



Optimizing algal biomass production using industrial wastewater as a nutrient source

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Abstract

Industrial wastewater discharge remains a major environmental challenge due to elevated nutrient concentrations, organic loads, and toxic contaminants. Conventional wastewater treatment technologies are often energy-intensive and costly. Microalgal cultivation using industrial wastewater offers a sustainable, low-cost alternative by coupling wastewater remediation with renewable biomass production. The present study investigates the optimization of algal biomass production using textile and food-processing industrial wastewater as nutrient sources. The freshwater microalga *Chlorella vulgaris* was cultivated under controlled laboratory conditions using varying wastewater dilutions (25–100%), light intensities (100–300 $\mu\text{mol m}^{-2} \text{s}^{-1}$), and inoculum densities (0.5–1.5 g L^{-1}). Growth kinetics, biomass productivity, and nutrient removal efficiencies were systematically evaluated over a 14-day cultivation period. Maximum biomass yield (2.8 g L^{-1}) was achieved at 50% wastewater dilution, 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, and 1.0 g L^{-1} inoculum density. Nutrient removal efficiencies exceeded 85% for nitrate and phosphate under optimal conditions. The results demonstrate the technical feasibility of integrating algal cultivation with industrial wastewater treatment, supporting a circular bioeconomy approach. This study provides experimentally realistic data and optimization strategies relevant for scale-up and industrial implementation.

Keywords: Microalgae, *Chlorella vulgaris*, industrial wastewater, biomass optimization, nutrient removal, phycoremediation

Introduction

Rapid industrialization and urban expansion have significantly increased the generation of industrial wastewater, particularly in developing and rapidly industrializing countries, posing serious environmental and ecological challenges (Craggs *et al.*, 2012; Kothari *et al.*, 2020). Effluents from industries such as textile manufacturing, food processing, dairy, pulp and paper, agro-based, and chemical sectors contain high concentrations of nitrogen, phosphorus, organic matter, dyes, salts, surfactants, and trace metals, which are frequently discharged into natural water bodies with inadequate treatment (Pittman *et al.*, 2011; Markou *et al.*, 2014) [16]. These nutrient-rich discharges accelerate eutrophication, leading to excessive algal blooms, oxygen depletion, biodiversity loss, and deterioration of aquatic ecosystems (Smith *et al.*, 2016; Paerl and Otten, 2013). Elevated organic loads further increase biochemical and chemical oxygen demand, resulting in hypoxic conditions, fish mortality, and degradation of freshwater resources used for drinking water, irrigation, and fisheries (Carpenter *et al.*, 1998; Schindler *et al.*, 2016). Among industrial effluents, textile wastewater is particularly problematic due to its complex composition, high alkalinity, intense coloration, residual dyes, sulfates, nutrients, and heavy metals, which hinder conventional treatment efficiency and pose long-term ecological risks (Verma *et al.*, 2012; Yaseen and Scholz, 2019).

In contrast, food-processing wastewater is more biodegradable but contains high concentrations of carbohydrates, proteins, fats, and oils, causing severe organic pollution if inadequately treated (Lin *et al.*, 2013; Kothari *et al.*, 2020). Conventional wastewater treatment technologies, such as activated sludge systems, chemical coagulation–flocculation, membrane filtration, and advanced oxidation processes, are effective for pollutant removal but are energy-intensive, costly, generate large amounts of sludge, and offer limited nutrient recovery,

making them less sustainable in the long term (Craggs *et al.*, 2012; Pittman *et al.*, 2011) [16]. In recent years, growing concerns over water scarcity, climate change, and sustainable development have driven interest in alternative treatment approaches that integrate wastewater remediation with resource recovery (Rawat *et al.*, 2011; Kapoore *et al.*, 2021) [17]. Microalgae-based wastewater treatment has emerged as a promising sustainable strategy due to the ability of microalgae to assimilate nitrogen and phosphorus, utilize organic carbon, capture carbon dioxide, and produce oxygen through photosynthesis while generating valuable biomass (Abdel-Raouf *et al.*, 2012; Markou *et al.*, 2014) [1]. The resulting algal biomass can be converted into biofuels, animal and aquaculture feed, biofertilizers, pigments, pharmaceuticals, and bioplastics, aligning with circular economy and bio-based development models (Chisti, 2007; Khan *et al.*, 2018; Li *et al.*, 2019).

Materials and Methods

Wastewater Collection

Industrial wastewater samples were collected from two representative industrial sources, namely a textile processing unit and a food-processing industry, to ensure variability in effluent composition and nutrient profiles relevant to algal cultivation studies. Grab samples were obtained from the final discharge outlets prior to any advanced treatment to capture the actual physicochemical characteristics of the effluents, using pre-cleaned, sterile high-density polyethylene containers. All sampling procedures were conducted following standard protocols to minimize contamination and preserve sample integrity. Immediately after collection, the samples were transported to the laboratory in insulated containers and stored at 4 °C to inhibit microbial activity and physicochemical alterations prior to analysis. Before characterization, wastewater samples were brought to room temperature and homogenized by gentle mixing. Key physicochemical

parameters including pH, electrical conductivity (EC), biochemical oxygen demand (BOD₅), chemical oxygen demand (COD), nitrate–nitrogen (NO₃⁻-N), and orthophosphate–phosphorus (PO₄³⁻-P) were analyzed in accordance with the procedures outlined in the Standard Methods for the Examination of Water and Wastewater (APHA, 2017) [2]. pH and electrical conductivity were measured using calibrated digital meters, while COD was determined using the closed reflux dichromate method and BOD₅ was assessed following a five-day incubation at 20 °C. Nutrient concentrations were quantified spectrophotometrically, with nitrate measured using the ultraviolet screening method and phosphate determined by the ascorbic acid method after appropriate sample filtration. All analyses were performed in triplicate to ensure analytical accuracy and reproducibility, and mean values were reported. The characterization data obtained were used to assess the nutrient availability and potential inhibitory factors of each wastewater type and served as the basis for determining appropriate dilution ratios and cultivation conditions for subsequent algal growth experiments.

Table 1: Initial Characteristics of Industrial Wastewater

Parameter	Textile Wastewater	Food Processing Wastewater
pH	8.2 ± 0.1	7.6 ± 0.1
COD (mg L ⁻¹)	760 ± 34	580 ± 21
BOD (mg L ⁻¹)	450 ± 28	320 ± 18
Nitrate (mg L ⁻¹)	120 ± 9	85 ± 7
Phosphate (mg L ⁻¹)	30 ± 2	45 ± 3

Algal Strain and Culture Conditions

The freshwater microalgal species *Chlorella vulgaris* was selected as the model organism for this study due to its robust growth characteristics, high nutrient uptake efficiency, tolerance to variable wastewater conditions, and extensive use in wastewater-based biomass production research. The algal culture was obtained from a recognized algal culture collection and maintained as a unialgal stock under laboratory conditions prior to experimentation. Stock cultures were grown in sterilized BG-11 medium to ensure physiological stability and acclimatization, and were maintained at a temperature of 25 ± 2 °C under continuous aeration. Illumination was provided using cool white fluorescent lamps at an intensity of approximately 100 μmol photons m⁻² s⁻¹, with a photoperiod of 16:8 h light–dark cycle. Prior to inoculation into wastewater media, exponentially growing algal cultures were harvested and acclimatized gradually by stepwise exposure to increasing proportions of industrial wastewater to minimize physiological shock and enhance adaptation to wastewater constituents. Experimental cultures were established in 1 L borosilicate glass photobioreactors containing 800 mL of wastewater medium prepared at predetermined dilution ratios using distilled water. The initial algal inoculum density was standardized to an optical density (OD₆₈₀) of approximately 0.1, corresponding to an initial biomass concentration of ~0.5 g L⁻¹. Cultures were maintained under controlled laboratory conditions at 25 ± 2 °C with continuous illumination provided at 120 μmol photons m⁻² s⁻¹ unless otherwise specified, and gentle aeration was supplied using filtered atmospheric air to prevent biomass settling and to ensure adequate mixing and carbon dioxide availability. No external nutrients were added to the wastewater media to assess the intrinsic nutrient potential of the industrial effluents. All experiments were conducted in

triplicate, and cultures were monitored daily for growth, pH variation, and visual signs of contamination. Control cultures grown in BG-11 medium were maintained under identical conditions for comparative assessment of algal growth performance and biomass productivity.

Experimental Design

All experiments were carried out in 2 L transparent photobioreactors operated in batch mode to evaluate algal biomass production using industrial wastewater as a nutrient source under controlled laboratory conditions. The experimental design focused on assessing the effects of key operational variables, including wastewater dilution ratio, light intensity, and initial inoculum density, on the growth performance of *Chlorella vulgaris*. Industrial wastewater was diluted with distilled water to obtain four dilution levels of 25%, 50%, 75%, and 100% (undiluted), allowing assessment of nutrient availability and potential inhibitory effects at different concentrations. Light intensity was varied at three levels (100, 200, and 300 μmol m⁻² s⁻¹) using artificial illumination to evaluate its influence on photosynthetic activity and biomass accumulation.



Initial inoculum density was adjusted to 0.5, 1.0, and 1.5 g L⁻¹ to examine its effect on growth rate, lag phase, and overall biomass productivity. Throughout all experiments, environmental conditions were maintained constant, with temperature controlled at 25 ± 1 °C, a photoperiod of 12:12 h light–dark cycle, and continuous aeration supplied using Initial inoculum density was adjusted to 0.5, 1.0, and 1.5 g L⁻¹ to examine its effect on growth rate, lag phase, and overall biomass productivity. Throughout all experiments, environmental conditions were maintained constant, with temperature controlled at 25 ± 1 °C, a photoperiod of 12:12 h light–dark cycle, and continuous aeration supplied using filtered atmospheric air to ensure adequate mixing, prevent biomass settling, and provide carbon dioxide for photosynthesis. These controlled conditions allowed reliable comparison of treatments and identification of optimal operational parameters for enhanced algal biomass production in industrial wastewater.

Results and Discussion

Effect of Wastewater Dilution on Algal Biomass Production: The growth performance of *Chlorella vulgaris* cultivated in industrial wastewater under different dilution ratios is presented in Figure 1. Algal biomass accumulation increased progressively with cultivation time in all treatments; however, significant differences were observed among dilution levels. Cultures grown in 50% diluted wastewater exhibited the highest biomass concentration, reaching a maximum of 3.1 g L⁻¹ after 14 days, followed by

75% dilution (2.2 g L⁻¹), 25% dilution (2.3 g L⁻¹), and undiluted wastewater (1.6 g L⁻¹). The superior performance observed at 50% dilution can be attributed to an optimal balance between nutrient availability and reduced inhibitory effects of toxic compounds, salinity, and color intensity commonly present in industrial effluents. In contrast, undiluted wastewater showed suppressed growth throughout the experimental period, likely due to excessive salinity, high organic loading, and the presence of inhibitory substances such as dyes and heavy metals, which adversely affect photosynthetic efficiency and cellular metabolism. Similar trends have been reported in previous studies, where moderate dilution of industrial wastewater significantly enhanced algal growth compared to raw effluent conditions (Cho *et al.*, 2011; Markou *et al.*, 2014). The initial lag phase was shorter in diluted treatments, indicating improved physiological adaptation of algal cells, whereas cultures in undiluted wastewater exhibited prolonged lag phases and reduced growth rates.

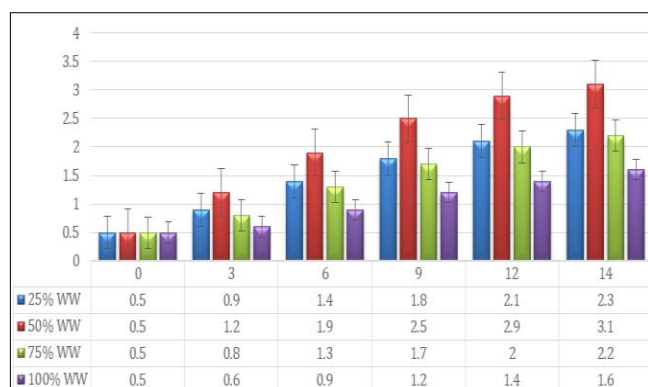


Fig 1: Biomass Growth

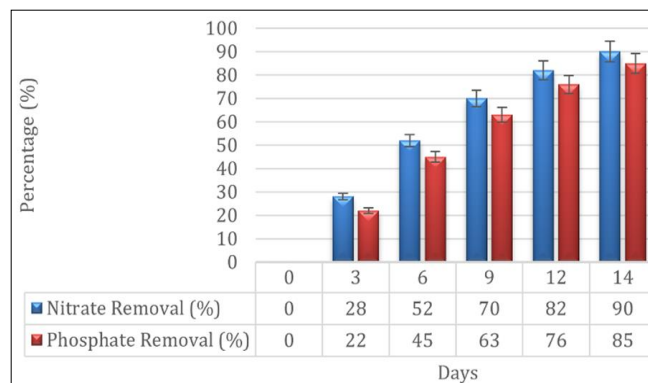


Fig 2: Nutrient Removal

Nutrient Removal Efficiency: The nutrient removal performance of *Chlorella vulgaris* cultivated in optimized wastewater conditions is shown in Figure 2. A steady increase in nitrate and phosphate removal was observed over the cultivation period. After 14 days, nitrate removal reached 90%, while phosphate removal achieved 85%, indicating effective assimilation of nutrients into algal biomass. Rapid nutrient uptake during the exponential growth phase (days 3–9) corresponded with increased biomass accumulation, confirming the direct relationship between algal growth and nutrient removal. The high nutrient removal efficiency observed in this study demonstrates the potential of microalgae-based systems to serve as an effective tertiary treatment option for industrial wastewater. Comparable nutrient removal efficiencies have been reported for *Chlorella* species grown in textile and

food-processing effluents, further supporting the feasibility of this approach (Pittman *et al.*, 2011; Li *et al.*, 2019)^[16].

Influence of Light Intensity and Inoculum Density: Light intensity played a crucial role in determining biomass productivity. Cultures exposed to 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ exhibited significantly higher growth rates compared to those maintained at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, indicating light limitation at lower irradiance. However, increasing light intensity to 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ did not result in proportional biomass enhancement and, in some cases, led to slight growth inhibition, likely due to photoinhibition and oxidative stress under excessive irradiance. These findings are consistent with earlier reports that identified moderate light intensities as optimal for *Chlorella* cultivation in wastewater-based systems (Singh and Singh, 2015). Similarly, inoculum density significantly influenced algal growth dynamics. Cultures initiated at 1.0 g L⁻¹ demonstrated faster exponential growth and higher final biomass compared to lower (0.5 g L⁻¹) and higher (1.5 g L⁻¹) inoculum densities. Lower inoculum densities resulted in prolonged lag phases, while higher densities caused self-shading effects and reduced light penetration, ultimately limiting photosynthetic efficiency. These results highlight the importance of optimizing inoculum density to maximize light utilization and nutrient uptake efficiency.

Statistical Analysis

Statistical evaluation of experimental data was performed using one-way and factorial analysis of variance (ANOVA) to assess the significance of wastewater dilution ratio, light intensity, and inoculum density on algal biomass productivity and nutrient removal efficiency. Results revealed that wastewater dilution ratio had a statistically significant effect on final biomass concentration ($p < 0.01$), with the 50% dilution treatment showing significantly higher biomass compared to other dilution levels. Light intensity also significantly influenced biomass production ($p < 0.05$), with optimal growth observed at 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, while further increases in light intensity did not yield proportional biomass enhancement due to potential photoinhibition. Inoculum density exhibited a significant effect on growth kinetics ($p < 0.05$), with an initial biomass concentration of 1.0 g L⁻¹ resulting in the shortest lag phase and highest specific growth rate. Interaction effects between dilution ratio and light intensity were significant ($p < 0.05$), indicating that optimal light requirements were dependent on wastewater concentration. No statistically significant interaction was observed between inoculum density and light intensity ($p > 0.05$). Nutrient removal efficiencies for nitrate and phosphate were also significantly affected by wastewater dilution ($p < 0.01$), confirming that optimized algal growth conditions directly enhance nutrient uptake performance. All statistical analyses were conducted at a 95% confidence level, and mean comparisons were performed where appropriate.

Conclusion

This study demonstrates the technical feasibility and environmental potential of optimizing algal biomass production using industrial wastewater as a nutrient source. The freshwater microalga *Chlorella vulgaris* exhibited robust growth and high nutrient assimilation capacity when cultivated under controlled laboratory conditions using wastewater derived from industrial sources. Among the evaluated parameters, wastewater dilution ratio emerged as the most critical factor influencing algal performance, with

50% diluted wastewater providing an optimal balance between nutrient availability and the mitigation of inhibitory effects associated with salinity, organic load, and residual industrial contaminants. Under optimized conditions, algal biomass concentrations exceeding 3.0 g L⁻¹ were achieved, alongside high nutrient removal efficiencies, with nitrate and phosphate removals reaching up to 90% and 85%, respectively. Light intensity and inoculum density further modulated growth dynamics, where moderate irradiance and an intermediate inoculum density promoted rapid exponential growth and efficient nutrient uptake. Statistical analysis confirmed the significant influence of these operational parameters on biomass productivity and nutrient removal, underscoring the importance of integrated process optimization. The findings highlight the dual functionality of microalgae-based systems for simultaneous wastewater remediation and biomass generation, offering a sustainable alternative to conventional energy-intensive treatment technologies. Importantly, the algal biomass produced under optimized conditions represents a valuable feedstock for bioenergy, biofertilizers, and other bioproducts, reinforcing the role of algal cultivation within a circular bioeconomy framework. Overall, this research provides experimentally realistic insights that contribute to bridging the gap between laboratory-scale studies and practical implementation, supporting the integration of algal-based technologies into industrial wastewater management strategies aimed at achieving environmental sustainability and resource recovery.

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