



## OPEN Impact of mixing duration on growth and nutrient removal efficiency of *Scenedesmus* sp. in a novel raceway pond system

Muhammad Umer Abbas<sup>1</sup>, Rashid Iftikhar<sup>1✉</sup>, Sahar Saleem<sup>2</sup>, Sarah Bader Alotaibi<sup>3</sup>, Nadeem Ullah<sup>1</sup>, Mashael M. Alfge<sup>3</sup>, Muhammad Ali Inam<sup>4</sup>, Faras Ahmad Shahbaz<sup>1</sup> & Ahmad Aakash<sup>5</sup>

Raceway ponds are regarded as a popular and cost-effective method for microalgae cultivation; however, their performance is strongly influenced by hydrodynamic conditions. Conventional paddlewheel driven systems are restricted to low operating velocities to avoid culture spilling out which often leads to reduced mixing and stagnant zone formation. In this study, a raceway pond was designed with the inclusion of curved slits at the bent zones and submersible pump as an alternative mixing device to prevent culture overflow, improve flow stability and minimize dead zones. This novel integration of structural modifications and pump-based mixing represents a significant advancement over traditional paddlewheel systems by providing higher velocities, enhanced circulation and more uniform algal growth conditions. Experiments were conducted to evaluate the effect of mixing durations in 6 L raceway ponds under identical environmental conditions. The raceway systems permitted a broader velocity range (0.10–0.45 m s<sup>-1</sup>) without spillage. The system with continuous 24 h mixing compared to 20 and 16 h mixing resulted in the highest biomass productivity of 1.01 g L<sup>-1</sup> d<sup>-1</sup> and maximum nutrient removal rates of 5.18 mg L<sup>-1</sup> d<sup>-1</sup> and 3.41 mg L<sup>-1</sup> d<sup>-1</sup> for NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>3-</sup>, respectively. Submersible-pump configured open raceway pond achieved comparable or higher biomass yield, lower energy consumption with a net energy efficiency of 62%, demonstrating its practicality and cost-effectiveness as a viable alternative to conventional paddlewheel driven systems for large-scale *Scenedesmus* sp. cultivation.

**Keywords** Nutrient recovery; evaporation loss; energy efficiency, Submersible pump, Reactor design, Hydrodynamics

The expanding global economy and growing world population require the development of cost-effective and environmentally friendly energy production systems, as well as sustainable food sources<sup>1</sup>. Microalgal biomass is currently regarded as a sustainable source of various products, including biofuels, high-value chemicals, food, and animal feed, due to its distinct advantages over terrestrial plants and its inherent potential<sup>2,3</sup>. Nevertheless, the cultivation of microalgae for commodity products like biofuels remains limited due to high production costs and low biomass productivity<sup>4</sup>. For successful commercial cultivation of microalgal biomass, several factors must be considered when selecting the appropriate cultivation setup and operating conditions. These factors include the capital and operating costs, desired final product, land and water availability, the biology and intrinsic properties of the selected microalgae, as well as operational factors such as mixing and culture depth<sup>5</sup>.

Among cultivation systems, open pond systems such as paddle wheel driven raceway ponds are currently preferred for large-scale microalgae production due to their significantly lower costs, reasonably high biomass

<sup>1</sup>Institute of Environmental Sciences and Engineering (IESE), School of Civil and Environmental Engineering (SCEE), National University of Sciences and Technology (NUST), Islamabad 44000, Pakistan. <sup>2</sup>Department of Sciences, School of Interdisciplinary Engineering & Science (SINES), National University of Science and Technology (NUST), Islamabad, Pakistan. <sup>3</sup>Department of Teaching and Learning, College of Education and Human Development, Princess Nourah Bint Abdulrahman University, Riyadh 11671, Saudi Arabia. <sup>4</sup>Institute of Water Resources and Water Supply, Hamburg University of Technology (TUHH), Am Schwarzenberg-Campus 3, 21073 Hamburg, Germany. <sup>5</sup>Department of Chemistry "Giacomo Ciamician", University of Bologna, Via Selmi 2, Bologna 40126, Italy. ✉email: rashid.iftikhar@iese.nust.edu.pk; rashid.iftikhar@hotmail.com

production efficiency, and larger capacity<sup>6</sup>. Mixing in raceway ponds is crucial, as optimal mixing can increase algal biomass productivity by nearly tenfold. It serves several purposes, including the removal of oxygen generated during photosynthesis, keeping cells in suspension, ensuring nutrient availability to the algal cells, and increasing their exposure to sunlight. Additionally, efficient light utilization is enhanced by avoiding photoinhibition and photo limitation through uniform mixing<sup>7</sup>. However, a major challenge in raceway ponds is maintaining adequate mixing.

In raceway ponds, culture flow velocity for mixing typically ranges between 20 and 30 cm s<sup>-18</sup>. Increasing the mixing rates requires more energy but may induce culture overflow, whereas reducing them results in laminar flow, which significantly decreases biomass productivity<sup>9,10</sup>. In raceway systems, power consumption and operational costs are major concerns due to the need for turbulent mixing<sup>11</sup>. Powering paddle wheels in these ponds can account for ~25% of the annual operating costs for microalgal production<sup>9</sup>. Therefore, reducing energy consumption while optimizing turbulent mixing represents a potential cost cutting option for raceway ponds in commercial scale cultivation.

For a long time, there had been little change to the structure of raceway ponds. However, scientists have recently started exploring various ways to improve productivity and reduce energy consumption. Kusmayadi et al.<sup>12</sup> compared the performance of paddle wheels alone with paddle wheels combined with CO<sub>2</sub> spargers in raceway ponds in terms of mixing and microalgae growth. They found that the combination of paddle wheels and CO<sub>2</sub> spargers had no significant effect on mixing efficiency compared to paddle wheels alone. However, the addition of CO<sub>2</sub> spargers effectively reduced low-velocity zones within the raceway pond. Despite this, the design is not suitable for large-scale microalgae production as the low liquid levels lead to decreased carbon utilization by the microalgae. Cheng et al.<sup>13</sup> designed a raceway pond with a combination of upward and downward baffle plates. A clockwise and counterclockwise vortex flow is generated to enhance liquid mixing as it passes through successive upward and downward baffle plates. However, this structure has limitations, as it leads to cell aggregation in the baffle's backflow areas, which hinders effective mixing. Although numerous studies have examined the mixing performance of raceway ponds, improving the mass transfer efficiency of microalgae remains a key area of research.

Modifying raceway ponds with paddle wheels fails to simultaneously reduce energy demands and improve mixing efficiency<sup>14</sup>. In the present study, it was hypothesized that the hydrodynamic performance of raceway ponds could be enhanced through the introduction of energy efficient submersible pumps for mixing purposes. The submersible regime induces turbulent thrust (velocity, 0.60–1.0 m s<sup>-1</sup>) that can improve mixing efficiency and reduce power requirements, providing an efficient alternative to the conventional paddle wheel raceway pond systems. Furthermore, fully submerged submersible pumps overcome the restricted flow velocity and risk of culture loss associated with the partial immersion of conventional paddlewheels by enabling higher velocity operation without overflow ensuring hydraulic stability. At channel bends, slits were incorporated to redirect flow, thereby enhancing turbulence, improving circulation, and minimizing stagnant zone formation. The batch cultivation experiments investigate the effect of different submersible pump-induced mixing durations (24 h, 20 h, and 16 h) on the removal of nitrate nitrogen and phosphate phosphorus and the growth of *Scenedesmus* sp. in Bold Basal Medium to establish a deeper understanding of the optimal mixing duration for efficient nutrient utilization and increased biomass production. By integrating reactor design based hydrodynamic analysis with cultivation performance metrics, this study provides guidance for optimizing energy savings and demonstrates the potential of submersible pumps to address limitations of previous raceway pond studies.

## Materials and methods

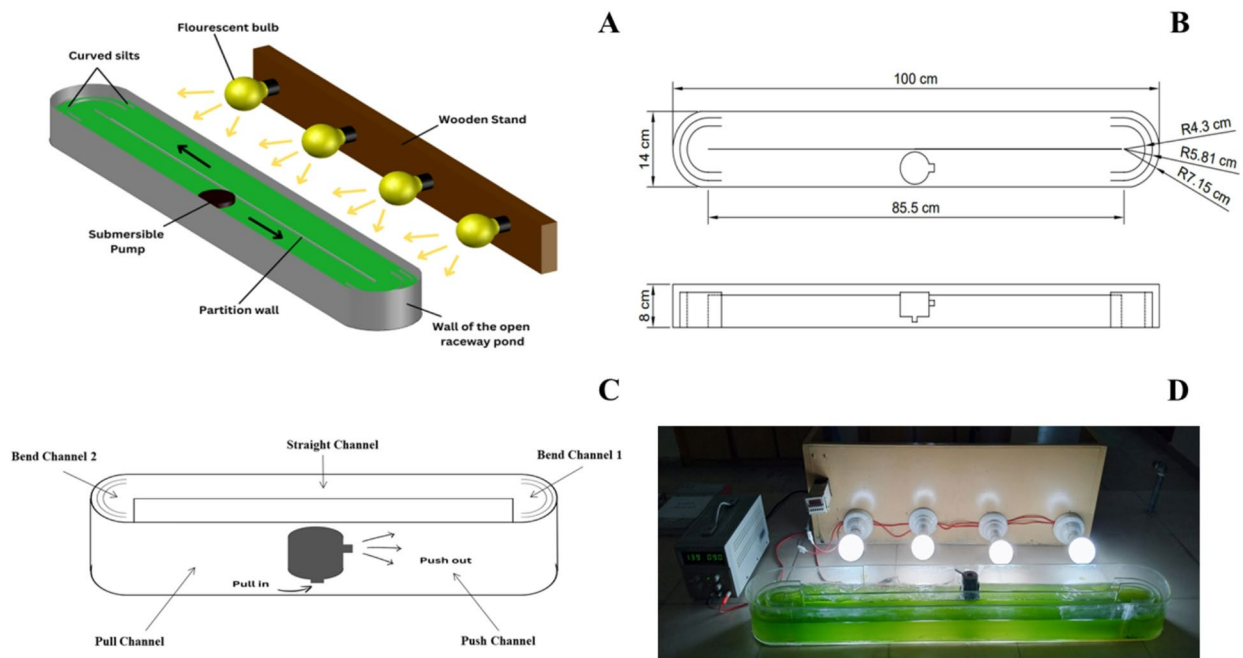
### Inoculum preparation

The *Scenedesmus* sp. Niva-Chl 99 used in the experiment was obtained from the Norwegian Culture Collection of Algae, NORCCA, Oslo, Norway. In the present study *Scenedesmus* sp. was used because of its rapid growth under diverse environmental conditions, tolerance to nutrient fluctuations, and ease of harvesting through natural sedimentation. These characteristics make it a suitable candidate for evaluating improved mixing strategies and energy efficiency in the present system<sup>15</sup>. A sterilized standard BBM media was used to promote the initial inoculum growth of microalgae in a Pyrex bottle (1 L), and the composition of the media was the same as mentioned by Chakraborty<sup>16</sup> (Section S1.1). The *Scenedesmus* sp. culture was allowed to grow under a 14:10 h light-dark cycle, room temperature 25 ± 2 °C, and constant light intensity (200 μmol photons m<sup>-2</sup> s<sup>-1</sup>) maintained through four 20-Watt LED lights (Race Technology, China). An aeration pump with a flow rate (52.5 L h<sup>-1</sup>) was utilized to maintain the culture in suspension, and syringe filters made of 0.2 μm cellulose acetate (Sartorius, 1784B) were used to maintain the sterilized conditions<sup>7</sup>.

*Scenedesmus* sp. biomass was harvested via centrifugation at 1500 x g for 5 min. Deionized water was used to wash the harvested algae and centrifuged a second time at 1500 x g for 5 min to avoid interference from the nutrient media before being used for experimentation. Algae pellets were then evaluated in terms of optical density at 680 nm (OD<sub>680</sub>) spectrophotometrically (PG Model T60U, PG Instruments, UK) and introduced into an open raceway pond to provide an initial biomass concentration of 0.2 g L<sup>-1</sup> based on Eq. 3.

### Design and fabrication of open raceway pond

Figure 1 presents the schematic illustration of the laboratory-scale novel raceway system, showing (A) the diagrammatic labeling of the experimental setup, (B) the top and side views, (C) the flow directions and flowrate measuring channels, and (D) the visual representation of the complete setup. An open raceway pond made up of acrylic (5 mm, thickness) was used in this experiment. The pond consists of two straight flow channels (length: 80 cm, width: 14 cm, depth: 9.8 cm) and two bend flow channels (radius: 11 cm, depth: 9.8 cm) created through a center acrylic wall partition. Each bend flow channel also has two acrylic slits (5 mm, thickness) of radius 5.81 cm and 4.3 cm, separated by a channel of 1.5 cm from each other, respectively. A locally manufactured



**Fig. 1.** Schematic illustration of the laboratory scale novel raceway system. (A) diagrammatic labeling of the experimental setup, (B) top and side views of the system, (C) flow directions and flowrate measuring channels in the pond, and (D) visual representation of the setup.

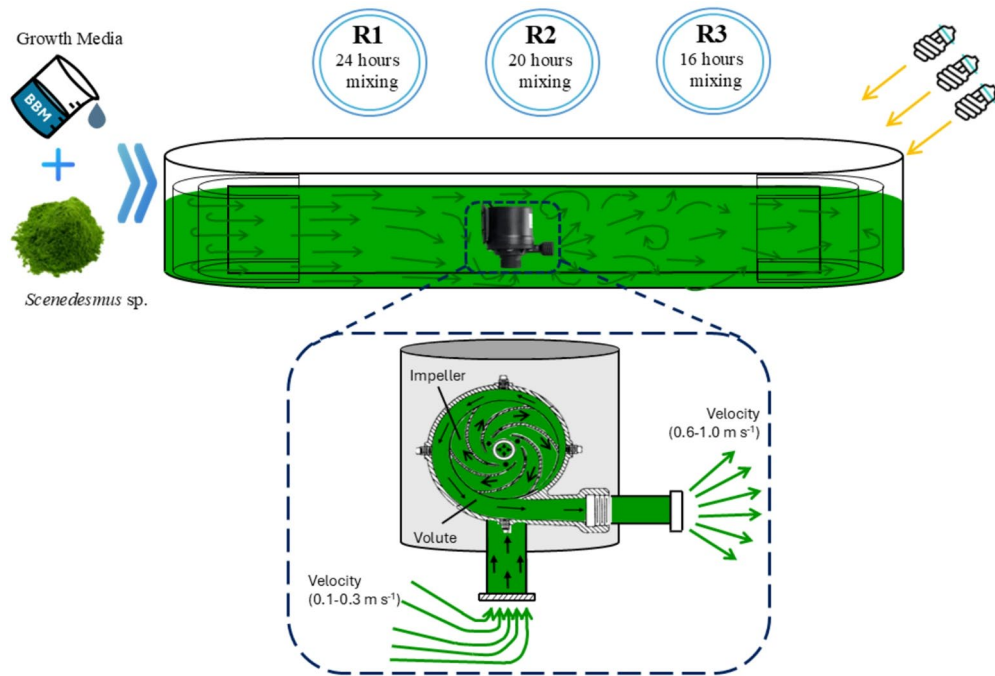
submersible pump (SHAFI) of power (8 W, DC 12 V) was installed in one of the straight flow channels to promote the circulation of microalgae culture and simulate outdoor open raceway systems, where cost-effectiveness and operational simplicity rely on wind and surface aeration supplemented by mechanical paddlewheels, the study was conducted with submersible pumps assuming they could provide comparable aeration while enabling higher velocity ranges. In this setup, additional CO<sub>2</sub> supplementation was avoided to mimic outdoor conditions. The pump employed in this system functions by drawing the microalgal culture through an inlet at the bottom, situated at a height (1 cm) from the bottom surface of the pond, and subsequently propelling it through an outlet located along the channel parallel to the base as shown in Fig. 1C. No spargers were used for air bubbling and turbulence generated by the culture flow was the sole supplier of pure air. A DC power supply (Matrix) was used to operate the submersible pump at constant voltage (9 V) to maintain a mean flow velocity of 0.35 m s<sup>-1</sup> inside the open raceway pond, ensuring turbulent mixing and no splash out. Light intensity was maintained at 200 μmol photons m<sup>-2</sup> s<sup>-1</sup> using four 9 W fluorescent bulbs about 24 cm apart and was measured using the quantum sensor (Apogee quantum sensor, MQ 500, USA).

### Experimental phases and conditions

The experiments were conducted in the Algal Biotechnology Lab located inside the Environmental Engineering Department (IESE), NUST (N 33° 38' 44.0592", E 72° 59' 25.242"). To explore the impacts of mixing duration on *Scenedesmus* sp. biomass production, experiments were conducted in three distinct raceway systems (R1, R2, and R3), each having the same configuration but different operating conditions in terms of daily mixing duration as shown in Fig. 2. In system R1, the submersible pump operated continuously to permit mixing for 24 h per day. In system R2, the pump was activated for 20 h and was not operational for 4 h per day, causing the culture not to mix during the interruption period. In system R3, the pump was activated for 16 h per day only, halting the culture mixing for 8 h per day. The experiment was performed for a duration of 10 days, and all experiments were performed in triplicate. The cultivation of microalgae in all systems was conducted with a 6 L working volume containing algae inoculum and standard BBM. The working volume of 6 L was opted as the optimum operating volume for the system to impart efficient mixing and to avoid overflowing. The pH and electric conductivity were measured daily using a multimeter (inoLab pH/Cond 720, Germany), and pH was adjusted initially at 6.5 ± 0.1 by adding either 8% NaOH or 2% HCl solution as required<sup>18</sup>.

### Biomass settling rate

The settlement of *Scenedesmus* sp. in an open raceway pond was observed independently from the primary experiments by switching off the pump for 4 h and 8 h, respectively. For this purpose, the same concentration of algae was transferred into the raceway pond, allowing mixing in R2 for the first 20 h and in R3 for the first 16 h. The *Scenedesmus* sp. cells were also observed under the microscope (Olympus CKX41) before and after the experiment to investigate the potential impacts of submersible pump thrust on algal cells (Section S1.2).



**Fig. 2.** The experimental setup shows the raceway pond configuration. The inputs are highlighted on the left and right of the pond and mixing regimes at the top (R1–R3). The submersible panel below shows that the impeller of a submersible pump raises fluid's velocity and pressure imparting mixing through turbulent eddies.

#### Biomass mixing efficiency due to the submersible pump

The mixing efficiency of the submersible pump used in an open raceway system for *Scenedesmus* sp. cultivation was evaluated through a separate experiment. *Scenedesmus* sp. biomass ( $0.20 \text{ g L}^{-1}$ ) in BBM was introduced into the raceway pond and mixed for 12 h at a mean velocity of  $0.35 \text{ m s}^{-1}$ . Working volume was kept at 6 L and the experimental conditions were kept similar to the mixing duration experiment. A composite sample based on four subsamples of 20 mL each was collected from different heights (0, 1.5, 3, and 4.75 cm), where 0 cm represents a sample from the bottom surface of the bioreactor and 4.75 cm represents the surface sample within the working volume of the pond. These samples were analyzed for biomass production to assess the mixing uniformity throughout the pond. The raceway system consists of five distinct zones as shown in Fig. 1C. The fluid velocity in each zone was measured by using a water flow probe at working culture volume as well as the maximum culture volume that the raceway system could sustain to observe the impacts of velocity on system operations.

#### Evaporative losses

To counter the impact of evaporation on biomass outcome, evaporative losses were compensated with the addition of distilled water after every 24 h. The volume of culture loss through evaporation was determined by evaluating the working depth of the open raceway pond. The initial working depth of the open raceway pond was 4.75 cm, observed through the measuring scale present on one side of the system. The change in depth was multiplied by the area of the open raceway pond to calculate the loss volume of culture through evaporation.

$$V_{evap} = \frac{(H_i - H_f) 1000}{(T_f - T_i)} A_{SRP} \quad (1)$$

where  $V_{evap}$  is the loss of water from the culture due to evaporation in open raceway pond ( $\text{L d}^{-1}$ ),  $H_f$  and  $H_i$  are the final and initial depth of the working area of the open raceway pond (m),  $T_f$  and  $T_i$  are the final and initial time duration of water loss through evaporation (d), and  $A_{SRP}$  is the total area of the open raceway pond ( $\text{m}^2$ ).

The  $V_{evap}$  value obtained from Eq. 1 was verified by the evaporation rate calculations mentioned by Malek et al.<sup>19</sup> and Matanguihan et al.<sup>20</sup>.

$$F_{evp} = \frac{L W Ev_h}{\rho L_{evp}} \quad (2)$$

Where  $F_{evp}$  is volumetric flow of water leaving the open raceway pond ( $\text{m}^3 \text{ d}^{-1}$ ),  $Ev$  is the evaporation heat flux ( $\text{MJ m}^{-2} \text{ d}^{-1}$ ),  $\rho$  is the density of water ( $\text{kg m}^{-3}$ ),  $L$  is the length and  $W$  is the width of open raceway pond (m),  $L_{evp}$  is the latent heat of evaporation ( $\text{kJ kg}^{-1}$ ).

### Biomass growth analysis

A correlation between biomass concentration and optical density was developed first to observe *Scenedesmus* sp. growth in an open raceway pond. For this purpose, 5, 10, 15, 20, and 25 mL samples of *Scenedesmus* sp. culture were collected and diluted up to 50 mL with distilled water. A UV visible spectrophotometer (PG Model T60U, PG Instruments, UK) operated at 680 nm was used to observe the optical densities of each sample<sup>21,22</sup>. The corresponding samples with the same amount of culture were placed in china dishes and oven dried at 80 °C for 8 h to observe the biomass concentration. The biomass concentration was calculated through the difference in the final weight of the china dish containing algae residue and the initial weight of the empty petri dish. The linear regression established between the OD<sub>680</sub> and biomass concentration (g<sub>dw</sub> L<sup>-1</sup>) for the calculation of *Scenedesmus* sp. dry biomass concentration is as shown in Eq. 3.

$$C_b = 0.9698 a + 0.0403 \quad (R^2 = 0.993) \quad (3)$$

Where  $C_b$  is the biomass concentration (g L<sup>-1</sup>) and  $a$  is the absorbance at OD<sub>680</sub>.

For experimental purposes, culture sampling was done at 24-hour intervals. To avoid bias, 5 mL samples were collected from each of the four sides (two straight flow channels and two bend flow channels) and combined to make a representative sample of 20 mL. The samples were subjected to spectrophotometry at a wavelength of 680 nm using the UV visible spectrophotometer.

### Growth kinetics and nutrients removal of *Scenedesmus* Sp.

The specific growth rate was calculated using Eq. 4 and the doubling time was determined by using the first-order kinetics in Eq. 5 according to a Liu et al.<sup>23</sup>.

$$\mu_{nm} = \frac{\ln(C_{b(f)}) - \ln(C_{b(i)})}{t_f - t_i} \quad (4)$$

Where  $\mu_{nm}$  is specific growth rate, and  $C_{b(i)}$  and  $C_{b(f)}$  are the initial and final biomass concentration of *Scenedesmus* sp. at initial ( $t_i$ ) and final ( $t_f$ ) days, respectively.

$$t_{Doubling} = \frac{\ln(2)}{\mu_{nm}} \quad (5)$$

Where  $t_{Doubling}$  is doubling time and  $\mu_{nm}$  is the specific growth rate of microalgae.

The biomass productivity was calculated according to<sup>24</sup> as shown in Eq. 6.

$$P = \frac{C_{b(f)} - C_{b(i)}}{T_f - T_i} \quad (6)$$

Where  $C_{b(i)}$  and  $C_{b(f)}$  are the initial and final biomass concentration of microalgae at time, initial ( $T_i$ ) and final ( $T_f$ ) days, respectively.

The biomass samples were collected on every alternative day and centrifuged at 1500 x g for 5 min<sup>25</sup>. The obtained supernatant was filtered through a 0.45 μm filter paper using a vacuum filtration assembly and analyzed for orthophosphate (PO<sub>4</sub><sup>3-</sup>), nitrate (NO<sub>3</sub><sup>-</sup>), and ammonia (NH<sub>4</sub><sup>+</sup>) following the standard methods<sup>1</sup>. Removal parameters were calculated as mass removal (R, mg L<sup>-1</sup>) by using Eq. 7, removal efficiency (%R) by using Eq. 8 and nutrient removal rates (RR<sub>x</sub>, mg L<sup>-1</sup> d<sup>-1</sup>) were calculated by using Eq. 9.

$$R = C_{in} - C_{eff} \quad (7)$$

$$\%R = \frac{C_{in} - C_{eff}}{C_{in}} 100 \quad (8)$$

$$RR_x = \frac{(C_{in} - C_{eff})}{t} \quad (9)$$

where  $C_{in}$  and  $C_{eff}$  are the initial and final concentrations of the nutrient, and  $t$  is the time duration of the experimental phase.

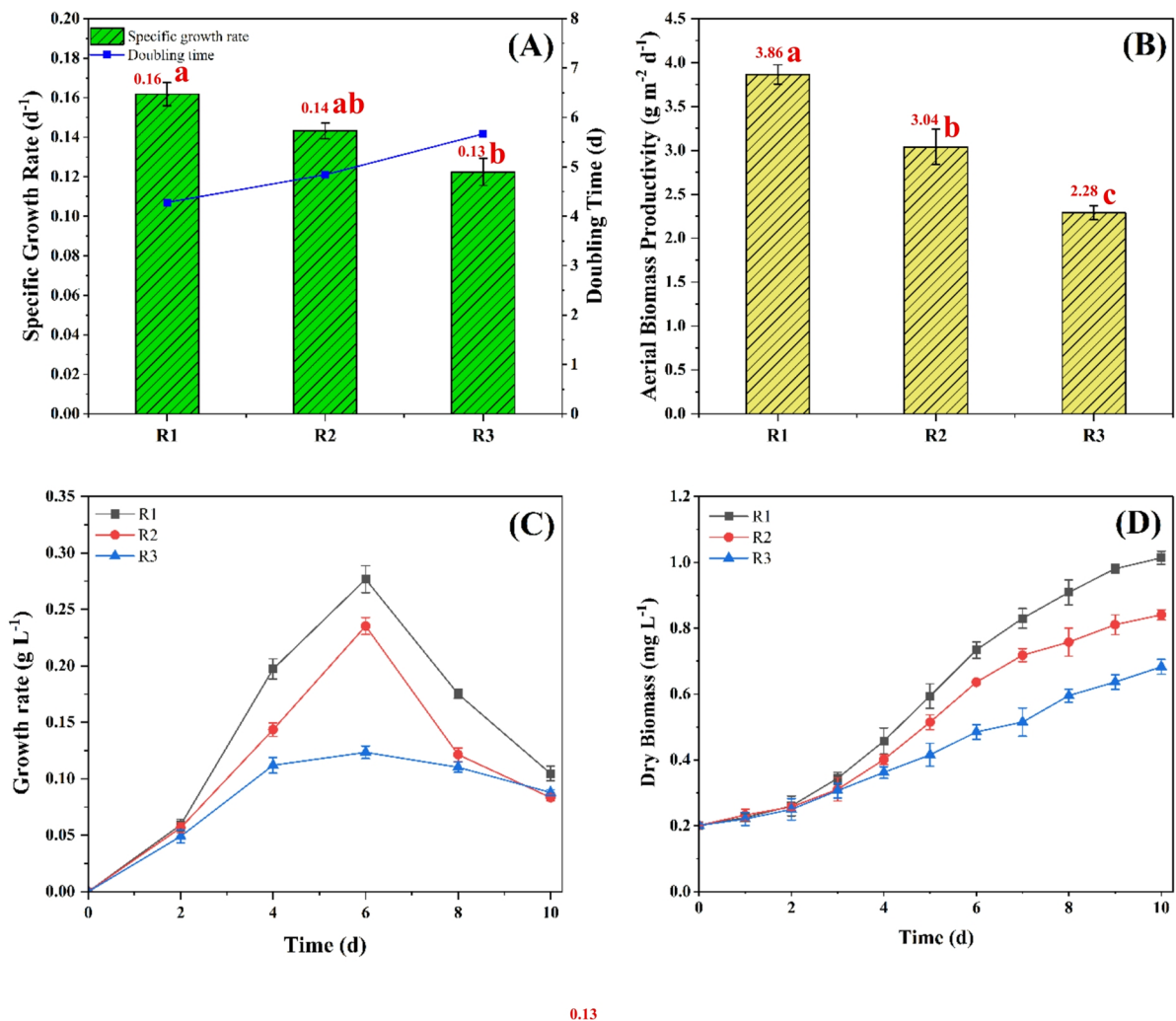
### Statistical analysis

All experiments were performed in triplicate for the growth and nutrient measurement throughout each phase. The results obtained are presented in the form of arithmetic means ± standard deviation. The difference in results and measurement was computed and determined using analysis of variance (ANOVA) with a  $p$ -value of < 0.05 on statistical software Origin 8 (Origin Lab Corporation, USA). The Tukey method was employed to further investigate the variations among biomass production and nutrient removal.

### Results and discussions

#### Growth kinetics and biomass production of *Scenedesmus* Sp.

The specific growth rate, biomass productivity and dry biomass of *Scenedesmus* sp. cultivated in BBM demonstrate the impact of different mixing durations in the raceway pond (Fig. 3A, B and D). Green, robust, and stable *Scenedesmus* sp. cultures were observed in all three experiments, indicating the productivity advantages



**Fig. 3.** Effect of different mixing duration on *Scenedesmus* sp. growth. (A) specific growth rate with doubling time, (B) aerial biomass productivity, (C) growth rate over time, and (D) dry biomass concentration over time. R1 = 24 h/day mixing; R2 = 20 h/day mixing; R3 = 16 h/day mixing. Different superscript letters (a, b, c) indicate statistically significant differences ( $p < 0.05$ ), while values sharing the same letter are not significantly different.

offered through controlled environment in raceway systems. All three mixing duration systems (R1, R2 and R3) showed an initial lag phase of two days where growth was stationary. This might be because *Scenedesmus* sp. was not acclimatized to the gas exchange dynamics based on natural air entrapment due to turbulence generated by liquid flow, since the cultures before the experiment initiation were aerated by bubbling pure air. Hemalatha et al.<sup>26</sup> reported that microalgae need two days to adjust to their new environment before the biomass of algae begins to increase.

*Scenedesmus* sp. specific growth rate increased with an increase in the mixing duration as the system R1 achieved the highest specific growth rate compared to the R2 and R3 systems, respectively, as shown in Fig. 2A. The highest dry biomass yield of 1.01 g L<sup>-1</sup> and biomass productivity of 0.08 g L<sup>-1</sup> d<sup>-1</sup> were observed in the R1 system. The biomass productivities observed in R2 and R3 systems were 20.45% and 48.31% lower than that obtained in the R1 system and the probable reason might be the absence of *Scenedesmus* sp. culture mixing leading to the creation of stagnant zones<sup>27</sup>. The static flow of culture in the pond limits the growth of microalgae generating layers of settled algal cells in the stagnant zone, which further promotes the formation of anaerobic conditions<sup>27,28</sup>. Another reason for reduced growth in R2 and R3 systems might be the development of a nutrient and oxygen gradient around the *Scenedesmus* sp. cells in response to the absence of mixing. The oxygen gradient develops when mixing within the algae culture becomes interrupted, which becomes problematic during the dark regime when algae switch from photosynthesis to respiration. The accumulated oxygen around the algae cell limits the proper supply of carbon dioxide (CO<sub>2</sub>), leading to stress and biomass loss in algae culture<sup>29</sup>. The continuous mixing disperses this gradient by providing a continuous supply of nutrients to algae and uniform distribution and removal of oxygen in the culture. The continuous mixing ensures that the algal cells continuously change their positions, which results in even distribution of algal cells in the culture and prevents the production of stagnant dead zones in the pond<sup>30</sup>. The biomass outcomes obtained in this study also correspond to the

literature where biomass productivity for daytime mixing ( $12 \text{ h d}^{-1}$ ) was 41% lower compared to the continuous mixing of *Nannochloropsis* sp., when cultivated in an outdoor open raceway pond<sup>1</sup>. The Tukey HSD test was used to perform comparisons between the dry biomass of different mixing duration systems. A statistically significant difference existed for the dry biomass of *Scenedesmus* sp. in all three mixing duration systems.

The temperature, oxygen, and pH profiles were also monitored and are described in addition to the growth kinetics in Section S2.1. The positive growth rates for all three systems during the entire experiment suggest the suitability of environmental conditions for biomass growth as shown in Fig. 3C. The drop-in growth rates for the last four days of the experiment might have resulted from the shielding effect of the cells, and elevated pH in systems might have converted dissolved  $\text{CO}_2$  into carbonate and bicarbonates, which remained unconsumed and slowed down the growth of microalgae<sup>31</sup>. A comparison of the current system with other studies in terms of biomass productivity and environmental conditions indicates the potential of the submersible regime for enhanced biomass output under optimal environmental conditions (e.g., aeration,  $\text{CO}_2$  supply, and high light intensity) as shown in Table 1. No statistically significant difference was found in the specific growth rate of *Scenedesmus* sp. between R1 and R2 or between R2 and R3 mixing durations ( $p > 0.05$ ). However, a significant difference was observed between R1 and R3 ( $p < 0.05$ ), with the specific growth rate being higher under continuous mixing duration.

### Effects of submersible regime

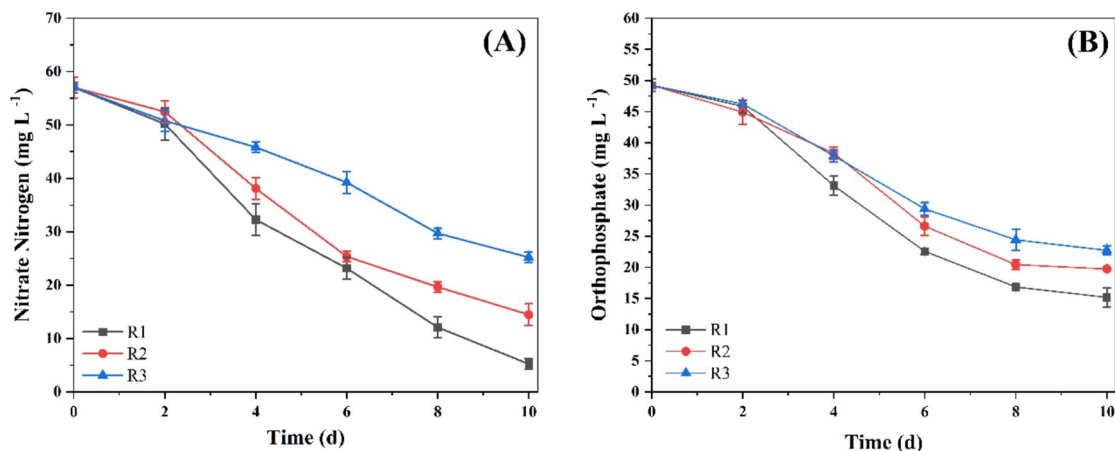
The mixing efficiency experiment analysis indicated that the biomass production varied only slightly among the samples ( $\pm 0.32 \text{ mg L}^{-1}$ ) taken from different heights (Section S2.2). This small variation indicates that the mixing system was highly efficient, as it ensured a uniform distribution of *Scenedesmus* sp. along the entire area of the pond. The liquid velocity initiated by the submersible pump in the push channel was  $0.74 \text{ m s}^{-1}$ , resulting in a turbulent flow necessary for homogeneity (Fig. 1(C, D)). The flow velocity greatly reduces in the bent channels and along the straight channel due to pressure loss from clockwise movement and head loss associated with liquid frictional resistance (range, 0.25 to  $0.29 \text{ m s}^{-1}$ ). The mean flow velocity of  $0.35 \text{ m s}^{-1}$  maintained in submersible regime exceeds the operational flow velocities (range,  $0.2$  to  $0.3 \text{ m s}^{-1}$ ) employed for mixing of culture in traditional raceway ponds, ensuring efficient mixing and avoiding formation of dead zones<sup>8</sup>. The pump inlet in the pull channel draws liquid from the bottom layer and propels it into the top culture layers through an outlet that allows further agitation and mixing as indicated in Fig. 1C.

The estimated evaporation rate was  $0.40 \text{ L d}^{-1}$  in the R1 system, which represents 6.75% of the total working volume of 6 L. The evaporation rate values were  $0.34 \text{ L d}^{-1}$  and  $0.27 \text{ L d}^{-1}$  in R2 and R3 systems, corresponding to 5.6% and 4.5% of total working volume, respectively. The higher evaporation rate in the R1 system might be due to high surface turbulence compared to the R2 and R3 systems. The consistent agitation in the culture promotes better heat transfer from the culture surface to the air, speeding up the evaporation losses<sup>37</sup>. The surface turbulence replaces the layer of humid air with fresh air on the surface of the open raceway pond, leading to accelerated evaporation of culture<sup>38</sup>. In R2 and R3 systems, the mixing was stopped for 4 and 8 h, respectively, causing fewer disruptions to the air-liquid interface, ultimately leading to slower evaporation rates. The evaporation rate was estimated as the ratio of culture volume height to time multiplied by the area, as shown in Eq. 7, and was verified with the evaporation losses estimated from Eq. 2 proposed by previous study<sup>19,20</sup>. There was a difference of  $\pm 20 \text{ mL d}^{-1}$  in culture volume corresponding to 0.33% error, suggesting that the evaporation loss can be expressed as a simplified function of height for controlled raceway ponds.

A few studies have suggested intermittent mixing during cultivation as an energy saving alternative to continuous mixing<sup>9,39</sup>. However, the biomass settling experiment indicated that 8 h of mixing cessation resulted in 27% settlement of biomass compared to the initial concentration of algae in the R3 system, suggesting sinking of *Scenedesmus* sp. under the influence of gravity. Similarly, after 4 h of stoppage, 14% of biomass was found to be sinking to the bottom of the R2 system. In the previous study, the flagella of *Terselmis suecica* ceased to sink to the bottom of the pond after switching off the pump for a night, as fast moving and lively cells were observed under the microscope<sup>39</sup>. Since the *Scenedesmus* sp. is nonmotile due to a lack of flagella, this is more susceptible to settling due to natural sedimentation at the bottom, when there is no mixing<sup>40,41</sup>. The stoppage of mixing in R2 and R3 systems might have led to the development of stagnant or dead zones in the raceway ponds. These zones reduce effective pond volume and contribute to biomass loss through the creation of anaerobic conditions and respiration in dark zones<sup>42,43</sup>. This might be the reason for reduced biomass production in R2 and R3 systems as compared to the R1 system.

### Nutrient recovery in response to growth Nitrate removal

The growth and biomass production of microalgae is directly linked with nitrogen uptake, which is an important requirement for the synthesis of nucleic acid, cellular protein and phospholipids<sup>44</sup>. In this study, nitrate as the source of nitrogen was studied throughout the experimental period due to it being the readily available source of nitrogen. The concentration of  $\text{NO}_3^-$  showed a decreasing trend in all three different mixing duration systems as indicated in Fig. 4(A). The mass removal of nitrate was observed to be  $51.81 \text{ mg L}^{-1}$  in the R1 system. After the initial 2 days, large drops in nitrate concentration were observed for the R1 system, leading to a 90.82% reduction at the end of the experiment. The removal efficiency of nitrate nitrogen was 75.16% in R2 and 55.81% in R3 systems, suggesting that the continuous mixing in the R1 system prevented nutrient stratification, ensuring uniform nitrate distribution and consistent algal access for optimal uptake, ultimately leading to higher nitrate removal<sup>45</sup>. Additionally, the low initial pH of 6.5 stabilized the nitrate nitrogen at the beginning of all mixing systems. However, as the pH increased from 6.5, microbial processes including denitrification and dissimilatory nitrate reduction to ammonia became more active, promoting the conversion of nitrate nitrogen into ammonia nitrogen ( $\text{NH}_3 - \text{N}$ ). The R2 and R3 systems had 8.5 and 8.4 pH values at the end of the experiment, which



**Fig. 4.** Nutrient removal performance of *Scenedesmus* sp. in raceway system under three different mixing duration systems: (A) nitrate-nitrogen concentration, and (B) orthophosphate concentration. R1 = 24 h/day mixing; R2 = 20 h/day mixing; R3 = 16 h/day mixing.

are less than the 8.9 pH observed in the R1 system. Below 8.5 pH, the rate of nitrate nitrogen reduction to ammonium ( $\text{NH}_4^+$ ) and to ammonia release is lower<sup>46</sup>. As the pH reaches 8.5 and above, the ammonium rapidly converts into ammonia gas, leading to significant nitrogen loss through volatilization<sup>47</sup>. A statistically significant difference ( $p < 0.05$ ) was found in the nitrates mass removal, removal rate, and removal efficiency between *Scenedesmus* sp. cultivated under 16 h, 20 h and 24 h mixing duration conditions.

#### Orthophosphate removal

Phosphorus is an essential nutrient along with nitrogen for the cultivation of microalgae. According to<sup>48</sup>. The dry biomass of microalgae constitutes 0.6–0.8% of phosphorus in its overall weight. A considerable reduction of orthophosphate was observed in all three raceway systems as shown in Fig. 4(B). The maximum removal of orthophosphate, 69.17%, was observed in the R1 system, which was 9.27% and 15.34% higher than the R2 and R3 systems. A statistically significant difference ( $p < 0.05$ ) was found in the phosphate mass removal for R1, R2 and R3 mixing durations. The continuous mixing in the R1 system reduced stratification within the open raceway pond, which avoids the accumulation of phosphorus at the lower level<sup>21</sup>. The mixing was stopped for 4 h and 8 h in R2 and R3 systems, the phosphorus might have settled down and become less available for the algal uptake. The rate of orthophosphate reduction at the end of the experiments became steady and gradual reductions were observed for the last two days in all three systems. The lower levels of nitrogen could change the phosphate reduction patterns in the media<sup>17,49</sup>. The removal of phosphate was reduced at the end of the experiment due to the reduced level of nitrogen, contributing to the incomplete removal of inorganic phosphorus as the microalgae consumes the essential nutrient in a balanced concentration to perform the optimum regulation of their biological processes<sup>50</sup>. Table 1 shows the nutrient parameters including the mass removal (R), average removal rate (RR) and efficiency of inorganic phosphorus (%R). Mixing duration directly correlates with the nutrient removal in the growth media. A statistically significant difference ( $p < 0.05$ ) was found in the phosphate removal rate and removal efficiency for all three mixing duration systems.

#### Implications, energy efficiency and upscaling

The present study entails a promising approach of utilizing submersible pumps in raceway systems to improve the design and operations of raceway ponds. The traditional raceway systems are largely dependent on the immersion depth of the paddle wheel, where an increase in depth can lead to culture volume spilling out by the paddle wheel, allowing a maximum operating velocity in the range of 0.15–0.3  $\text{m s}^{-1}$ <sup>51</sup>. Furthermore, the energy consumption of the paddle wheel also increases with an increase in culture volume depth as the power directly depends on the culture load<sup>52</sup>. The inclusion of a submersible pump in place of a paddle wheel for raceway ponds permits a wider range of operating velocities (0.10–0.45  $\text{m s}^{-1}$ ) without causing spill out. In the current study, it was observed that the pump impeller caused the diffuser to generate pressure and propel the culture volume turbulently, such that the flow caused the culture volume to be uniformly mixed throughout the raceway system, as shown in Fig. 2. Additionally, a sparger connected with a compressor allows pure air bubbling in conventional open raceway ponds. In the current study, no spargers were used and the system received air through flow turbulence generated at the surface by submersible pump output thrust. The presence of two slits at each bent zone created three narrow channels that stabilized the flow and improved mixing. These curved slits enhance the mixing of microalgae in culture and prevent the formation of dead zones in areas with minimal water flow<sup>53</sup>. Nevertheless, further studies are required for optimization of operational conditions in submersible regime systems to maximize energy utilization and enhance biomass productivity.

The submersible pumps are also known for energy efficiency as they are placed directly in water and can operate at variable frequency drives. The net energy efficiency ( $\eta$ ) for the submersible pump was estimated as the ratio of the theoretical power ( $W_h$ ) to the actual measured energy consumption ( $W_m$ ) as shown in section S2.3.

| Algae                           | Scale | Reactor   | Growth media                  | Specific growth rate | Biomass productivity  | Growth conditions  |                        |   | References |
|---------------------------------|-------|---|-------------------------------|----------------------|---|--------------------|------------------------|---|------------|
|                                 |       |   |                               |                      |   | Aeration           | CO <sub>2</sub> supply | Light Intensity                             |            |
| <i>Scenedesmus</i> sp.          | Lab   | Open raceway pond   | Synthetic media (BBM)         | 0.16 d <sup>-1</sup> | 0.08 g L <sup>-1</sup> d <sup>-1</sup> or 3.9 g m <sup>-2</sup> d <sup>-1</sup> | No                 | No                     | 200 μmol m <sup>-2</sup> s <sup>-1</sup>    | This study |
| <i>Scenedesmus</i> sp.          | Lab   | Open raceway pond (batch)                                     | Synthetic media (BBM)         | –                    | 0.085 g L <sup>-1</sup> d <sup>-1</sup>   | Yes                | 1% supplement          | 110 μmol m <sup>-2</sup> s <sup>-1</sup>    | 32         |
|                                 |       |   |                               | 0.14 d <sup>-1</sup> | 0.055 g L <sup>-1</sup> d <sup>-1</sup>   |                    | 0.035% supplement      |   |            |
| <i>Nannochloropsis oculata</i>  | Lab   | Open raceway pond (conventional)                              | Synthetic media (f/2)         | 0.14 d <sup>-1</sup> | 0.043 g L <sup>-1</sup>   | Yes                | Yes                    | 800 μmol m <sup>-2</sup> s <sup>-1</sup>    | 33         |
|                                 |       | Open raceway pond (built-in planar waveguide modules (43 mm)) |                               | 0.19 d <sup>-1</sup> | 0.08 g L <sup>-1</sup>  |                    |                        |   |            |
| <i>Scenedesmus</i> sp.          | Lab   | Indoor open raceway pond (Raceway simulating reactor)         | Centrate                      | –                    | 7.80 g m <sup>-2</sup> d <sup>-1</sup>  | Magnetic agitators | For pH adjustments     | 200 μmol m <sup>-2</sup> s <sup>-1</sup>    | 15         |
| <i>Scenedesmus</i> sp.          | Pilot | Outdoor open raceway pond                                     | Secondary domestic wastewater | –                    | 13 g m <sup>-2</sup> d <sup>-1</sup>  | Yes                | No                     | Sunlight                                    | 34         |
| <i>Nannochloropsis gaditana</i> | Pilot | Outdoor open raceway pond                                     | Centrate                      | –                    | 0.1 g L <sup>-1</sup> d <sup>-1</sup> or 5.5 g m <sup>-2</sup> d <sup>-1</sup>  | Yes                | Yes                    | Sunlight                                    | 35         |
| <i>Chlorella</i>                | Lab   | Open Raceway Pond (fitted with flow deflectors)               | Synthetic media               | –                    | 4.95 g m <sup>-2</sup> d <sup>-1</sup>  | Yes                | Yes                    | 192.24 μmol m <sup>-2</sup> s <sup>-1</sup> | 36         |
|                                 |       | Open Raceway Pond (Conventional)                              |                               |                      | 6.24 g m <sup>-2</sup> d <sup>-1</sup>  |                    |                        | 445.14 μmol m <sup>-2</sup> s <sup>-1</sup> |            |

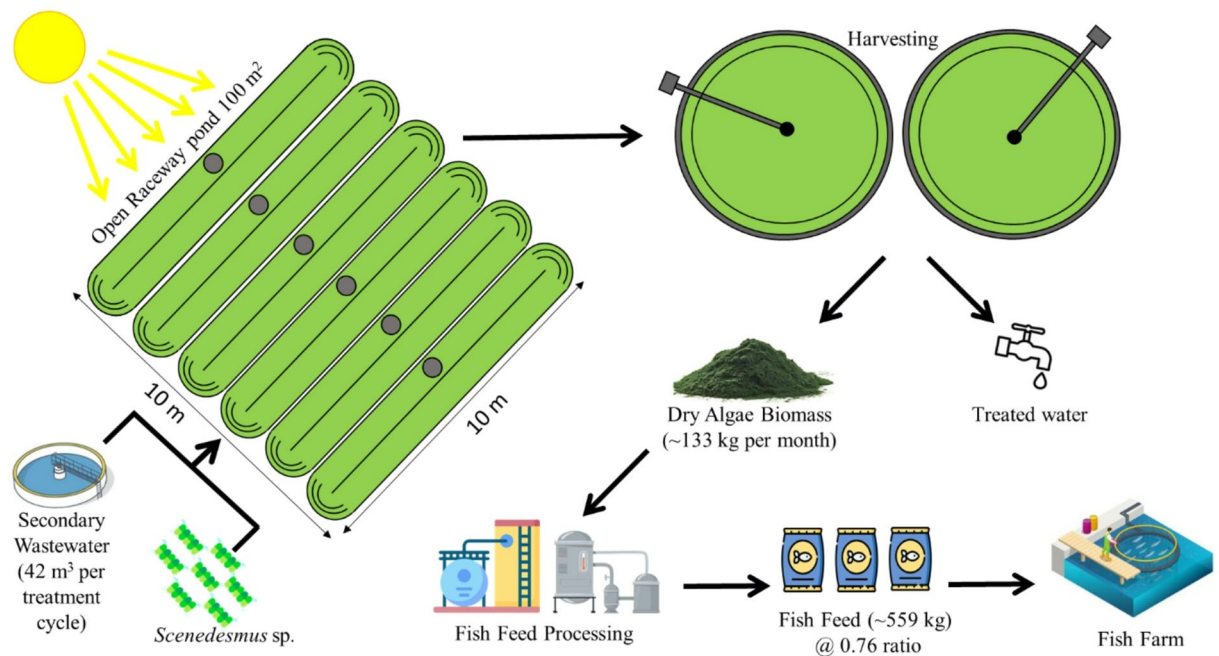
**Table 1.** Comparative assessment of submersible based raceway system with conventional raceway ponds in terms of biomass productivity and specific growth rate.

| Systems | Nutrient Parameters | Nitrate (NO <sub>3</sub> <sup>-</sup> -N) | Orthophosphate (PO <sub>4</sub> <sup>3-</sup> -P) |
|---------|---------------------|---|---|
| R1      | R                   | 51.81 ± 2.37 <sup>a</sup>                 | 34.05 ± 0.44 <sup>a</sup>                         |
|         | RR <sub>x</sub>     | 5.18 ± 0.24 <sup>a</sup>                  | 3.41 ± 0.04 <sup>a</sup>                          |
|         | % R                 | 90.82 ± 3.09 <sup>a</sup>                 | 69.17 ± 0.65 <sup>c</sup>                         |
| R2      | R                   | 42.56 ± 2.74 <sup>b</sup>                 | 29.49 ± 0.19 <sup>b</sup>                         |
|         | RR <sub>x</sub>     | 4.26 ± 0.27 <sup>b</sup>                  | 2.95 ± 0.02 <sup>b</sup>                          |
|         | % R                 | 74.62 ± 0.94 <sup>b</sup>                 | 59.90 ± 0.32 <sup>c</sup>                         |
| R3      | R                   | 31.84 ± 0.32 <sup>c</sup>                 | 26.50 ± 1.16 <sup>c</sup>                         |
|         | RR <sub>x</sub>     | 3.18 ± 0.03 <sup>c</sup>                  | 2.65 ± 0.12 <sup>c</sup>                          |
|         | % R                 | 55.81 ± 0.72 <sup>c</sup>                 | 53.83 ± 1.75 <sup>c</sup>                         |

**Table 2.** Nutrient recovery analysis for *Scenedesmus* sp. cultivated in raceway pond under different mixing duration systems. R denotes mass removal (mg L<sup>-1</sup>), RR<sub>x</sub> removal rate (mg L<sup>-1</sup> d<sup>-1</sup>), %R removal efficiency (%). R1 = 24 h/day mixing; R2 = 20 h/day mixing; R3 = 16 h/day mixing. Different superscript letters (a, b, c) indicate statistically significant differences ( $p < 0.05$ ) determined among removal efficiency, removal rate, and biomass.

The net energy efficiency corresponds to 62% for a mean velocity maintained at 0.35 m s<sup>-1</sup>. The energy efficiency of the raceway system dropped to 11% when estimated for the maximum working volume (~ 9 L), providing 0.35 m s<sup>-1</sup> mean velocity indicating the energy demand increases alongside increasing culture depth and mixing duration. It is estimated that paddle wheels contribute to about ~ 25% of the total power consumption for raceway cultivation systems<sup>52</sup>. The net energy ratio (NER), 0.049, was calculated based on the difference between the energy produced from biomass to the energy consumed for its production, as described in section S2.3. The input energy supplied by the submersible pump was 720 KJ d<sup>-1</sup> whereas the theoretical energy recovery for *Scenedesmus* sp., 19.5 MJ kg<sup>-1</sup>, was estimated based on calorific values evaluated by previous study<sup>54</sup>. The NER values in the range of 0.04–0.09 are typical of open raceway systems and differ by the category of harvesting method, microalgae secondary products, economies of scale and inclusion of solar panels<sup>55</sup>.

In algal cultivation systems, priority should be on reducing the energy requirements as well as focusing on optimizing the mixing. Considering the experimental outcomes of the present study, an R1 based raceway system can be used to treat 42 m<sup>3</sup> of working volume of wastewater by installing raceway systems in an area of 100 m<sup>2</sup>. R1 system with 24 h mixing was considered for scale-up as the system produced the highest biomass production corresponding to the largest nutrient recovery. It is assumed that six submersible regime ponds will be operated with a 65% working volume compared to the total capacity of 6528 m<sup>3</sup> each having an area of 13.6 m<sup>2</sup>. Based on the experimental results achieved for nutrient removal and biomass production in the R1 system, the average removal for nitrate and phosphate would be 65.2 kg and 31.2 kg per year while the *Scenedesmus* sp. production would be 1.6 × 10<sup>3</sup> kg per year. According to the scale, a 1.0HP submersible pump (Flow 300



**Fig. 5.** Upscaling of submersible regime-based raceway system for *Scenedesmus* sp. cultivation in 100 m<sup>2</sup> area with continuous mixing (24 h) operation.

L min<sup>-1</sup>, Power 0.75 KW, Pipe diameter 50.8 mm) can be used for each raceway pond to circulate the culture liquid with a mean velocity of about 0.35 m s<sup>-1</sup>. The total pump driven energy required to produce 1 kg biomass would be 24.8 MJ, resulting in energy efficient biomass production. The proposed upscaling scheme indicated that 100 m<sup>2</sup> will be able to produce 1657.6 kg per year dry biomass of *Scenedesmus* sp., which can be utilized for further applications including biofuels, nutraceuticals and animal feed<sup>56</sup>, as shown in Fig. 5. The protein content of *Scenedesmus* strain generally falls in the range of 30–50% of dry biomass, demonstrating its potential as a sustainable protein source for fish meal<sup>54,57–59</sup>, as shown in Fig. 5. However, these are theoretical values that are calculated based on the results of a lab-scale study and must not be extrapolated to largescale systems without further validation.

The economic feasibility of algae-based systems is influenced not only by capital investment and associated operation and maintenance costs but also by critical factors such as strain selection, environmental conditions, reactor design, process optimization, and system scalability, all of which collectively determine the competitiveness of algae-derived products. Large-scale open raceway ponds configured with paddlewheels usually have 10–20% overall electrical hydraulic efficiency<sup>55</sup>. In comparison, this study presents that the submersible pump achieved 62% net energy efficiency under controlled laboratory conditions, demonstrating relative reduction in energy demand ranging 68–84%. Although lab-scale open raceway pond demonstrated higher energy efficiency, scaling to industrial level open raceways will raise specific energy demand as frictional and hydraulic head losses increases with the system dimensions, so maintaining the same mean flow rate would require higher pump power<sup>60</sup>. The submersible pump-driven raceway pond enabled a broader velocity range (0.10–0.45 m s<sup>-1</sup>), thereby improving mixing quality with minimal dead zones and cell disruption, however, this system is expected to involve higher maintenance demands due to continuous submergence compared to paddlewheel regime<sup>61</sup>. The incorporation of slit baffles further enhanced flow distribution and nutrient availability, supporting superior cultivation performance<sup>62</sup>. Although evaporative losses play a role in culture cooling, covering raceways can reduce excessive evaporation while simultaneously minimizing the risk of contamination<sup>63</sup>. The fully submerged configuration of submersible system allows ease of covering and low evaporative losses due to reduced liquid surface area exposed to air compared to paddlewheels having partial immersion. However, uncertainties regarding scale-up performance where frictional losses and energy demands may differ significantly from laboratory conditions, demand investigations under pilot- and full-scale outdoor settings to validate long-term performance, operational reliability, and economic feasibility. Addressing these limitations through design optimization and operational refinements will be critical to realizing the large-scale applicability of this technology for sustainable microalgae cultivation.

## Conclusion

This study highlights that submersible pump-driven raceway ponds can overcome key limitations of conventional paddlewheel systems by enabling broader velocity ranges and preventing culture overflow. The improved surface hydrodynamics allowed for the uniform distribution of cells and intercepted the formation of stagnant or dead zones. Continuous mixing delivered the highest biomass productivity (1.01 g L<sup>-1</sup> d<sup>-1</sup>) and nutrient removal efficiency, while the system achieved optimal mixing and a net energy efficiency of 62%, underscoring

its potential as a practical and cost-effective strategy for large-scale *Scenedesmus* cultivation. These findings highlight the novelty and promise of submersible pump integration in raceway pond design; however, the results remain preliminary, demanding validation at larger scales, under outdoor conditions, and across different algal strains with optimization of operational conditions and sensitivity analysis remaining critical to the large-scale application of this technology.

### Data availability

Data will be made available on request. Requests for data should be directed to Dr. Rashid Iftikhar (rashid.iftikhar@iese.nust.edu.pk; rashid.iftikhar@hotmail.com).

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### References

- Vadiveloo, A. & Moheimani, N. Effect of continuous and daytime mixing on *Nannochloropsis* growth in raceway ponds. *Algal Res.* **33**, 190–196. <https://doi.org/10.1016/j.algal.2018.05.018> (2018).
- Wijffels, R. H., Kruse, O. & Hellingwerf, K. J. Potential of industrial biotechnology with cyanobacteria and eukaryotic microalgae. *Curr. Opin. Biotechnol.* **24**, 405–413. <https://doi.org/10.1016/j.copbio.2013.04.004> (2013).
- Vanthoor-Koopmans, M., Wijffels, R. H., Barbosa, M. J. & Eppink, M. H. M. Biorefinery of microalgae for food and fuel. *Bioresour. Technol.* **135**, 142–149. <https://doi.org/10.1016/j.biortech.2012.10.135> (2013).
- Valverde, F., Romero-Campero, F. J., León, R., Guerrero, M. G. & Serrano, A. New challenges in microalgae biotechnology. *Eur. J. Protistol.* **55**, 95–101. <https://doi.org/10.1016/j.ejop.2016.03.002> (2016).
- Borowitzka, M. Large-scale algal culture systems: the next generation. *Australasian Biotechnology* (1994).
- Jorquera, O., Kiperstok, A., Sales, E. A., Embiruçu, M. & Ghirardi, M. L. Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors. *Bioresour. Technol.* **101**, 1406–1413. <https://doi.org/10.1016/j.biortech.2009.09.038> (2010).
- Kumar, K., Mishra, S. K., Shrivastav, A., Park, M. S. & Yang, J. W. Recent trends in the mass cultivation of algae in raceway ponds. *Renew. Sustain. Energy Rev.* **51**, 875–885. <https://doi.org/10.1016/j.rser.2015.06.033> (2015).
- Borowitzka, M. A. & Moheimani, N. R. Open Pond Culture Systems. in *Algae for Biofuels and Energy* (eds Borowitzka, M. A. & Moheimani, N. R.) 133–152 (2013). [https://doi.org/10.1007/978-94-007-5479-9\\_8](https://doi.org/10.1007/978-94-007-5479-9_8)
- Lundquist, T. J., Woertz, I. C., Quinn, N. W. T. & Benemann, J. R. A Realistic Technology and Engineering Assessment of Algae Biofuel Production. (2010).
- Rogers, J. N. et al. A critical analysis of paddlewheel-driven raceway ponds for algal biofuel production at commercial scales. *Algal Res.* **4**, 76–88. <https://doi.org/10.1016/j.algal.2013.11.007> (2014).
- Stephens, E. et al. An economic and technical evaluation of microalgal biofuels. *Nat. Biotechnol.* **28**, 126–128. <https://doi.org/10.1038/nbt0210-126> (2010).
- Kusmayadi, A., Philippidis, G. P. & Yen, H. W. Application of computational fluid dynamics to raceways combining paddlewheel and CO<sub>2</sub> spargers to enhance microalgae growth. *J. Biosci. Bioeng.* **129**, 93–98. <https://doi.org/10.1016/j.jbiosc.2019.06.013> (2020).
- Cheng, J., Yang, Z., Ye, Q., Zhou, J. & Cen, K. Enhanced flashing light effect with up-down chute baffles to improve microalgal growth in a raceway pond. *Bioresour. Technol.* **190**, 29–35. <https://doi.org/10.1016/j.biortech.2015.04.050> (2015).
- Huang, J. et al. Design and optimization of a novel airlift-driven sloping raceway pond with numerical and practical experiments. *Algal Res.* **20**, 164–171. <https://doi.org/10.1016/j.algal.2016.09.023> (2016).
- Jebali, A., Acién, F. G., Sayadi, S. & Molina-Grima, E. Utilization of centrate from urban wastewater plants for the production of *scenedesmus* sp. in a raceway-simulating reactor. *J. Environ. Manage.* **211**, 112–124. <https://doi.org/10.1016/j.jenvman.2018.01.043> (2018).
- Chakraborty, B., Gayen, K. & Bhowmick, T. K. Transition from synthetic to alternative media for microalgae cultivation: A critical review. *Sci. Total Environ.* **897**, 165412. <https://doi.org/10.1016/j.scitotenv.2023.165412> (2023).
- Saleem, S. et al. Operation of microalgal horizontal twin layer system for treatment of real wastewater and production of lipids. *J. Water Process. Eng.* **48**, 102932. <https://doi.org/10.1016/j.jwpe.2022.102932> (2022).
- Bulykina, S. S., Ziganshina, E. E. & Ziganshin, A. M. Growth Efficiency of *Chlorella sorokiniana* in Synthetic Media and Unsterilized Domestic Wastewater. *BioTech* **12**, 53 (2023). <https://doi.org/10.3390/biotech12030053>
- Malek, A., Zullo, L. C. & Daoutidis, P. Modeling and dynamic optimization of microalgae cultivation in outdoor open ponds. *Ind. Eng. Chem. Res.* **55**, 3327–3337. <https://doi.org/10.1021/acs.iecr.5b03209> (2016).
- Matanguihan, A. E. D. et al. Design, fabrication, and performance evaluation of open raceway ponds for the cultivation of *Chlorella vulgaris* Beijerinck in the Philippines. *Philippine J. Sci.* **149**, 353–362. <https://doi.org/10.56899/149.02.13> (2020).
- Ahmad, S., Pathak, V. V., Kothari, R., Kumar, A. & Naidu Krishna, S. B. Optimization of nutrient stress using *C. pyrenoidosa* for lipid and biodiesel production in integration with remediation in dairy industry wastewater using response surface methodology. *3 Biotech.* **8**, 326. <https://doi.org/10.1007/s13205-018-1342-8> (2018).
- Xu, C., Wang, L., Liu, Z., Cai, G. & Zhan, J. Nitrogen and phosphorus removal efficiency and algae viability in an immobilized algae and bacteria symbiosis system with Pink luminescent filler. *Water Sci. Technol.* **85**, 104–115. <https://doi.org/10.2166/wst.2021.606> (2022).
- Liu, Y. et al. Treatment of real aquaculture wastewater from a fishery utilizing phytoremediation with microalgae. *J. Chem. Technol. Biotechnol.* **94**, 900–910. <https://doi.org/10.1002/jctb.5837> (2019).
- Saleem, S., Sheikh, Z., Iftikhar, R. & Zafar, M. I. Eco-friendly cultivation of microalgae using a horizontal twin layer system for treatment of real solid waste leachate. *J. Environ. Manage.* **351**, 119847. <https://doi.org/10.1016/j.jenvman.2023.119847> (2024).
- APHA. Standard Methods for the Examination of Water and Wastewater. (2017).
- Hemalatha, M., Sravan, J. S., Min, B. & Venkata Mohan, S. Microalgae-biorefinery with cascading resource recovery design associated to dairy wastewater treatment. *Bioresour. Technol.* **284**, 424–429. <https://doi.org/10.1016/j.biortech.2019.03.106> (2019).
- Richmond, A. Biological Principles of Mass Cultivation of Photoautotrophic Microalgae. in *Handbook of Microalgal Culture* 169–204 (2013). <https://doi.org/10.1002/9781118567166.ch11>
- Hadiyanto, H., Elmore, S., Van Gerven, T. & Stankiewicz, A. Hydrodynamic evaluations in high rate algae pond (HRAP) design. *Chem. Eng. J.* **217**, 231–239. <https://doi.org/10.1016/j.cej.2012.12.015> (2013).
- Kazbar, A. et al. Effect of dissolved oxygen concentration on microalgal culture in photobioreactors. *Algal Res.* **39**, 101432. <https://doi.org/10.1016/j.algal.2019.101432> (2019).
- Chisti, Y. Large-Scale Production of Algal Biomass: Raceway Ponds. in *Algae Biotechnology: Products and Processes* (eds Bux, F. & Chisti, Y.) 21–40 (2016). [https://doi.org/10.1007/978-3-319-12334-9\\_2](https://doi.org/10.1007/978-3-319-12334-9_2)
- Sutherland, D. L., Howard-Williams, C., Turnbull, M. H., Broady, P. A. & Craggs, R. J. The effects of CO<sub>2</sub> addition along a pH gradient on wastewater microalgal photo-physiology, biomass production and nutrient removal. *Water Res.* **70**, 9–26. <https://doi.org/10.1016/j.watres.2014.10.064> (2015).

32. Ketheesan, B. & Nirmalakhandan, N. Feasibility of microalgal cultivation in a pilot-scale airlift-driven raceway reactor. *Bioresour. Technol.* **108**, 196–202. <https://doi.org/10.1016/j.biortech.2011.12.146> (2012).
33. Sun, Y. et al. Boosting *Nannochloropsis oculata* growth and lipid accumulation in a lab-scale open raceway pond characterized by improved light distributions employing built-in planar waveguide modules. *Bioresour. Technol.* **249**, 880–889. <https://doi.org/10.1016/j.biortech.2017.11.013> (2018).
34. Posadas, E., Morales, M. M., Gomez, C., Acien, F. G. & Muñoz, R. Influence of pH and CO<sub>2</sub> source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways. *Chem. Eng. J.* **265**, 239–248. <https://doi.org/10.1016/j.cej.2014.12.059> (2015).
35. Ledda, C., Romero Villegas, G. I., Adani, F. & Acien Fernández, F. G. Molina Grima, E. Utilization of centrate from wastewater treatment for the outdoor production of *Nannochloropsis Gaditana* biomass at pilot-scale. *Algal Res.* **12**, 17–25. <https://doi.org/10.1016/j.algal.2015.08.002> (2015).
36. Huang, J. et al. Investigation on the performance of raceway ponds with internal structures by the means of CFD simulations and experiments. *Algal Res.* **10**, 64–71. <https://doi.org/10.1016/j.algal.2015.04.012> (2015).
37. Friedrich, K. et al. Reservoir Evaporation in the Western United States: Current Science, Challenges, and Future Needs 99, (2018). <https://doi.org/10.1175/BAMS-D-15-00224.1>
38. Carrier, O. et al. Evaporation of water: evaporation rate and collective effects. *J. Fluid Mech.* **798**, 774–786. <https://doi.org/10.1017/jfm.2016.356> (2016).
39. Cuello, M. C., Cosgrove, J. J., Randhir, A., Vadiveloo, A. & Moheimani, N. R. Comparison of continuous and day time only mixing on tetraselmis Suecica (Chlorophyta) in outdoor raceway ponds. *J. Appl. Phycol.* **27**, 1783–1791. <https://doi.org/10.1007/s10811-014-0420-5> (2015).
40. Trainor, F. R. Reproduction in *scenedesmus*. *Algae (The Korean J. Phycology)*. **11** (2), 183–201 (1996).
41. Barsanti, L. & Gualtieri, P. *Algae: Anatomy, Biochemistry, and Biotechnology, Second Edition*. (2014). <https://doi.org/10.1201/b16544>
42. Rayen, F., Behnam, T. & Dominique, P. Optimization of a raceway pond system for wastewater treatment: a review. *Crit. Rev. Biotechnol.* **39**, 422–435. <https://doi.org/10.1080/07388551.2019.1571007> (2019).
43. Chuka-ogwude, D. et al. Effect of medium recycling, culture depth, and mixing duration on *D. salina* growth. *Algal Res.* **60**, 102495. <https://doi.org/10.1016/j.algal.2021.102495> (2021).
44. Zhu, L. et al. Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. *Water Res.* **47**, 4294–4302. <https://doi.org/10.1016/j.watres.2013.05.004> (2013).
45. Marra, J., Bidigare, R. R. & Dickey, T. D. Nutrients and mixing, chlorophyll and phytoplankton growth. *Deep Sea Res. Part. Oceanogr. Res. Papers.* **37**, 127–143. [https://doi.org/10.1016/0198-0149\(90\)90032-Q](https://doi.org/10.1016/0198-0149(90)90032-Q) (1990).
46. Lachmann, S. C., Mettler-Altmann, T., Wacker, A. & Spijkerman, E. Nitrate or ammonium: influences of nitrogen source on the physiology of a green Alga. *Ecol. Evol.* **9**, 1070–1082. <https://doi.org/10.1002/ece3.4790> (2019).
47. Jiang, R. et al. The joint effect of ammonium and pH on the growth of *Chlorella vulgaris* and ammonium removal in artificial liquid digestate. *Bioresour. Technol.* **325**, 124690. <https://doi.org/10.1016/j.biortech.2021.124690> (2021).
48. Tan, F. et al. Nitrogen and phosphorus removal coupled with carbohydrate production by five microalgae cultures cultivated in biogas slurry. *Bioresour. Technol.* **221**, 385–393. <https://doi.org/10.1016/j.biortech.2016.09.030> (2016).
49. Beuckels, A., Smolders, E. & Muylaert, K. Nitrogen availability influences phosphorus removal in microalgae-based wastewater treatment. *Water Res.* **77**, 98–106. <https://doi.org/10.1016/j.watres.2015.03.018> (2015).
50. Pena, A. C. C., Agustini, C. B., Trierweiler, L. F. & Gutterres, M. Influence of period light on cultivation of microalgae consortium for the treatment of tannery wastewaters from leather finishing stage. *J. Clean. Prod.* **263**, 121618. <https://doi.org/10.1016/j.jclepro.2020.121618> (2020).
51. Barboza-Rodríguez, R., Rodríguez-Jasso, R. M., Rosero-Chasoy, G., Rosales Aguado, M. L. & Ruiz, H. A. Photobioreactor configurations in cultivating microalgae biomass for biorefinery. *Bioresour. Technol.* **394**, 130208. <https://doi.org/10.1016/j.biortech.2023.130208> (2024).
52. Li, Y., Zhang, Q., Wang, Z., Wu, X. & Cong, W. Evaluation of power consumption of paddle wheel in an open raceway pond. *Bioprocess. Biosyst. Eng.* **37**, 1325–1336. <https://doi.org/10.1007/s00449-013-1103-3> (2014).
53. Yadala, S. & Cremaschi, S. A. Dynamic Optimization Model for Designing Open-Channel Raceway Ponds for Batch Production of Algal Biomass. *Processes* **4**, 10 (2016). <https://doi.org/10.3390/pr4020010>
54. El-Chaghaby, A., Rashad, G., Abdel-Kader, S. F. & Rawash, S. A. Abdul Moneem, M. Assessment of phytochemical components, proximate composition and antioxidant properties of *scenedesmus obliquus*, *chlorella vulgaris* and *spirulina platensis* algae extracts. *Egypt. J. Aquat. Biology Fisheries.* **23**, 521–526. <https://doi.org/10.21608/ejabf.2019.57884> (2019).
55. Chen, M., Chen, Y. & Zhang, Q. A. Review of energy consumption in the acquisition of Bio-Feedstock for microalgae biofuel production. *Sustainability* **13**, 8873. <https://doi.org/10.3390/su13168873> (2021).
56. Bhatt, P. et al. Algae in wastewater treatment, mechanism, and application of biomass for production of value-added product. *Environ. Pollut.* **309**, 119688. <https://doi.org/10.1016/j.envpol.2022.119688> (2022).
57. Amorim, M. L., Soares, J., Vieira, B. B., Batista-Silva, W. & Martins, M. A. Extraction of proteins from the microalga *scenedesmus obliquus* BR003 followed by lipid extraction of the wet deproteinized biomass using hexane and Ethyl acetate. *Bioresour. Technol.* **307**, 123190. <https://doi.org/10.1016/j.biortech.2020.123190> (2020).
58. Patnaik, R., Singh, N. K., Bagchi, S. K., Rao, P. S. & Mallick, N. Utilization of *scenedesmus obliquus* protein as a replacement of the commercially available fish meal under an algal refinery approach. *Front. Microbiol.* **10** <https://doi.org/10.3389/fmicb.2019.02114> (2019).
59. Olsen, M. F. L. et al. Outdoor cultivation of a novel isolate of the microalgae *scenedesmus* sp. and the evaluation of its potential as a novel protein crop. *Physiol. Plant.* **173** (2), 483–494. <https://doi.org/10.1111/ppl.13532> (2021).
60. Skifa, I., Chauchat, N., Cocquet, P. H. & Guer, Y. L. Microalgae cultivation in raceway ponds: Advances, challenges, and hydrodynamic considerations. *EFB Bioeconomy* **J.** **5**, 100073. <https://doi.org/10.1016/j.bioeco.2024.100073> (2025).
61. Fakher, S., Khlaif, A., Hossain, M. E. & Nameer, H. Rigorous review of electrical submersible pump failure mechanisms and their mitigation measures. *J. Petroleum Explor. Prod. Technol.* **11**, 1507–1525. <https://doi.org/10.1007/s13202-021-01271-6> (2021).
62. Zhang, Q. et al. Installation of flow deflectors and wing baffles to reduce dead zone and enhance flashing light effect in an open raceway pond. *Bioresour. Technol.* **198**, 150–156. <https://doi.org/10.1016/j.biortech.2015.08.144> (2015).
63. Hoeniges, J., Zhu, K., Pruvost, J. & Legrand, J. Impact of Dropwise condensation on the biomass production rate in covered raceway ponds. *Energies* **14**, 268 (2021).

## Author contributions

Muhammad Umer Abbas: Writing—original draft, Writing—review & editing, Methodology, Validation, Conceptualization. Rashid Iftikhar: Writing—review & editing, Conceptualization, Validation, Project administration, Funding acquisition, Supervision. Sahar Saleem: Writing—review & editing and Methodology. Sarah Bader Alotaibi: Funding, resources & review. Nadeem Ullah: Resources and Review. Mashael M. Alfge: Funding & review. Muhammad Ali Inam: Methodology, Supervision, Validation, Investigation, final approval. Faras Ahmad Shahbaz: Writing—review & editing. Ahmad Aakash: Writing—review & editing.

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## Declarations

### Competing interests

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### Additional information

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**Correspondence** and requests for materials should be addressed to R.I.

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