes in effluent	Effluent changes require significant changes to the process system
CO_2, O_2 and nutrients obtained from natural processes and wastewater sources	Require artificial methods in the treatment systems.
No drastic addition of chemicals to cultivation processes	Large amounts of chemicals required for flocculation processes
Single-step process eliminates pollutants and heavy metals	Multi-step process to percentages of each pollutant parameters
Process integration and waste integration have a dual purpose in wastewater treatment and energy production	Sludge ends up in landfill sites
Algal bioenergy production produces large volumes of biofuel, biogas and biodiesel.	Requires a large consumption of energy

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 (bench-scale) exhibited the highest mono-culture growth rate (0,752 ± 0,03 mg d^{-1}), 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 slightly, 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 ± 0,05 mg d^{-1}), surpassing both C1 (0,65 ± 0,08 mg d^{-1}) and C3 (0,68 ± 0,03 mg d^{-1}). 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 visible dead zones were constantly present, inhibiting uniform growth.
- The widest column (C3: 15 cm), despite having a larger gas-liquid interface and better CO2 retention, 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.
- The smallest column (C1: 5 cm) showed the lowest mono-culture growth rate (0,585 mg d^{-1}), likely due to higher turbulence and rapid CO2 stripping, preventing sustained carbon availability for microalgal uptake. However, co-cultivation improved growth rates (0,65 mg d^{-1}), 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 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, Fig. 10a illustrates how the co-cultivation served as an attached cultivation methodology in the study, while Fig. 10b depicts the harvested biomass obtained from the co-cultivation of *Scenedesmus sp.* and *Lemna sp*. The integration of naturally attached cultivation within the bubble column reactors facilitated algal attachment onto the roots of Lemna sp. This significantly increased 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, attached cultivation provides 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 PBRs.

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 (Fig. 10a) 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.

Fig. 10b further reinforces the advantages of attached cultivation by displaying the significantly higher biomass accumulation achieved through this approach. The harvested biofilm exhibited dense algal attachment, highlighting the effectiveness of surface immobilization in improving microalgal retention and reducing harvesting costs. The combination of attached and suspended growth modes resulted in a cumulative biomass yield of 700,12 ± 4,12 mg over 18 days, compared to 369,27 ± 2,28 mg during monoculture: demonstrating a nearly 90 % 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 from Fig. 10a and 10b emphasize the potential of attached cultivation in enhancing biofuel production efficiency by addressing key limitations of traditional PBRs. 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

Table 2
The effect of upscaling reactors during mono-culturing and co-culturing periods.

	30 L reactor	Bubble Column C1	Bubble Column C2	Bubble Column C3
Column diameter (cm)	30	5	10	15
Column height (cm)	50	100	100	100
No. of sparger holes	50 holes x 2 spargers	18	30	45
Size of holes (mm)	0,1	0,45	0,35	0,25
Maximum monoculture growth rate (mg d^{-1})	0,752 ± 0,03	0,585 ± 0,03	0,664 ± 0,04	0,602 ± 0,05
Maximum co-culture growth rate (mg d^{-1})	0,829 ± 0,07	0,65 ± 0,08	0,74 ± 0,05	0,68 ± 0,03

Table 3
Final optimised bubble column data with maximum growth rate and biomass productivity.

	10 cm diameter column optimised runs (Scenedesmus sp. monoculture)	10 cm diameter column optimised runs (Scenedesmus sp. and duckweed co-culture)
Growth rate (mg d^{-1})	= 0,664	= 0,74
Biomass Produced (mg L^{-1} d^{-1})	= 49,50	= 93, 85
Cumulative biomass after 18 days (mg)	= 49,50 mg L^{-1} d^{-1} = 369,27 mg	= 93,85 mg L^{-1} d^{-1} = 700,12 mg

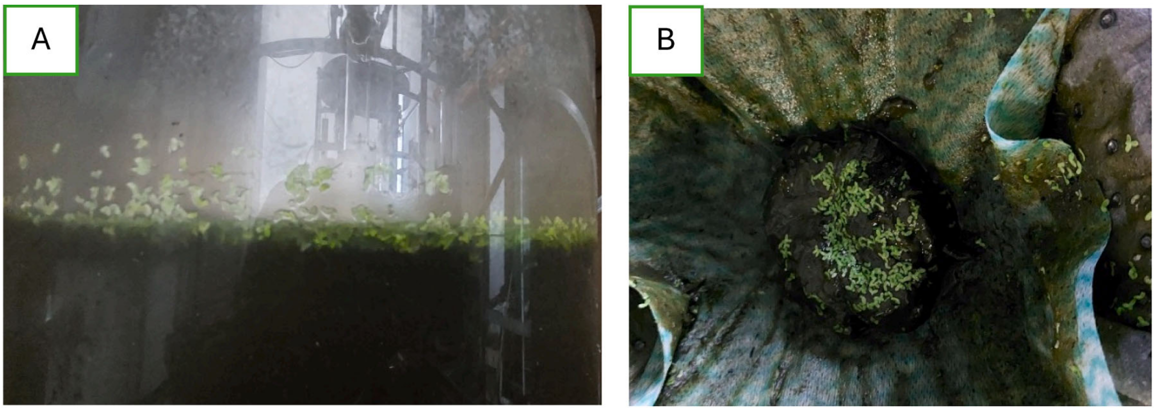

Fig. 10. (a) Illustration of co-cultivation attached surface methodology; (b) Harvested biomass.

designs. Future research should explore further optimizations in attachment surfaces, biofilm stability and nutrient recycling mechanisms to maximize the efficiency of attached cultivation systems for commercial bioenergy applications.

4.3.2. Optimized growth performance in the 10 cm bubble column

Table 3 presents the final optimized data for the 10 cm diameter column (C2), comparing monoculture and co-culture conditions in terms of growth rate and biomass productivity. The results reinforce the superiority of co-cultivation, demonstrating higher cumulative biomass production and sustained growth over 18 days.

- Mono-culture cultivation (Scenedesmus sp.) achieved a growth rate of 0,664 ± 0,04 mg d^{-1}, with biomass productivity of 49,50 ± 2,28 mg L^{-1} d^{-1}, accumulating 369,27 mg over 18 days.
- Co-cultivation resulted in a higher growth rate (0,74 ± 0,05 mg d^{-1}), with increased biomass productivity of 93,85 ± 4,12 mg L^{-1} d^{-1}, leading to a total cumulative biomass yield of 700,12 mg, almost 90 % higher than the mono-culture system.
- The increase in biomass productivity under co-culture conditions can be attributed to multiple factors, including enhanced nutrient recycling by Lemna sp., increased CO2 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.
- The optimized reactor configuration in C2 allowed for efficient gas-liquid mass transfer, minimizing shear stress while maintaining sufficient turbulence to prevent sedimentation. Additionally, repetitive visual observations showed that the moderate sparger hole size (0,35 mm) and sparger distribution ensured uniform bubble dispersion, preventing localized CO2 depletion or oxygen saturation stress, which were issues observed in C1 (high turbulence) seen in Fig. 11a, compared to C3 which had multiple stagnant areas within the column (dead zones) as seen in Fig. 11b below.

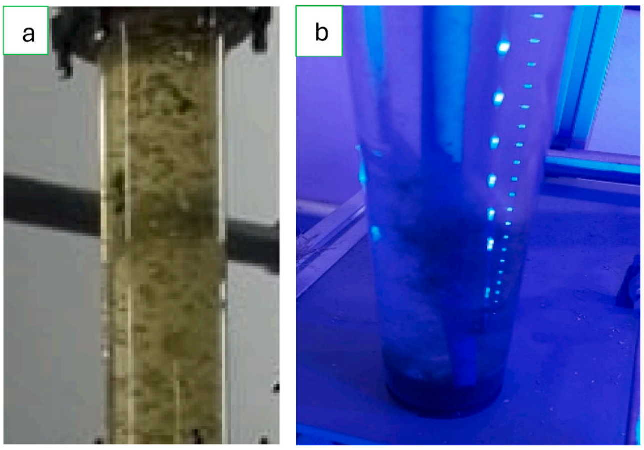

Fig. 11. Visual observations of (a) turbulence experienced in column C1 and (b) dead zones present in column C3.

These results validate the 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 comparative analysis in Tables 2 and 3 underscores the necessity of optimizing both reactor dimensions and cultivation strategies to maximize growth rates, gas exchange efficiency, and biomass accumulation. Co-cultivation consistently outperformed monoculture across all reactor scales, demonstrating its potential to improve nutrient uptake, CO_2 sequestration, and overall system stability. Co-cultivation improved growth rates by 11 % and nearly doubled biomass. Duckweed acted as a natural biofilm support, aiding in harvesting, CO_2 capture and nutrient assimilation.

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 (700,12 mg over 18 days). These findings highlight the importance of reactor-scale design considerations in improving microalgal biofuel production, setting the foundation for further research into aeration strategies, sparger design modifications, and optimized mixing conditions to enhance large-scale biomass cultivation for sustainable bioenergy applications.

5. Discussion

This is the first study to experimentally integrate hydrodynamic optimization with Duckweed assisted attached cultivation in sewage wastewater fed bubble columns, with varying diameters. 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, DO 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 centre 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. Unlike previous hydrodynamic studies that primarily examined bubble column mixing and gas–liquid transfer, this study demonstrates how co-cultivation with Lemna sp. simultaneously improves biomass stability, moderates pH/DO fluctuations, and provides an integrated harvesting mechanism. This dual approach advances the field by linking reactor-scale design with biological process intensification, offering a practical framework for scaling phycoremediation systems.

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.* [31] reported that gas hold-up and increased pressure significantly impact mass transfer, mirroring this study's results. Ahmad et al. [32] 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. The higher growth rate observed in this study compared to Ahmad et al. [32] is likely due to the stabilizing effect of Duckweed on DO and pH. Additionally, reduced turbulence stress in the 10 cm reactor improved algal productivity.

Gemelolo *et al.* [33] 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. Kumar et al. [34] 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, a key aspect observed in this study's experiments. Depra *et al.* [35] analyzed large-scale challenges in PBR 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.* [36] reported that raceway pond cultivation is more environmentally sustainable than open ponds, with 80 % lower global warming potential. Uyar *et al.* [11] compared different cultivation systems and concluded that bubble columns exhibit the highest volumetric mass transfer and growth efficiency, which further supports this study's results. The identification of the 10 cm diameter column as an optimal diameter provides insight for scaling. Maintaining similar gas holdup and light penetration ratios could guide the design of pilot and industrial-scale bubble columns. This study demonstrates that optimizing reactor diameter enhances hydrodynamic stability and biomass yield. Ahmad et al. [32] and Gemello *et al.* [33] highlighted the influence of bubble size, gas holdup and mixing on algal productivity. Our results extend this knowledge by providing empirical evidence that mid-range diameters minimize both turbulence-related stress and dead-zone formation. The integration of duckweed co-cultivation is novel compared to single-species studies. Lemna sp. contributed to nutrient recycling and surface attachment, enhancing both productivity and harvesting efficiency.

Beyond the observed increases in total biomass, the hydrodynamic regime also influences interspecies interactions in the co-culture system. In smaller columns (C1), enhanced mixing promoted closer physical contact between Scenedesmus and Lemna roots, facilitating algal attachment but also intensifying competition for dissolved nutrients. In contrast, the intermediate diameter (C2) appeared to balance mixing and stratification, creating microenvironments that favoured metabolic complementarity rather than direct competition. For instance, Scenedesmus released DO during photosynthesis, which supported Lemna root respiration, while root exudates from Lemna may have provided readily available organic substrates to the algae. These reciprocal exchanges likely stabilized pH and DO in C2, leading to enhanced productivity. In the largest column (C3), mixing inefficiencies could have reduced the frequency of such beneficial interactions, instead favouring nutrient-depleted zones where competition dominated. Thus, hydrodynamic conditions not only control mass transfer but also shape the ecological balance of co-cultured species, influencing whether interactions are synergistic or competitive. Compared to earlier monoculture bubble column studies (Pegallapati & Nirmalakhandan, 2012; Nauha & Alopaeus, 2013), our co-culture system delivered substantially higher growth rates, confirming the synergistic advantage. Practical implications of this study include (i) scalable wastewater treatment with simultaneous biofuel feedstock generation, (ii) reduced harvesting costs due to pre-attached biomass and (iii) improved CO_2 utilization efficiency in bubble column reactors.

At larger scales, bubble size distribution is often broader due to increased coalescence, resulting in reduced specific interfacial area and lower mass transfer efficiency compared to small columns. Similarly, the simple circulation loops observed in narrow laboratory reactors can evolve into complex flow patterns with multiple recirculation zones in larger units, leading to heterogeneities in light, nutrient, and dissolved gas availability. The identification of the 10 cm reactor as the most productive scale in this study suggests that an intermediate balance between mixing and light penetration is critical. To translate this to larger systems, scale-up should consider maintaining equivalent superficial sparger designs that limit bubble coalescence and using CFD modelling to predict circulation patterns. Pilot-scale testing in a 240 L Airlift reactor will be tested later on in this study, to validate whether the observed synergistic effects of geometry and co-cultivation can be preserved under industrially relevant operating conditions.

6. Conclusion

This study demonstrates that optimizing reactor diameter and integrating Lemna-assisted co-cultivation substantially improved biomass productivity in bench-scale bubble columns, and simplified harvesting in wastewater-fed systems. The optimal 10 cm diameter column (C2) provided the best mixing, gas retention and light distribution, while duckweed improved nutrient assimilation and stabilised culture conditions. Together, these factors produce almost 90 % more cumulative biomass in co-culture than in monoculture. Co-cultivation also enhanced pH and DO stability and provided a novel attached growth harvesting pathway.

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 (10 cm and 15 cm) demonstrated better mixing, agitation and homogenous air supply, leading to more stable pH and DO levels. The 10 cm column (C2) 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. Although light distribution was initially a limiting factor, it was effectively mitigated with adjusted aeration and optimised CO_2 supply, ensuring sustained photosynthetic activity.

To our knowledge, this represents the first experimental integration of reactor geometry optimisation with duckweed-assisted phycoremediation. Beyond advancing scientific understanding, the results provide practical guidance for scaling bubble column reactors in industrial wastewater treatment and biofuel production. By bridging hydrodynamic design with ecological synergy, this work offers a pathway toward more efficient, sustainable and economically viable algal-based bioprocesses.

Future research should explore whether the reactor-scale optimization demonstrated here can be strengthened by integrating advanced control and design tools. Real-time aeration and pH/DO feedback control could be applied to maintain optimal mixing and gas–liquid transfer, while CFD simulations can assist in predicting flow fields, minimizing dead zones and guiding scale-up. Combining empirical optimization with such control strategies would accelerate the transition of bubble column co-cultivation systems from bench-scale experiments to robust, industrially relevant platforms for wastewater treatment and bioresource recovery. Additional reactor configurations and aeration techniques, biochemical profiling of lipid extract, techno-economic feasibility and pilot-scale implementations can be investigated to further optimize large-scale production. These findings contribute novel insights into reactor engineering, cultivation strategies and the broader goal of sustainable bioenergy production and wastewater treatment through efficient algal cultivation systems.

Aim

Evaluate how bubble columns of different diameters (5, 10 and 15 cm) and co-cultivation with Lemna sp. influence hydrodynamic stability and biomass productivity in wastewater-fed bioreactors.

CRediT authorship contribution statement

Nikita Singh: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Prof Manimagalay Chetty:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Prof Sudesh Rathilal:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

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