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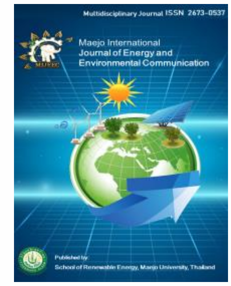

ARTICLE

Sustainable treatment of dairy wastewater using a selective microalgal consortium of *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Chlorococcus minutus*

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ABSTRACT

Dairy effluents are characterised by high concentrations of organic matter, nitrogen, phosphorus, suspended solids, and chemical additives, making them among the most challenging industrial wastewaters to treat. This study evaluates the potential of indigenous microalgae *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Chlorococcus minutus* for the phycoremediation of dairy wastewater. The untreated wastewater, characterised by high biological oxygen demand (BOD: 768 mg/L) and chemical oxygen demand (COD: 945 mg/L), as well as nitrate (9.31 mg/L), phosphate (4.13 mg/L), and ammonium (0.68 mg/L), was subjected to microalgal treatment. All cultures significantly improved water quality, with the consortium consistently outperforming monocultures. By day 15, BOD and COD reductions exceeded 90%, nitrate removal reached 99.5%, phosphate removal reached 98.1%, and ammonium removal reached 92.6%. Biomass productivity increased steadily, with the consortium achieving the highest yield (1.70 g/L DW), indicating synergistic nutrient assimilation and enhanced growth stability. The findings demonstrate that native microalgal consortia provide an eco-friendly, cost-effective, and scalable approach for dairy wastewater treatment, while generating biomass suitable for biofuel and biofertilizer applications.

1. Introduction

Dairy industries generate large volumes of wastewater that are typically characterised by high biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids, fats, oils, and nutrient loads, particularly nitrogen and phosphorus (Chandra et al., 2021). The variability and complexity of these effluents pose significant challenges for treatment, especially in sensitive ecosystems such as riverine systems, where untreated discharge accelerates eutrophication and threatens aquatic life. Conventional treatment processes, including activated sludge, anaerobic digestion, and chemical precipitation, have been widely applied to dairy effluents. While these methods can reduce pollutant loads, they often suffer from limitations such as high operational costs, the generation of secondary sludge, and inefficiency under fluctuating influent quality (Velmurugan et al., 2024). Among the various technologies for dairy wastewater treatment, including physical, chemical, and biological methods, biological methods stand out as environmentally friendly, cost-effective, and effective at reducing pollutants. This method utilises various micro-organisms, including bacteria, fungi, and microalgae, to naturally clean dairy wastewater (Woertz et al., 2009; Shete et al., 2013; Slavov, 2017).

Microalgae-based phycoremediation has emerged as a particularly promising approach for environmental remediation. Microalgae can simultaneously assimilate nutrients, degrade organic matter, and sequester CO_2, while generating biomass rich in lipids, proteins, pigments, and carbohydrates that can be utilised for biofuel, biofertilizer, nutraceuticals, and feed applications (Bhuyar et al., 2019a; Rathinam et al., 2025). These numerous benefits of microalgae-

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based wastewater treatment systems have garnered considerable attention in recent years. The use of chemicals in wastewater treatment decreases when microalgae are used (Razzak et al., 2017; Bhuyar et al., 2019b). Wastewater is a suitable and sustainable medium for cultivating microalgae. These microorganisms, renowned for their high photosynthetic efficiency, can rapidly adapt and grow in a wide range of wastewater types. They are particularly effective at absorbing nutrients, especially nitrogen and phosphorus, making them well-suited for wastewater treatment (Cheng et al., 2018; Palanisamy et al., 2022).

Previous studies explored the use of monocultures in dairy effluents, demonstrating the ability of individual species to remove nutrients and accumulate valuable biomass (Singh et al., 2024). The *Chlorococcum* sp. cultivated under mixotrophic and heterotrophic conditions showed enhanced biomass and lipid productivity while reducing COD and BOD (Ummalyma & Sukumaran, 2014). Similarly, *Spirulina* sp. achieved substantial reductions in nitrates, phosphates, sulphates, and organic pollutants, highlighting its dual role in nutrient removal and biomass valorisation (Saud et al., 2014). Building on these efforts, researchers began investigating native consortia and integrated processes. Hena et al. (2015) reported that an indigenous consortium grown in dairy wastewater achieved more than 98% nutrient removal and produced lipid-rich biomass convertible to biodiesel. Daneshvar et al. (2019) highlighted *Scenedesmus quadricauda* and *Tetraselmis suecica* maintained high nutrient removal efficiency and lipid production across multiple cultivation cycles, underscoring the feasibility of effluent reuse in algal cultivation systems. Choi et al. (2018) further demonstrated that *Chlorella* sp., isolated directly from dairy wastewater, exhibits natural resilience and adaptability to complex effluents, sustaining robust growth and lipid synthesis.

Therefore, the present study focuses on evaluating the potential of the indigenous microalgae *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Chlorococcus minutus*, both as monocultures and in consortium, for phycoremediation of raw dairy wastewater. By isolating and characterising native strains from the Alaknanda River ecosystem and employing controlled laboratory cultivation, this research aims to investigate their efficiency in nutrient removal, organic load reduction, and biomass production. The study also examines the feasibility of utilising dairy effluent as a growth medium, thereby reducing operational costs and environmental impact. This work seeks to contribute to the advancement of microalgae-based bioremediation technologies that integrate wastewater treatment with bioresource generation, supporting sustainable dairy industry practices.

2. Materials and methods

2.1 Collection and isolation of microalgae

Different microalgal samples were collected from two primary sources: the Alaknanda River and the Fishery Department, H.N.B. Garhwal University, Srinagar Garhwal, Uttarakhand, geographically located at coordinates 30°48′30.996″ N and 78°12′21.456″ E (Figure 1). Microscopic examination of the collected samples was conducted using a binocular compound light microscope (Olympus MLX-B SN-17C0080), and the species were identified based on their morphological characteristics by following the literature of Karthick et al. (2013) and Liu (2023), and the online database AlgaeBase (http://www.algaebase.org) (Figure 2). From these, three species, *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Chlorococcus minutus* were selected for further study. Isolation of pure strains was achieved through the serial dilution and further streak plating method on BG-11 agar medium. The inoculated plates were incubated for a week under controlled conditions, with a 16:8 h light–dark photoperiod at 25 ± 2 ºC. Distinct colonies were then aseptically picked and subcultured in BG-11 liquid medium to establish monocultures. Additionally, a mixed consortium of the three selected strains was prepared and maintained in BG-11 liquid medium for use in subsequent experiments.

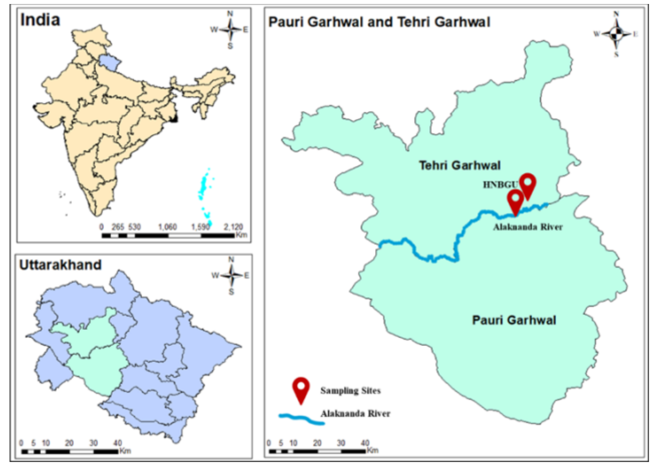

Figure 1. Sampling Station: Chauras Bridge-Alaknanda River and Chauras Campus-HNBGU, Uttarakhand.

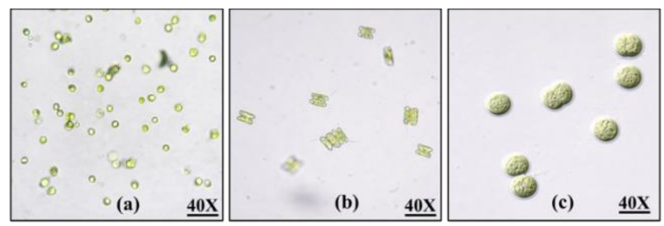

Figure 2: Microscopic images (Olympus MLX-B SN-17C0080) at 40x of selected microalgal strains: (a) *Chlorella vulgaris*, (b) *Scenedesmus obliquus*, (c) *Chlorococcus minutus*.

2.2 Cultural conditions for microalgae

The cultures were maintained in a temperature-controlled chamber at 25 ± 2 °C, with a 16:8 light: dark photoperiod, under white LED light at 300 µmol m^{-2} s^{-1} for 15 days, in a 500 mL conical flask with a working volume of 400 mL (Mogale, 2016). Continuous aeration was provided using sterile air pumps, ensuring proper mixing and sufficient carbon dioxide availability through gentle bubbling. The culture's pH was initially adjusted to 7.0 ± 0.2 and monitored regularly to track any shifts resulting from algal metabolism.

2.3 Characterisation and pre-treatment of raw DWW

Raw dairy wastewater (DWW) was collected from the Aanchal Milk Processing and Packaging Unit, Srinagar Garhwal, Uttarakhand, which generates approximately 120-150 tonnes of wastewater per day during routine operations. Prior to experimental use, the wastewater was allowed to settle naturally to facilitate the removal of coarse suspended solids. The resulting supernatant was subsequently filtered through a double layer of sterile gauze to eliminate remaining particulates. The filtered wastewater was then sterilised by autoclaving at 121 °C for 20 min to ensure experimental consistency and to prevent microbial interference during microalgal treatment and nutrient removal studies (Bhuyar et al., 2021; Gopalakrishnan et al., 2024).

2.4 Cultivation of microalgae in DWW

Monocultures of *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Chlorococcus minutus*, along with a mixed microalgal consortium comprising these species, were cultivated in pre-treated dairy wastewater. For each experimental setup, 500-mL Erlenmeyer flasks containing 400 mL of wastewater were inoculated with 10% (v/v) of actively growing algal cultures (Tang et al., 2023). The cultivation was conducted for 15 days, during which approximately 10 mL of culture was aseptically withdrawn at 3-day intervals. The collected samples were immediately centrifuged at 4,000 rpm for 10 min to separate the microalgal biomass from the supernatant for subsequent analyses (Nithin et al., 2020).

2.5 Physicochemical parameter analysis

Dairy wastewater samples were physicochemically characterised in collaboration with Uttarakhand Jal Sansthan (Water Supply Centre), Srinagar Garhwal, to establish baseline water quality and to monitor nutrient transformations during microalgal treatment. Inorganic nitrogen species were quantified by determining nitrate (NO_3^-) using the phenol-disulfonic acid method and ammonium (NH_4^+) via the indophenol blue colourimetric assay, which produces a characteristic blue chromophore. Phosphate (PO_4^{3-}) concentrations were measured employing the stannous chloride method, with absorbance recorded at 690 nm using a UV-visible spectrophotometer. Organic pollution levels were assessed by measuring chemical oxygen demand (COD) using the closed reflux dichromate method, and biological oxygen demand (BOD_5) was determined after a 5-day incubation at 20 ± 1 °C under dark conditions. The pH of the wastewater was measured using a calibrated digital pH meter. Collectively, these analyses provided essential baseline data for evaluating nutrient removal efficiency and elucidating the effectiveness of microalgal cultures in dairy wastewater remediation.

2.6 Algal growth and biomass productivity

Algal biomass productivity was monitored at regular intervals by determining the dry weight (DW) of the cultures. Briefly, harvested algal cells were collected by centrifugation and washed twice with sterile distilled water to remove residual salts. The resulting biomass was dried in a hot-air oven at 105 °C until a constant weight was obtained. Biomass concentration was expressed as grams per litre (g/L).

$$\text{Biomass concentration } X(mg/L) = Wd/V$$

Where, Wd = dry weight of biomass (g); V = culture volume used for drying (L).

$$\text{Biomass productivity } P(g/L/day) = (X_t - X_i)/t$$

Where, X_t = biomass concentration at time t (g/L); X_i = initial biomass concentration (g/L); t = cultivation time (days).

Qin et al. (2016) employed this approach to improve algal biomass productivity in wastewater-based systems, enabling a systematic evaluation of growth rates and biomass yields of different algal strains and consortia cultivated in dairy wastewater.

2.7 Nutrient removal efficiency

To determine the efficacy of nutrient uptake by the microalgal cultures, percentage removal was calculated for each parameter using the following formula:

$$\text{Percentage Removal (\%)} = \frac{C_i - C_t}{C_i} \times 100$$

Where C_i is the initial concentration at day 1 (mg/L); C_t is the concentration at any given time point t (mg/L)

Nutrient removal efficiency was evaluated using this method by applying the equation to nitrate, phosphate, and ammonium to determine their depletion kinetics over time (Qin et al., 2016).

3. Results and discussion

3.1 Algal biomass productivity

Chlorella vulgaris, *Scenedesmus obliquus*, and *Chlorococcus minutus* were evaluated for growth performance and nutrient removal in wastewater. In addition to monoculture treatments, a mixed algal consortium composed of the three species was established to assess potential synergistic effects. Biomass productivity was monitored over a 15-day cultivation period to assess algal growth and nutrient assimilation (Figure 3).

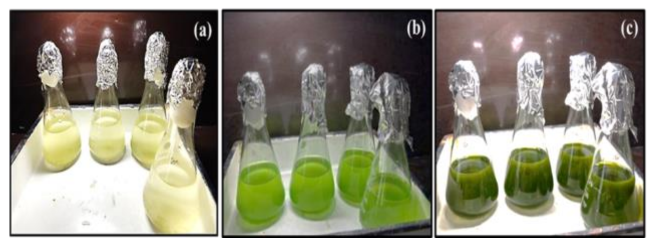

Figure 3: Progression of microalgal growth at (a) day 1, (b) day 6, and (c) day 15.

The consortium consistently accumulated more biomass than the individual strains throughout the experimental duration. By day 6, consortium biomass reached 0.81 g/L, surpassing *C. vulgaris* (0.61 g/L), *S. obliquus* (0.71 g/L), and *C. minutus* (0.55 g/L). This trend was maintained until the end of cultivation, with the consortium achieving the highest final biomass concentration of 1.70 g/L on day 15. In comparison, final biomass concentrations of 1.62 g/L and 1.51 g/L were recorded for *C. vulgaris* and *S. obliquus*, respectively, whereas *C. minutus* produced the lowest biomass (1.19 g/L). Comparable biomass accumulation has been reported in previous studies (Figure 4). Asmare et al. (2013) observed a dry biomass concentration of 0.209 g/L over 14 days using a natural microalgal consortium dominated by *Scenedesmus* and *Chlorella* species during dairy wastewater treatment. Similarly, Álvarez-Montero et al. (2025) reported a biomass productivity of 0.22 ± 0.05 g/L/d for *Scenedesmus* sp. cultivated in 80% dairy wastewater, reaching a maximum biomass concentration of 3.35 ± 0.45 g L^{-1} after 11 days.

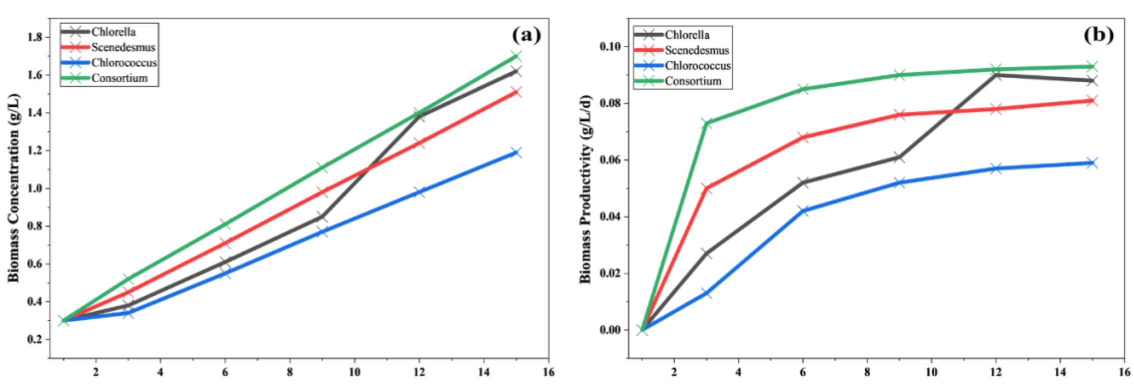

Figure 4: Temporal variation in microalgal biomass accumulation **(a)** and biomass productivity **(b)** during the 15-day cultivation period.

3.2 Analysis of physicochemical characteristics of dairy wastewater

At the onset of the experiment, the wastewater exhibited an acidic pH (5.1) and high organic and nutrient loads, with BOD and COD values of 768 and 945 mg/L, respectively, along with nitrate (9.31 mg/L), phosphate (4.13 mg/L), and ammonium (0.68 mg/L) concentrations (Table 1). During the cultivation period, a progressive increase in pH was observed across all treatments, attaining alkaline values (8.2-8.3) by day 15. This trend indicates enhanced photosynthetic activity and nitrate assimilation by microalgae, reducing dissolved CO_2 availability and promoting hydroxyl ion release. Comparable trends have been reported in earlier studies. Asmare et al. (2013) documented substantial reductions in organic load, with BOD and COD decreasing by 72.8% and 59.4%, respectively, following 14 days of treatment using a microalgal consortium. Likewise, Moustafa et al. (2023) reported BOD and COD removal efficiencies of 29-51% and 46.9%, respectively, in pilot-scale systems employing layered microalgal configurations, further highlighting the efficacy of microalgae-based treatment processes. More recently, Álvarez-Montero et al. (2025) demonstrated robust growth of Scenedesmus sp. in nutrient-rich wastewater, achieving a biomass productivity of 0.22 ± 0.05 g/L/d, slightly exceeding that obtained in standard BG11 medium. In addition, efficient nutrient assimilation was observed, with approximately 79% of total nitrogen and 77% of phosphate removed after 11 days of cultivation. These findings corroborate the strong bioremediation potential and operational adaptability of microalgal systems, particularly consortia-based approaches, for the effective treatment of dairy wastewater under diverse cultivation conditions.

Figure 6: (a) Phosphate removal (mg/L) and **(b)** removal efficiency (%), from dairy wastewater by microalgal monocultures and consortium over 15 days.

Days	Treatment	pH	BOD (mg/L)	COD (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)	Ammonia (mg/L)
1	*Chlorella*	5.1	768	945	9.31	4.13	0.68
	Scenedesmus	5.1	768	945	9.31	4.13	0.68
	Chlorococcus	5.1	768	945	9.31	4.13	0.68
	Consortium	5.1	768	945	9.31	4.13	0.68
3	*Chlorella*	7.5	420	542	6.9	2.8	0.25
	Scenedesmus	7.1	468	564	6.9	3.1	0.26
	Chlorococcus	6.8	488	584	7.5	2.9	0.31
	Consortium	7.5	320	478	6.1	2.4	0.13
6	*Chlorella*	7.6	198	304	4.2	1.6	0.17
	Scenedesmus	7.3	215	368	4.7	1.9	0.19
	Chlorococcus	7.1	286	397	4.9	2.1	0.21
	Consortium	7.9	120	285	3.1	1.03	0.09
9	*Chlorella*	7.8	98	243	2.4	1.5	0.11
	Scenedesmus	7.7	132	267	2.8	1.8	0.13
	Chlorococcus	7.6	144	288	3.2	1.9	0.14
	Consortium	8.1	37	89	0.9	0.7	0.05
12	*Chlorella*	8.2	52	88	0.8	0.5	0.08
	Scenedesmus	8.1	65	92	0.9	0.7	0.09
	Chlorococcus	8.1	68	94	1.3	0.9	0.11
	Consortium	8.2	27	32	0.05	0.09	0.05
15	*Chlorella*	8.3	40	68	0.8	0.5	0.08
	Scenedesmus	8.2	58	73	0.9	0.7	0.09
	Chlorococcus	8.3	63	75	0.9	0.7	0.09
	Consortium	8.3	27	32	0.05	0.08	0.05

3.3 Nutrient removal performance by microalgae

The main aspect of this study was to assess the efficiency of nitrate, ammonium, and phosphate removal by the cultivated microalgal systems. The findings demonstrate that both monocultures and the consortium effectively reduced nutrient concentrations in the wastewater, albeit with varying removal efficiencies across treatments. Notably, the consortium consistently demonstrated superior nutrient removal performance, underscoring its enhanced capacity for nutrient assimilation and utilisation compared with individual strains.

3.3.1 Nitrate removal

The initial nitrate concentration in the wastewater (9.31 mg/L) decreased steadily across all treatments over the 15-day cultivation period. The highest nitrate removal was observed in the consortium, which reduced concentrations to 0.05 mg/L, corresponding to a removal efficiency of 99.5%. Comparable reductions were achieved by the monocultures, with *Chlorella vulgaris* lowering nitrate levels to 0.8 mg/L (91.4%), while *Scenedesmus obliquus* and *Chlorococcus minutus* exhibited similar efficiencies (Figure 5). Although differences among the monocultures were marginal, the consortium consistently demonstrated a slight yet reproducible advantage, indicating a higher nutrient uptake potential. These observations are consistent with the findings of Ortega-Blas et al. (2025), who reported approximately 87% nitrogen removal from municipal wastewater using a *Chlorella*-based microalgal–bacterial consortium, reinforcing the enhanced nutrient assimilation capacity of mixed-culture systems relative to single-strain approaches.

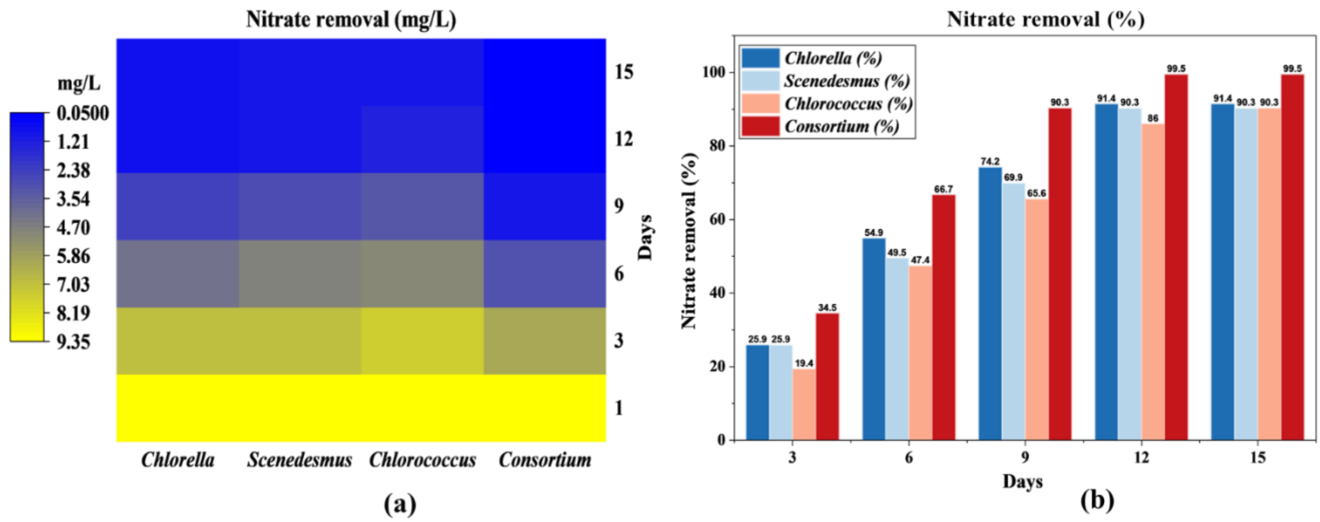

Figure 5: **(a)** Nitrate removal (mg/L) and **(b)** removal efficiency (%), from dairy wastewater by microalgal monocultures and consortium over 15 days.

3.3.2 Phosphate removal

The wastewater initially contained 4.13 mg/L of phosphate, which declined consistently across all treatments during the 15-day cultivation period. The consortium exhibited substantial phosphate removal, reducing concentrations to 0.08 mg/L and achieving a 98.1% removal efficiency. Among the monocultures, *Chlorella vulgaris* showed the highest phosphate uptake, lowering levels to 0.50 mg/L (87.9% efficiency), whereas *Scenedesmus obliquus* and *Chlorococcus minutus* reduced phosphate to 0.70 mg/L, corresponding to efficiencies of 83.1% (Figure 6). These findings align with those reported by Ortega-Blas et al. (2025), who achieved 94.4% phosphorus removal from municipal wastewater using a *Chlorella*-based microalgal–bacterial consortium, further supporting the enhanced nutrient uptake capacity of mixed-culture systems.

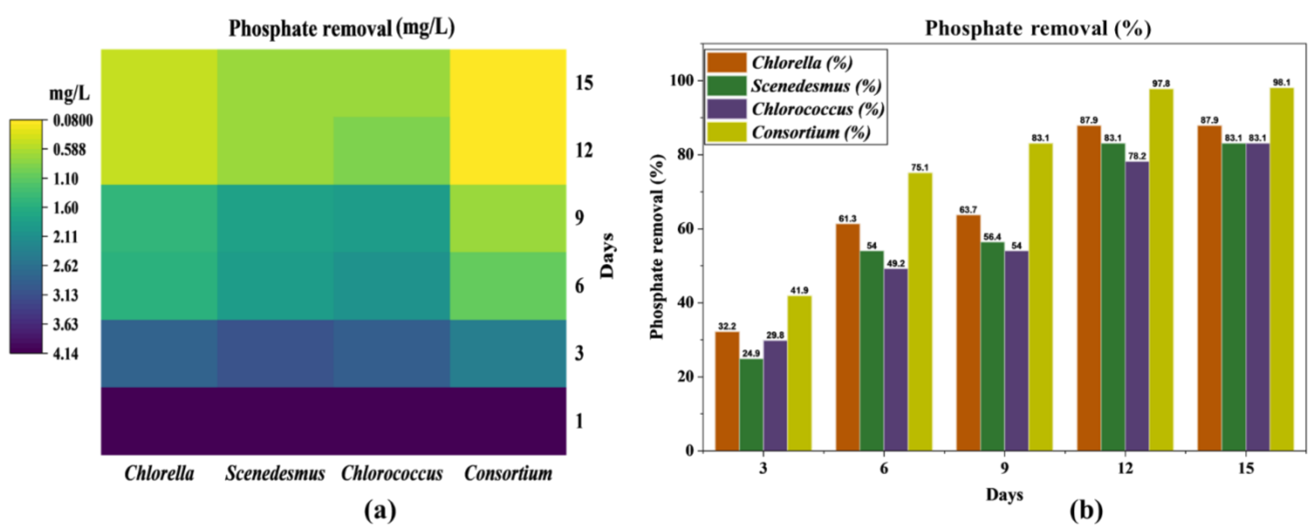

Figure 6: **(a)** Phosphate removal (mg/L) and **(b)** removal efficiency (%), from dairy wastewater by microalgal monocultures and consortium over 15 days.

3.3.3 Ammonium removal

Ammonium removal was highly efficient across all treatments, with residual concentrations approaching near-zero levels by the end of the cultivation period. The initial ammonium concentration of 0.68 mg/L declined progressively, reaching 0.08 mg/L in *Chlorella vulgaris*, 0.09 mg/L in both *Scenedesmus obliquus* and *Chlorococcus minutus*, and 0.05 mg/L in the consortium by day 15. The consortium exhibited the highest removal efficiency (92.6%), followed by *C. vulgaris* (88.2%), while *S. obliquus* and *C. minutus* each achieved 86.8% removal (Figure 7). These observations are consistent with previous reports by Jia et al. (2018), who documented high ammonium removal rates (>80 mg N L^{-1} day^{-1}) in algal–bacterial consortia, particularly under low-light conditions (~1000 lux), further emphasising the effectiveness of such systems for nitrogen removal.

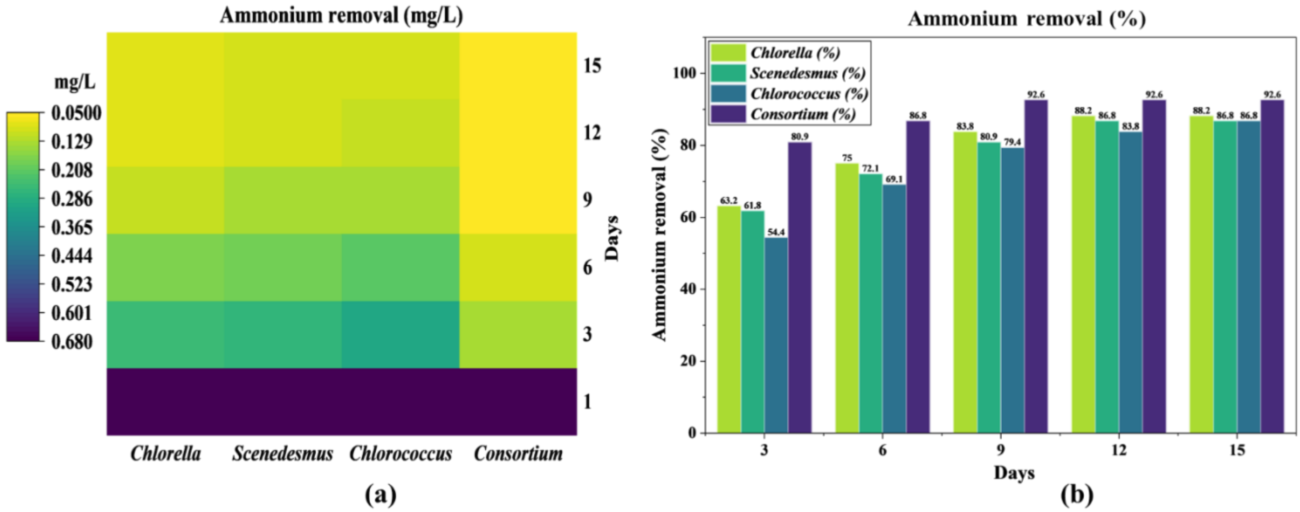

Figure 7: (a) Ammonium removal (mg/L) and **(b)** removal efficiency (%), from dairy wastewater by microalgal monocultures and consortium over 15 days.

4. Conclusion

This study systematically assessed the treatment potential of three microalgal species, *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Chlorococcus minutus,* and their consortium for the remediation of nutrient-rich dairy wastewater sourced from the Anchal Milk Processing Unit, Srinagar Garhwal, Uttarakhand. Microalgae were isolated from natural freshwater environments and institutional fish ponds using serial dilution and BG-11 agar plating, and subsequently cultivated under controlled laboratory conditions optimised for temperature, illumination, aeration, and pH. The raw wastewater was pre-treated through sedimentation, filtration, and sterilisation to ensure experimental consistency. Over the 15-day cultivation period, all microalgal treatments achieved substantial reductions in key pollutants, including biological oxygen demand (BOD), chemical oxygen demand (COD), nitrate, phosphate, and ammonium, with removal efficiencies frequently exceeding 90%. The consortium consistently outperformed monocultures, demonstrating superior nutrient removal and growth performance. A progressive shift in wastewater pH from acidic to alkaline values further reflected active photosynthetic metabolism and nutrient assimilation. Correspondingly, biomass productivity was highest in the consortium, which attained a maximum dry biomass yield of 1.70 g L^{-1}, indicating synergistic interactions that enhanced nutrient uptake and growth stability. Physicochemical analyses confirmed marked declines in contaminant concentrations, highlighting the dual functionality of microalgae in wastewater treatment and biomass generation. Efficient nitrogen and phosphorus assimilation underscores the potential of these systems not only for environmental remediation but also for nutrient recovery. The experimental framework, integrating assessments of nutrient removal and biomass productivity, provides robust evidence supporting microalgal consortia as an environmentally sustainable and economically attractive alternative to conventional dairy wastewater treatment methods.

CRediT authorship contribution statement

Ritika Pandey: Writing - original draft, Data curation, Conceptualization, and Formal Analysis. **Neha Kumari:** Writing - original draft, Data curation, Conceptualization, and Formal Analysis. **Pushpendra Singh Rawat:** Writing - review & editing. **Margdarshi Bhatt:** Writing - review & editing. **Nirmala Bhuvana Chandra Ramisetty:** Writing - review & editing. **Prakash Bhuyar:** Writing - review & editing. **Natanamurugaraj Govindan:** Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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