Comparative Performance of *Scenedesmus Sp.* Cultivated in Sewage Wastewater in 1L Bench Top Vessels and A 30L Reactor for Algal Growth and Biofuel Production

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***Abstract*—**Scaling up algal cultivation from bench-scale to pilot reactors is necessary for advancing biofuel production. Various operational parameters such as nutrient availability, light illumination, pH, aeration and several others are interdependent and influence algal growth; as well as the hydrodynamics of the growth environment such as the height and diameter of the vessel. This study compares the batch cultivation performance of *Scenedesmus sp.* in sewage wastewater using 1L bench-top vessels and a 30 L pilot-scale bubble column reactor, focusing on biomass productivity, growth rates and energy efficiency. Cultivation was conducted at pH of 8, with an 18-hour light: 6-hour dark cycle and a 2:2 Bold's Basal Medium (BBM) to sewage wastewater ratio, between 10 cm and 30 cm diameter reactor vessels. Ultraviolet (UV) sterilization of wastewater was performed to eliminate microbial competition within the wastewater source.

The aim is to analyse reactor hydrodynamics, the scalability of biofuel production and operational challenges encountered during scale-up. Necessary algal concentrations and inoculation volumes were established during the upscaling process. The 1 L reactor achieved an average biomass productivity of 64×10^4 cells/mL/day, and a growth rate of 0.79 day^{-1} under optimized conditions. In contrast, the 30 L reactor achieved a lower biomass productivity of 46.89×10^4 cells/mL/day and a growth rate of 0.577 day^{-1}, primarily due to inefficiencies in flow, culture depth, sheer stress, light intensities and other necessitating factors. However, the 30 L reactor demonstrated potential for higher biomass yield when adjustments to aeration and distance of light source were applied, with a projected improvement of 29 % in biomass recovery following system optimization. Additionally, CO_2 injections (3 %) at regular intervals maintained optimal pH concentration. The study highlights that hydrodynamics is crucial, with the 1 L benefiting from uniform mixing, while the 30 L reactor experienced localised shear forces that negatively impacted algal growth. Nonetheless, the 30 L vessel demonstrated higher biomass yield potential due to its ability to accommodate larger volumes. This research provides insights for optimizing algal cultivation at larger scales, contributing to the development of scalable and energy-efficient biofuel production processes.

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I. Introduction

The rapid depletion of natural resources, specifically fossil fuels, has created the urgent need to produce fossil fuels from renewable resources, hence making large-scale microalgae cultivation a significant topic of interest. Besides microalgae's capability to produce valuable biomass, algae can be utilised as a promising technique for global warming reduction/ carbon dioxide fixation as CO2 is globally recognised as a major source of global warming (Cui et al. 2023). Additionally, microalgae have repeatedly proven itself to absorb toxic components found in wastewater, successfully. Algal biofuel, particularly biodiesel appears as a conventional alternative compared to fossil fuel-derived diesel. Scenedesmus sp. is proven itself for its exceptional capability in producing lipids and hydrocarbon, required to produce biodiesel (Chen et al. 2015). Cultivating microalgae in wastewater addresses two critical global challenges, i.e. energy security (biofuel production) and environmental pollution (wastewater remediation). Although utilising wastewater for algae cultivation makes the process economically reliable, a primary problem that remains a problem is scaling up from lab scale to pilot plant/industrial scale systems (Ma and Wang, 2021).

Multiple abiotic and biotic factors affect algal growth and adaptability, which also in turn affect the overall yield and quality of biomass produced. Similar to all plant-like organisms, microalgae require energy, water, carbon and a nutrient source to continue its photosynthetic activity (Maresco et al. 2024). However, for profitable, efficient and optimised microalgae scale-up processes, optimum growth conditions, cultivation parameters, reactor geometry and configuration (hydrodynamics), light illumination regimes and nutrient supply/demand requirements need to be thoroughly established. Hence, reactor configurations are essential to ensure the feasibility of biofuel production at a commercial scale (Wu et al. 2013).

Several nutrients positively assist in microalgal growth, of

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which nitrogen (N) is the most important (Nauha and Alopaeus, 2013). The energy absorbed by the microalgal cells is supplied by a light source, influenced by the cell's intensity, distance and shadowing (Ferencz, Toporowska and Dawidek, 2023). CO2 availability depends on the input/injection rate, mixing speed and the rate of mass transfer. Too much light also causes photoinhibition, affecting the microbial cells (Cao et al. 2022). Proper light dispersion with proper mixing will allow cells to experience high illumination for short periods of time, depending on the reactor design. Hence, the cultivation of microalgae in larger volume types of equipment is not as straightforward as the smallest changes in fluid flow influence nutrient and energy movement through the system, necessitating the need for successful design, flow pattern and fluid dynamics to be considered in the photobioreactor for overall photosynthetic yield (Zhang et al. 2022).

II. Objective

The primary objective of this study was to compare the performance of Scenedesmus sp. cultivated in sewage wastewater using 1 L bench-top reactors and a 30 L pilot scale photobioreactor. The difference in growth rates, biomass productivity and energy efficiency between the two reactors were evaluated. Additionally, key operational challenges encountered were identified, whilst understanding the impact of hydrodynamic factors. Addressing these challenges provided valuable information to optimise large-scale algal reactors, contributing to the development of scalable and energy-efficient biofuel systems.

III. Materials And Methods

The present study evaluated the overall performance comparison between the 1 L reactor vessel and the 30 L (working volume of 25 L) bubble column photobioreactor for sewage effluent treatment using *Scenedesmus sp*. cultivated under conditions of pH concentration 8, 18-hour light: 6-hour dark illumination cycles and a 2:2 growth medium ratio of Bold's Basal Medium and sewage wastewater. The strain of microalgae used in this study was *Scenedesmus sp*., which was selected for its robust lipid production, making it ideal for biodiesel synthesis. *Scenedesmus sp.* was obtained from Nelson Mandela University (NMU), South Africa, whereas the sewage wastewater was collected from a treatment plant based in Durban, South Africa. The collected sewage wastewater underwent prior ultraviolet (UV) sterilization to eliminate microbial competition within the wastewater source.

BBM medium is prepared as follows: $NaNO_3$ (25.0 mg/L), K_2HPO_4 (75.0 mg/L), KH_2PO_4 (175.0 mg/L), $MgSO_4 \cdot 7H_2O$ (75.0 mg/L), $CaCl_2 \cdot 2H_2O$ (25.0 mg/L), NaCl (25.0 mg/L), EDTA disodium salt (50.0 mg/L), KOH (31.0 mg/L), $FeSO_4 \cdot 7H_2O$ (4.98 mg/L), H_2SO_4 (1 drop per liter), H_3BO_3 (11.42 mg/L), $ZnSO_4 \cdot 7H_2O$ (8.82 mg/L), $MnCl_2 \cdot 4H_2O$ (1.44 mg/L), MoO_3 (0.71 mg/L), $CuSO_4 \cdot 5H_2O$ (1.57 mg/L), and $Co(NO_3)_2 \cdot 6H_2O$ (0.49 mg/L). This combination provided the necessary nutrients to support optimal microalgal growth during the experiment.

A. Experimental/Reactor Set-up

The 1 L reactor flasks (internal diameter of 10 cm and a height of 20 cm, Figure 1) and the 30 L bubble column (diameter of 30 cm and height of 50 cm) were both illuminated by blue light, which was mounted circularly around the vessels. Aeration was initially provided at a rate of 5 L/hr using an air pump, with CO_2 injections of 3 % applied regularly at 4-hour intervals. In the 1 L reactor, air was supplied continuously through 6 cm air stones, whereas in the 30 L reactor, air was injected through fixed sparges with a 6 cm radius and 50 spout holes at the base. A blackout screen was used to contain the light source and ensure consistent light penetration.

Fig. 1 1 L reactor vessels used for microalgae cultivation

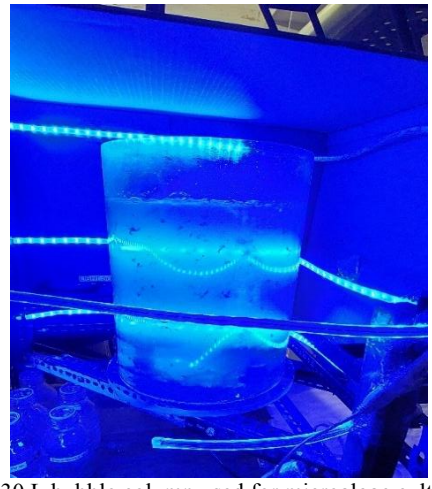

Fig. 2 30 L bubble column used for microalgae cultivation

B. Determination of biomass concentration

A linear relation between *Scenedesmus sp.* biomass concentration and optical density was measured by a Spectrophotometer (HACH DR 300) at a 650nm wavelength. Various algal concentration suspensions were made using dilution and used to calibrate the spectrophotometer. Biomass weight determination was accurately determined using

filtration through Whatmann filter paper, followed by drying of the *Scenedesmus sp.* biomass samples at 60 °C for 4 hours. All experimental data measurements were taken in triplicate with the average value used.

IV. Results

The cultivation of *Scenedesmus sp.* was carried out using the Design of Experiment (DoE) Box Behnken strategy, to optimize growth conditions. Prior to this, a one-factor-at-a-time (OFAT) analysis was conducted to establish baseline cultivation parameters. This preliminary analysis helped identify optimal conditions (pH, light illumination period and growth medium ratio) for small-scale cultivation. Firstly, the pH concentration range of 6.5 – 10 was tested. Table 1 below shows that a pH of 7.5 and 8 produced the highest growth rate and cell productivity. Since the aim is to achieve the highest cell productivity, a pH of 8 (39×10^4 cells/mL/day) was selected instead of pH of 7.5 (38.5×10^4 cells/mL/day). Growth rate and biomass productivity were also considered when establishing the optimum lighting period and growth medium ratios, leading to establishing that a 0.75 day (18-hour light: 6-hour dark) and a 2:2 ratio of sewage wastewater to BBM cultivation medium was selected. Although a 3:1 ratio slightly increased growth and productivity, the 2:2 ratio was preferred to align with the goal of maximizing wastewater treatment efficiency. Future optimizations, such as CO_2 injections, nutrient supplementation, and improved aeration, could help offset the slight difference in growth rate.

Under the optimised cultivation conditions (pH 8, 18-hour light: 6-hour dark and 2:2 cultivation medium), the maximum growth rate (GR) and cell productivity (CP) of Scenedesmus sp. achieved was 0.79/day and 64.2×10^4 cells/mL/day for an initial culture size of 0.2 g dry weight /L.

TABLE I
Growth rates and Biomass productivity of Scenedesmus cultivated in 1L reactor vessels with varying pH, illumination period and growth medium ratios.

pH	GR	CP	Light: dark	GR	CP	Medium: wastewater ratio	GR	CP
6.5	0.51	18.5	0.0	0.16	1.2	0.4	0.1	1.2
7	0.52	25.2	0.25	0.22	7.35	1.3	0.12	0.9
7.5	0.86	38.5	0.50	0.71	62.5	2.2	0.79	64.2
8	0.79	39	0.75	0.75	73.9	3.1	0.81	77.9
8.5	0.65	32.1	1.0	0.72	70.3	4.0	0.72	70.3
9	0.32	10.42						
9.5	0.24	5.23						
10	0.14	2.34						

Maintaining the same baseline characteristics when upscaling cultivation to the 30 L vessel brings about variation and sudden differences in the growth rate and cell productivity which is explained below.

TABLE II
Growth rates and Biomass productivity of Scenedesmus cultivated in 30 L bubble column with varying aeration rates and distance of light source.

Airation (l/hr) 1 sparger	GR	CP	Aeration (l/hr) 2 spagers	GR	CP	Distance of light (cm)	GR	CP
5	0.577	46.89	5	0.631	51.26	2	0.577	46.89
7	0.434	35.27	7	0.721	58.59	5	0.525	42.63
10	0.394	32.01	10	0.402	32.67	7	0.752	61.06
15	0.325	26.41	15	0.350	28.44	10	0.621	50.42

The 1 L bench-top reactor achieved an average biomass productivity of 64×10^4 cells/mL/day. In contrast, the 30 L pilot reactor produced a lower biomass productivity of 46.89×10^4 cells/mL/day at the same cultivation conditions. The difference in cell productivity between the two reactors can be attributed to the more efficient light penetration, smaller depth and better agitation/mixing in the smaller system, which allowed for more uniform growth conditions. The growth rate in the 1 L reactor was 0.79/day, significantly higher than the 0.577/day observed in the 30 L reactor. In the 30 L reactor, shear forces and dead zones contributed to a slower growth rate, as cells in certain areas received less light and fewer nutrients. In the 1 L reactor, light penetration was more uniform due to the shallower culture depth, ensuring that all algal cells received adequate illumination. In contrast, the 30 L reactor faced challenges with shadowing light, particularly in the deeper regions of the reactor. Cells at greater depths received less light, which limited their growth and photosynthetic efficiency as confirmed when the light was placed far (10cm) away. Adjusting the light intensity and using more efficient lighting configurations could mitigate these challenges in larger reactors. Energy input for aeration and mixing was higher in the 30 L reactor due to the larger volume and the need to maintain adequate CO_2 and nutrient distribution. Despite the higher energy input, the biomass yield in the 30 L reactor was lower, resulting in a reduced energy efficiency compared to the 1 L reactor, where smaller energy inputs yielded higher biomass. Scaling up increased energy demands as more power is needed for aeration but optimizing aeration and mixing could improve efficiency. In the 30 L reactor, localized shear forces and uneven mixing led to dead zones where algal cells experienced limited nutrient and CO_2 availability. This negatively affected overall biomass recovery.

The airflow rate was increased by 50 % (from 5 L/hr to 7.5 L/hr), ensuring better gas exchange and enhancing the distribution of CO_2 throughout the vessel. This increase in airflow not only helped maintain the pH balance more effectively but also improved the overall availability of CO_2, a

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critical factor for photosynthesis, growth rate (0.721/day) and biomass accumulation (58.59×10^4 cells/mL/day).

Additionally, an additional sparger was added and repositioned facing directly upwards within the reactor. The original placement of the spargers had led to uneven gas dispersion, contributing to the formation of dead zones where mixing and CO_2 distribution were insufficient. By adjusting their positioning, a more uniform flow of air and CO_2 bubbles was achieved, which helped to break up stagnant zones and improve circulation across the reactor's entire volume. This led to more consistent contact between the algae and the available nutrients, light and CO_2. The combined effect of increased airflow and optimized sparger placement resulted in enhanced mixing efficiency. This improvement ensured that cells in previously under-mixed regions now had greater access to light and nutrients, reducing the number of zones where growth was previously hindered. As a result, the overall biomass recovery improved by 25 % achieving 61.06×10^4 cells/mL/day, as cells throughout the reactor received a more uniform supply of the essential resources needed for optimal growth (0.752/day). These enhancements also led to better light distribution within the reactor. With improved mixing, algal cells were no longer confined to regions with limited light penetration. Cells were more consistently exposed to the light available in the reactor, increasing the overall photosynthetic efficiency. By reducing light attenuation and nutrient limitations, the growth conditions in the 30L reactor became more uniform, which contributed to an increase in algal biomass productivity.

CO_2 injections were used to maintain the optimal pH level of 8, which is critical for maximizing algal growth. In the 1 L reactor, pH was easily controlled due to the smaller volume and more homogeneous conditions. In the 30 L reactor, maintaining a stable pH required more frequent CO_2 injections due to the larger volume and slower gas exchange. However, with regular monitoring and adjustments, the pH in both systems remained within optimal ranges, contributing to stable algal growth.

V. Discussion

Calvo et al. (2022) and Shaoyi et al. (2021) findings support our findings during this comparison study between the 1 L and 30 L vessels. They also emphasized an increasing flow velocity in bioreactors accelerated the transportation of nutrients and promoted cellular metabolism and nutrient uptake, aiding in the successful growth of microalgae. Notably, as observed in our 30 L reactor, when the medium velocity is too high, the suspended particles are stripped and experienced shear stress forces/scouring, leading to the direct erosion and shedding of the cells, causing microbial cells to die (reducing biomass productivity as 10 – 15 L/hr aeration data proved). The 1L reactors appeared to have benefitted from the more uniform distribution of aeration, light and nutrients, therefore highlighting the challenges experienced with hydrodynamic inefficiencies as the reactor sizes increases.

A. Influence of Reactor Size, Hydrodynamics and Mixing

Hydrodynamics effects in larger reactors is critical to maintain consistent mixing, nutrient distribution and CO_2 availability. Scaling up from a 1 L bench-top reactor to a 30 L bubble column reactor presents both challenges and opportunities. One major challenge is the increased complexity of hydrodynamic limitations. The smaller reactor was less prone to hydrodynamic inefficiencies, allowing for more consistent growth conditions. Larger reactors, like the 30 L system often suffer from uneven mixing, resulting in localized shear forces and dead zones that impede algal growth. The presence of localized shear and dead zones within the reactor resulted in uneven nutrient and CO_2 distribution, which negatively impacted the overall growth of *Scenedesmus sp.* Additionally, energy requirements for aeration and mixing increase significantly as the reactor size grows, making it more difficult to maintain efficient operation. Light penetration is another critical factor, as the deeper culture depth in the 30 L reactor leads to poor light distribution, limiting algal photosynthesis and reducing overall biomass productivity. Despite these challenges, the larger reactor offers the opportunity to produce higher biomass yields when hydrodynamic and light distribution issues are addressed. The 30 L reactor's capacity to hold a larger culture volume provides greater potential for scalability, provided these operational hurdles can be overcome. Future studies may investigate alternative sparger head replacements or modified impeller designs that enhance mixing without any additional shear stress induced. Additionally, the limited light illumination experienced in larger reactors require innovative light arrangements to improve photosynthetic efficiency and productivity, such a light source between or within various depths throughout the reactor. This contrast highlights the importance of hydrodynamic optimization when scaling up to larger reactors.

B. Scalability of Algal Cultivation

The findings of our study indicate that careful optimization of larger reactors intended for pilot plant/ industrial scale cultivation are highly possible. When considering the scalability of algal cultivation, several essential key factors must be addressed to make large-scale biofuel production feasible, including reactor design, hydrodynamic

optimization, and nutrient distribution. The 30 L reactor demonstrates potential for further upscaling, but it will require improvements. Addressing these factors is crucial for achieving reliable and efficient algal cultivation on a larger, industrial scale.

The findings of this study have significant implications for the broader goal of biofuel production from microalgae. Improving reactor design to optimize hydrodynamics and light distribution could significantly boost biomass yield and energy efficiency, making the process more economically viable. By addressing the challenges encountered during scale-up, such as shear forces, dead zones, and light attenuation, the potential for large-scale biofuel production increases. This study contributes valuable insights into how reactor modifications and operational improvements can make algal biofuel production a more scalable and sustainable alternative to fossil fuels.

C. Economic and Environmental Impact

This study utlilised wastewater as a cultivation medium for microalgae, offering added environmental benefits by supporting wastewater treatment, nutrient recycling and biomass generation. The usual upscaling of microalgae cultivation holds significant economic implications, hence the need of this study to provide insights into the design modifications of reactors to make large-scale cultivation viable. By addressing these issues and improving the efficiency in large scale settings, production and operational costs can be reduced; hence making algal biofuels more competitive than conventional energy sources. Optimising large scale algal fuel has significant potential to replace fossil fuel and petroleum-fuel use, addressing both energy demands and environmental concerns.

D. Current limitations and Future directions

While this study achieved successful cultivation in the 30 L reactor, there were still several limitations noted. The specific geometry, design structure and mixing mechanisms does not represent all scalable systems. Hence, future work could explore different configurations including horizontal or multiple chamber designs that enhance light illumination and nutrient flow. Another option would be to introduce continuous monitoring techniques, such as real time pH, dissolved oxygen (DO) and CO_2 tracking as this would provide more detailed data into adjusting parameters dynamically for optimal growth and biomass productivity.

VI. Conclusion

This study identified and discussed the importance of hydrodynamic factors with regards to microalgal cultivation and scale up processes. Hydrodynamics plays a crucial part in algal biology and is influenced by the effects of current velocity, change of flow, water levels, aeration speed, depth of cultures, diameter and height of vessels. The hydrodynamics mechanisms mainly affect nutrient distribution, alter the transportation process affecting microbial cell functions and it destroy the integrity of the cell structure.

Water currents present within the reactor vessels are one of the primarily drivers of algal distribution and aggregation of the water. An insufficient propulsive driving force and changes in flow velocity have a significant effect on the characteristics of algal biomass physiology and biology. Microalgal cells sensitivity shows a single peak curve relationship between the flow velocity/aeration and algae growth.

This study highlights the challenges of scaling up algal cultivation from a 1 L bench-top reactor to a 30 L bubble column photobioreactor. The 1 L reactor demonstrated higher biomass productivity and growth rates due to better mixing and consistent light distribution, while the 30 L reactor faced challenges related to hydrodynamics, shear forces, dead zones and limited light penetration. However, after optimizing the aeration and mixing systems in the 30 L reactor, a 29 % improvement in biomass recovery was achieved, demonstrating the potential for higher biomass yields at larger scales. The findings from this study contribute to the understanding of how reactor design and operational parameters can be optimized for scalable and energy-efficient biofuel production. It is suggested that ideal reactor designs make use of enhanced mixing mechanisms and reduce shear forces within the column; hence maintaining the cell integrity in larger scale producing systems.

Furthermore, the information gained from this study contribute to the broader efforts in making algal biofuels more efficient, cost-effective and sustainable; potentially influencing future investment in bioenergy since microalgae can address both energy security and environmental concerns.

A. Experimental and Analytical Recommendations

To enhance the scalability and efficiency of algal cultivation in larger reactors, further experimentation is recommended. One key area for future research is improving hydrodynamic behavior in larger scale photobioreactors. This could be achieved by varying aeration rates to optimize mixing and CO_2 distribution, as well as modifying impeller designs to reduce localized shear forces that negatively impact microalgal growth. Another promising avenue is exploring alternative reactor configurations, such as horizontal photobioreactors with a middle chamber of light, which may offer improved light distribution and minimize issues related to light attenuation at greater culture depths. These changes could potentially lead to more uniform growth conditions and higher biomass productivity in larger reactors. Through continuous optimization of reactor design and implementation of these improvements, the feasibility of algae-based biofuels

as a sustainable energy source could significantly increase; making microalgae emerge as a key contributor to global energy solutions.

B. Analytical Recommendations

For a more comprehensive understanding of microalgal growth dynamics, it is recommended to implement more frequent and detailed growth measurements. Using continuous monitoring systems could provide real-time data on growth rates and biomass accumulation, offering insights into the influence of environmental variables on culture performance.

Additionally, to assess the long-term feasibility of scaling up algal cultivation systems, incorporating energy analysis methods such as Life Cycle Assessment (LCA) is crucial. LCA would evaluate the overall energy efficiency and sustainability of the process, identifying potential areas for improvement in resource use and minimizing environmental impacts as the scale of production increases. These analytical tools will provide a clearer path toward optimizing large-scale biofuel production from algae.

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Conflicts of Interest

The authors declare no conflict of interest.

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