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Original Research

Biotechnological Potential of *Oscillatoria sp.* for Acid Cheese Whey Remediation: Insights Into Mixotrophic Metabolism and Nutrient Removal

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Abstract

Background: The unsafe disposal of milk processing effluents has a negative impact on the environment due to their high content of nutrients and organic matter. Green alternatives can be applied to effectively manage and valorize these effluents, reducing their environmental footprint. **Methods**: The ability of the free-living cyanobacterium *Oscillatoria sp*. to grow in real cheese whey was evaluated as a potential strategy for integrating dairy wastewater treatment with biomass valorization. Autotrophic and mixotrophic cultures were maintained under controlled laboratory conditions and monitored over 28 days for growth, cell viability, biomass, pigment content, and physicochemical parameters, including pH, protein, carbohydrate, and chemical oxygen demand (COD). **Results**: *Oscillatoria sp*. successfully adapted to the initial acidic conditions of the effluent (pH 2.8–2.9), increasing the pH of the treated whey to levels suitable for industrial wastewater disposal (pH 6.0–9.0). A 5-fold increase in dehydrogenase activity was observed after a 28-day culture, with no signs of oxidative damage. Cyanobacterial biomass cultivated under mixotrophic conditions displayed a significant reduction (∼55%) in photosynthetic pigments, including chlorophyll a and total carotenoids, compared to autotrophic cultures. Notably, *Oscillatoria sp*. biomass increased by 2.3-fold under mixotrophy, compared to the autotrophic control. The higher biomass production was accompanied by a significant reduction in the whey COD from 35,250 mg/L to 8500 mg/L, along with a 65% and 80% decrease in protein and carbohydrate content, respectively. **Conclusions**: These findings provide new insights into the metabolic behavior of *Oscillatoria sp*. during cheese whey bioremediation, highlighting the potential of mixotrophic cyanobacteria for managing dairy wastewater management.

Keywords: dairy products; waste water; environmental sustainability; cyanobacteria; heterotrophic processes; metabolism; biomass

1. Introduction

The dairy industry generates milk processing waste streams that significantly impact the environment [1,2]. Cheese-making effluents, due to their high nutrient and organic content, contribute to resource depletion and greenhouse gas emissions during wastewater treatment processes [3]. The improper management of cheese whey can lead to microbial contamination, oxygen depletion, acidification, and eutrophication of natural and recreational waters, representing one of the most serious ecological consequences of cheese manufacturing [4,5]. The final disposal or industrial reuse of whey depends on its quality (i.e., microbial load and physicochemical composition), quantity, available technologies, and the economic costs associated with its treatment.

In recent years, many countries, including the United States, Canada, Australia, New Zealand, and several in the European Union, have implemented strict environmental regulations to mitigate the polluting impact of whey [6]. These new standards, introduced alongside strong circular bioeconomy policies, have encouraged the dairy industry to explore novel approaches and opportunities for reusing the generated effluents [2]. The reuse of cheese whey faces several challenges, including microbial spoilage, high transportation costs, regulatory restrictions, and variability in composition. Effective processing and infrastructure are also required to ensure its sustainable utilization [4,6,7]. Therefore, the *in situ* remediation presents an effective solution to face these challenges. By treating whey directly at the production site, its environmental impact can be miti-

gated while simultaneously generating bioenergy or microbial biomass. This localized approach enhances sustainability, optimizes resource recovery, and aligns with bioeconomy principles within the dairy industry.

In this context, the production of microalgal and cyanobacterial biomass using agri-food effluents as a nutrient source has gained significant attention [8,9]. Species such as *Chlorella sp*., *Scenedesmus sp*., *Nostoc sp*., and *Plectonema sp*. have shown great potential to reduce nutrient, metal, and carbonaceous compound loads in industrial wastewater to safe levels. Large-scale cultivation systems of autotrophic microorganisms can also contribute to carbon dioxide mitigation through photosynthesis [10,11]. However, these systems usually require nutrient supplementation, which increases costs. Wastewater with a high organic load, such as cheese-making effluents, could serve as an alternative nutrient source, offering both economic and environmental benefits.

Cyanobacteria are of particular interest due to their physiological robustness and ability to convert inorganic carbon into valuable by-products. These microorganisms are increasingly recognized worldwide for their diverse biotechnological applications, including green energy production, sustainable agriculture, wastewater remediation, and use as feedstock in the biopharmaceutical, nutritional, and nutraceutical industries [12–16]. Among cyanobacteria, *Oscillatoria* is a dominant genus that thrives in a wide variety of natural environments. Despite its high biomass yield and filamentous structure, which facilitate a more efficient harvesting process, this organism remains largely untapped for the production of bioactive molecules, including food-value pigments [17,18]. Additionally, limited research has focused on the eco-physiological adaptations that enable *Oscillatoria* to overcome stressful conditions and adapt to environmental changes. Its bioremediation potential for agri-food effluents, such as cheese whey, remains unexplored despite its known ability to capture excess nutrients. Therefore, a gap remains in understanding the full potential of this blue-green filamentous cyanobacterium.

This work evaluates the ability of *Oscillatoria sp.* to grow and survive in real cheese whey provided by a local company. The effects on oxidative damage, cell viability (dehydrogenase activity), biomass production, and photosynthetic pigment content (chlorophyll a and total carotenoids) during *Oscillatoria sp*. mixotrophism were compared with an autotrophic culture. The reduction in the pollutant load of the effluent through co-culturing with *Oscillatoria sp*. was also assessed by measuring chemical oxygen demand (COD) levels, carbohydrate, and protein content. To the best of our knowledge, this report is one of the first to describe the metabolic and physiological adaptations of this mixotrophic cyanobacterium during cheese whey remediation, as a first step toward its use in dairy effluent management.

2. Materials and Methods

2.1 Cheese Whey Collection and Characterization

The cheese whey sample used in the present study was kindly provided by a local company (Milkaut S.A., Rafaela, Argentina). This side-stream liquid cannot be re-introduced into the production chain during dairy manufacturing, and thus must be properly disposed of, imposing additional costs on the company (data provided by the supplier). A preliminary characterization of the acidic whey delivered by the company is presented in Table 1. Biological oxygen demand (BOD_5, mg/L) and chemical oxygen demand (COD, mg/L) were determined according to American Public Health Association Standard Methods for the Examination of Water and Wastewater (APHA) methods SM5210-D and SM5220-D, respectively [19]. Electrical conductivity (EC, mS/cm) and pH were measured using an AD1030 digital pH-meter (Adwa, Nușfalău, Romania). Density (g/mL) was determined by weight and volume measurements using a Mettler-Toledo balance (Model ML 201/01; accuracy, $\pm$100 µg; Mettler-Toledo, Columbus, OH, USA). Total fats (FOG, g/L) and total solids (TS, g/L) analyses were carried out following standard methods [19]. Total protein content was determined according to Lowry *et al.* [20]. Soluble carbohydrates were quantified according to Dubois *et al.* [21]. Total heterotrophs (cfu/mL) were quantified by plate counting on Plate Count Agar (PCA) (Britania S.A., Buenos Aires, Argentina) [19].

Table 1. Characterization of the industrial acid cheese whey used in the present study.

Parameter	Values
pH	2.85 $\pm$ 0.05
Density (g/mL)	1.0276 $\pm$ 0.0002
Conductivity (mS/cm)	6.55 $\pm$ 0.20
COD (mg/L)	75,500 $\pm$ 2600
BOD_5 (mg/L)	38,800 $\pm$ 2200
Protein content (g/L)	4.5 $\pm$ 0.3
Soluble carbohydrates (g/L)	32 $\pm$ 5
Total fats (g/L)	1.50 $\pm$ 0.05
Total solids (g/L)	46.5 $\pm$ 0.1
Total heterotrophs (cfu/mL)	$4.0 \pm 0.5 \times 10^5$

Data are reported as the mean value $\pm$ standard deviation (S.D.) of three replicates (n = 3). COD, chemical oxygen demand; BOD_5, biological oxygen demand.

2.2 Cyanobacterium Culture and Biomass Preparation

Oscillatoria sp. was kindly provided by INTA-Rosario (National Institute of Agricultural Technology, Santa Fe, Argentina). The cyanobacterial stock culture was grown and maintained in sterile modified Zarrouk medium (16.8 g/L $NaHCO_3$, 0.5 g/L K_2HPO_4, 2.5 g/L $NaNO_3$, 1.0 g/L K_2SO_4, 1.0 g/L NaCl, 0.2 g/L $MgSO_4$, 0.04 g/L

$CaCl_2$ and 1.0 mL solution A_5 prepared with 2.86 g/L H_3BO_3, 1.81 g/L $MnCl_2$, 0.222 g/L $CuSO_4 \cdot 5H_2O$ and 0.015 g/L MoO_3; pH 7.4). The culture was incubated at room temperature in a 3 L glass flask, equipped with aquarium air diffusers, under a 12-h photoperiod (light intensity of 100 µE/m^2·s), using a combination of dual-spectrum white LED panels (full-spectrum, 380–700 nm) and supplemental red LED lamps (peak emission at ~660 nm). This lighting configuration was selected to provide optimal support for cyanobacterial photosynthesis. Although molecular characterization via 16S rRNA gene sequencing is considered ideal for species-level identification, technical limitations associated with DNA extraction from filamentous *Oscillatoria* biomass currently hinder routine implementation. Morphological identification was instead conducted by a certified laboratory. Taxonomic analysis of the stock culture was routinely performed by *Instituto del Alimento* (Rosario, Argentina), and purity was confirmed based on the morphological characteristics of the cells. Protein content, soluble carbohydrates, total carotenoids, and chlorophyll a were determined according to Carralero Bon *et al.* [22]. The characteristics of the stock culture of *Oscillatoria sp*. used in this study are presented in Table 2.

Before the experiments, the biomass was harvested by centrifugation (3000 ×g, 10 min), and the pellet was washed twice with sterile phosphate-buffer solution (PBS, pH 7.4). Biomass dry weight was determined by filtering 50.00 mL of culture (n = 3) through previously tared filter papers (Whatman™ Grade 589/3, Maidstone, UK) and oven-drying the filter papers overnight at 80 °C to constant weight. An average initial cyanobacterial biomass of 0.55 ± 0.05 g/L (dry weight) was used in all experiments.

Table 2. Characterization of the *Oscillatoria sp*. stock culture used in the present study.

Parameter	Values
pH	10.0 ± 0.2
Conductivity (mS/cm)	16.5 ± 0.2
Protein content (% dry weight)	38.5 ± 4.2
Soluble carbohydrates (% dry weight)	21.3 ± 3.8
Total carotenoids (mg/L)	10.9 ± 0.6
Chlorophyll a (mg/L)	5.1 ± 0.3

Data are reported as the mean value ± S.D. of three replicates (n = 3).

2.3 Cyanobacteria Growth in the Presence of Whey

To compare the growth of *Oscillatoria sp*. under autotrophic and mixotrophic conditions, 500 mL of cyanobacterial culture (containing both mother microalgae and medium) was directly mixed with either 500 mL Zarrouk medium (Z) or 500 mL raw industrial acid whey (W). Prior to this, any intrinsic microflora in the whey sample was inactivated by UV light irradiation (40 W/cm^2, 45 min) [23] to prevent possible interference in subsequent assays. UV sterilization was selected over membrane filtration primarily due to the high viscosity and particulate content of the raw industrial acid whey, which clogged membrane filters and compromised flow rates, making filtration impractical at the volumes used in this study. Additionally, membrane filtration can unintentionally remove macromolecules and micronutrients, potentially altering the nutritional composition of the whey and affecting its ability to support cyanobacterial growth. In contrast, UV sterilization was chosen as a non-invasive, time-efficient, and cost-effective method capable of significantly reducing microbial load in the crude sample, with minimal impact on the overall physicochemical and nutritional profile of the whey.

The effectiveness of the UV treatment was confirmed by plating 1.0 mL of the treated sample on nutritive agar media (PCA), ensuring the complete absence of bacterial colony growth. The corresponding control was routinely included in all assays to ensure the reliability of the conclusions. Both cultures (Z and W) were maintained under controlled laboratory conditions (23 ± 2 °C, irradiance of 100 µE/m^2·s, and a 12-h light/dark cycle) with constant agitation (100 rpm) for 28 days. Periodically (every 48–72 h), aliquots were taken for microbiological and physicochemical analyses. Due to the turbidity of W, optical density measurements were not suitable for estimating cyanobacterial growth. Instead, dehydrogenase activity was used as a proxy for metabolic activity and cell viability over time (Section 2.5), while biomass accumulation was determined gravimetrically at the end of the 28-day period (Section 2.7).

2.4 Determination of Physiological Parameters of Cyanobacteria Cultures

The pH values of both Z and W cultures were measured using a digital pH-meter AD1030 (Adwa, Nușfalău, Romania). Chlorophyll a, total carotenoids, soluble carbohydrates and protein content were quantified by UV-Vis spectrophotometry following the procedure described by Carralero Bon *et al.* [22]. Data were reported as the mean value ± S.D. of three replicates (n = 3).

2.5 Evaluation of Cyanobacteria Cell Viability

To determine the viability of the cyanobacterial cultures, a colorimetric assay based on the enzymatic reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to 1,3,5-triphenyl formazan (TF, red color) was used [24]. An increase in the number of metabolically active cells correlates with higher overall activity of microalgal dehydrogenases responsible for converting TTC into the red-colored TF, which can be spectrophotometrically measured at 485 nm [22,25,26]. Briefly, 2.0 mL aliquots of cyanobacterial culture along with 0.10 mg/mL of TTC (Sigma-Aldrich, Catalog No. 108380, St. Louis, MO, USA; CAS No. 298-96-4)

in test tubes and incubated in the dark at room temperature for 1 h. After centrifugation at 7000 ×g for 10 min, the supernatants were discarded, and the pellets were resuspended in 1.0 mL of 70% (v/v) acetone to lyse the cells and release the red dye. Additionally, the samples were sonicated in a TB-04 bath for 10 min (Testlab S.R.L., Buenos Aires, Argentina) and vortexed at maximum speed for 15 sec to ensure complete release of the intracellular TF. Finally, the samples were centrifuged at 7000 ×g for 10 min, and the absorbance was measured at 485 nm using a UV-Vis Lambda 25 spectrophotometer (Perkin Elmer, Boston, MA, USA). All assays were performed in triplicate ($n = 3$). Data were reported as µmol/mL of TF according to the following equation:

$$\text{TF (µmol/mL)} = 0.0603 \times \text{Abs}_{485}$$

2.6 Determination of Lipid Peroxidation/MDA

Lipid peroxidation was evaluated by quantifying the amount of thiobarbituric acid reactive substances (TBARS) following the method described by Carralero Bon *et al.* [22]. Results were expressed as nmol of malondialdehyde (MDA) per liter (nmol/L) using an extinction coefficient of 155 mM/cm for calculation and reported as the mean ± S.D. of three replicates ($n = 3$).

2.7 Determination of Cyanobacterial Biomass

The cyanobacterial biomass was determined before and after 28 days of growth under autotrophic or mixotrophic conditions. For this, 10.00 mL samples from cultures Z and W were added to pre-weighed crystallizing dishes (previously dried at 105 °C for 24 h). The dishes were then oven-dried at 80 °C to constant weight, and the loss in weight was calculated as grams of dry weight per liter (g/L). To correct the data and avoid overestimation of the cyanobacterial biomass, the same method was applied to determine the dissolved and particulate solids initially present in the diluted whey, as well as the amount of dissolved salts in the Zarrouk medium. These values were adjusted to reflect the reduction in carbohydrates and proteins after 28 days of cyanobacterial treatment. Data were expressed as mean values ± S.D. from three independent assays ($n = 3$).

2.8 Statistical Analysis

All statistical analyses were performed using SigmaStat 3.5 (Systat Software Inc., San Jose, CA, USA). The Student's *t*-test (for comparison between two groups) or Tukey's Honest Significant Difference (HSD) *post-hoc* test (for comparison between multiple groups after ANOVA) were applied, with a confidence interval of 95% ($p < 0.05$).

3. Results and Discussion

3.1 Physiological Parameters of Oscillatoria sp. Under Autotrophic and Mixotrophic Growth

Fig. 1 shows the pH evolution in the autotrophic and mixotrophic cultures (Z and W, respectively) of *Oscillatoria sp.* over 28 days. The Z culture remained alkaline throughout the entire period, with pH values ranging from 9.4 to 10.1, as expected for an alkaliphilic cyanobacterium. Culture alkalinization is employed by cyanobacteria to reduce nutrient competition, as high pH inhibits the growth of non-alkaliphilic microorganisms [27,28]. This strategy underscores the ecological advantage that *Oscillatoria sp.* gains by modifying its environment, alleviating pressure on resources. In contrast, acidification of the medium can serve as an early indicator of microbial contamination, suggesting the presence of competing or harmful microorganisms that could affect culture stability and biomass productivity. Thus, monitoring pH during cyanobacterial growth is an effective tool for evaluating culture health and optimizing growth conditions in both industrial and research settings [27].

On the other hand, the pH of the mixotrophic W culture initially exhibited lower values (pH 6.0) compared to Z. This can be attributed to the intrinsic acidity of the whey (pH 2.8–2.9; see Table 1), primarily due to the presence of organic acids (e.g., lactic acid) produced by lactic acid bacteria during the cheese-making process. Over the following days, the pH gradually increased, becoming alkaline. Notably, two distinct phases were observed. The first (from day 1 to day 10) showed a rapid pH rise, reaching a value around 8.0, which is still suitable for safe industrial effluent disposal [29]. This behavior could be attributed to the consumption of carbohydrates and organic acids (major components of cheese whey), and to a lesser extent, the degradation of whey proteins (further explained in Section 3.2), which releases ammonia (NH_3) into the culture. The second stage (days 10 to 28) showed a more gradual pH increase, reaching a maximum between 9.0 and 9.5 (Fig. 1). The slower increase could reflect the cyanobacterium's prolonged adaptation to the presence of whey, where the depletion of nutrients slows the rate of degradation, leading to a more gradual pH rise. These results show that *Oscillatoria sp.* successfully adapted to mixotrophic conditions with a 1:1 ratio of crude whey as the primary substrate. This is particularly relevant, as the acidity of cheese-processing effluents often limits the use of biological methods for whey bioremediation, typically requiring prior chemical neutralization [4,5]. Minimizing additional chemical or water-based treatments (e.g., dilution) is crucial for reducing costs and the volume of effluent to be treated [30]. Further, the observed pH trends demonstrate that *Oscillatoria* can not only grow in the presence of acid whey but also generate an alkaline environment, which may help ensure that the resulting biomass remains free of pathogens.

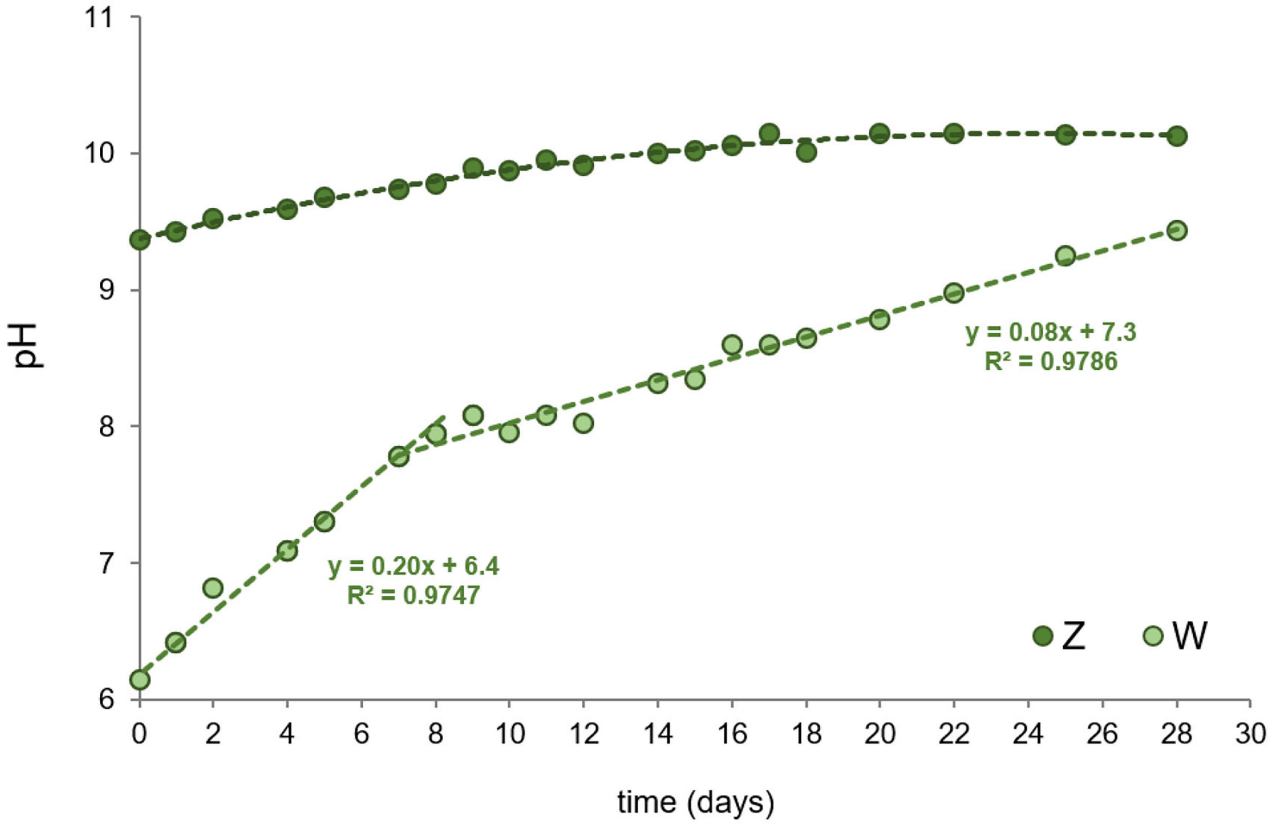

Fig. 1. Evolution of pH in *Oscillatoria sp.* cultures grown under autotrophic (Z) or mixotrophic (W) conditions.

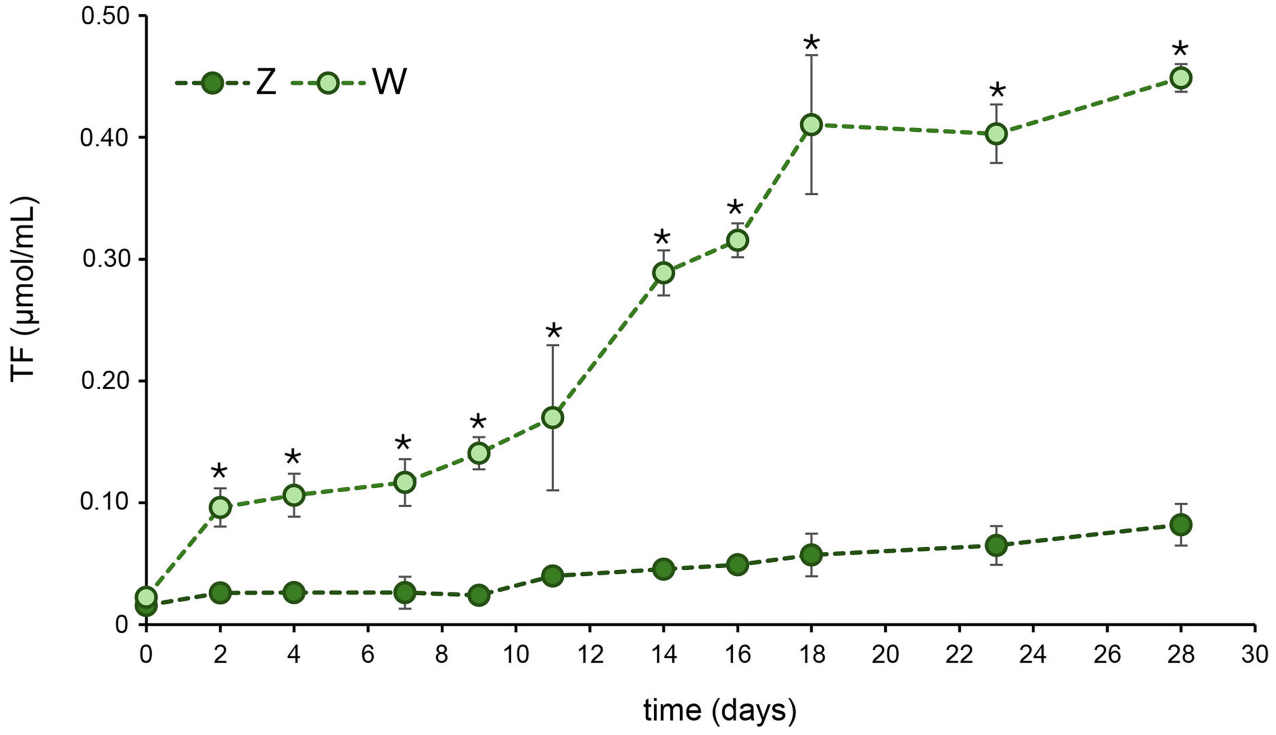

Fig. 2. Dehydrogenase activity in *Oscillatoria sp.* cultures grown under autotrophic (Z) or mixotrophic (W) conditions. Asterisks (*) indicate statistically significant differences ($p < 0.05$) in TF content between the cultures on each day. TF, 1,3,5-triphenyl formazan.

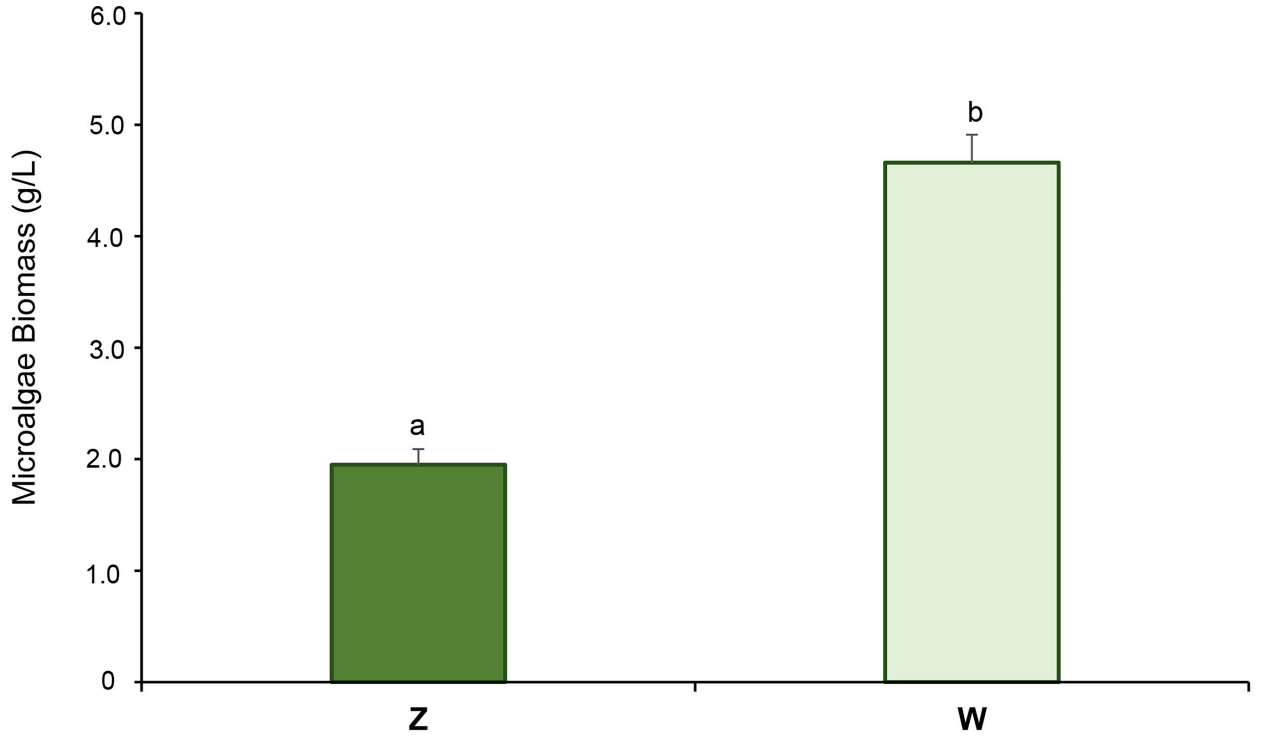

Fig. 3. Total cyanobacterial biomass production (g/L) after 28 days of growth under autotrophic (Z) and mixotrophic (W) conditions. Different letters indicate statistically significant differences (*t*-test, $p < 0.05$); e.g., "a" and "b" are statistically different from each other.

Although the pH evolution of the mixotrophic culture towards alkaline values can be considered a good indicator of healthy cyanobacterial growth [27,28], analyzing cellular metabolic activity offers a more precise assessment of *Oscillatoria sp.* viability [31].

In culture W, a parallel trend between the pH evolution (Fig. 1) and cell dehydrogenase activity (Fig. 2) was observed. The accumulation of TF in the biomass growing under mixotrophic conditions was lower during the first 10–12 days but began to increase from day 14 onward, continuing until the end of the experimental period (day 28). These results are consistent with the two stages of pH evolution observed for this culture in Fig. 1. During the initial days of growth, the suboptimal conditions caused by the high acidity of the cheese whey likely affected the enzymatic reduction of TTC to TF by the cyanobacterial cells, as this conversion is a metabolism-driven process [24].

The quantity of TF formed is also proportional to cell density [26]. Accordingly, Fig. 3 shows that under autotrophic conditions (Z), the amount of cyanobacterial biomass obtained after 28 days of culture was 2.3 times lower (2.0 ± 0.1 g/L) than the yield reached under mixotrophic growth (4.7 ± 0.3 g/L). Notably, no significant differences were observed in the quantification of TBARs, a parameter associated with cellular oxidative stress [31], between the two cultures (Fig. 4). Altogether, these results demonstrate the potential of linking industrial whey treatment with the production of healthy cyanobacterial biomass. Both biomass yield and the quality of the biomass produced are critical factors for any biorefinery concept. Cyanobacteria can produce a wide range of intracellular metabolites of interest, and their ability to accumulate these molecules is highly dependent on the growth medium composition and the physiological integrity of the cells, which ultimately supports the synthesis of valuable end-products [32].

3.2 Organic Load Reduction From Cheese Whey Using Oscillatoria sp.

The higher dehydrogenase activity observed in *Oscillatoria sp.* grown in the presence of whey, as shown in Fig. 2, provides compelling evidence of the cyanobacterium's heterotrophic metabolism. Dehydrogenases are involved in the aerobic catabolism of carbohydrates, primarily in glycolysis, as well as in the oxidative pentose phosphate pathway and the tricarboxylic acid (TCA) cycle. These enzymes also play a role in lipid and protein metabolism [33–36]. Consequently, the results in Fig. 2 align with the concomitant decrease in carbohydrate and protein content in the industrial whey when mixed with *Oscillatoria sp.* (Fig. 5). The concentration of soluble carbohydrates in the mixotrophic culture (W) significantly decreased ($p < 0.05$) during the first 9 days (Fig. 5a), corresponding to the period of rapid pH increase observed in this

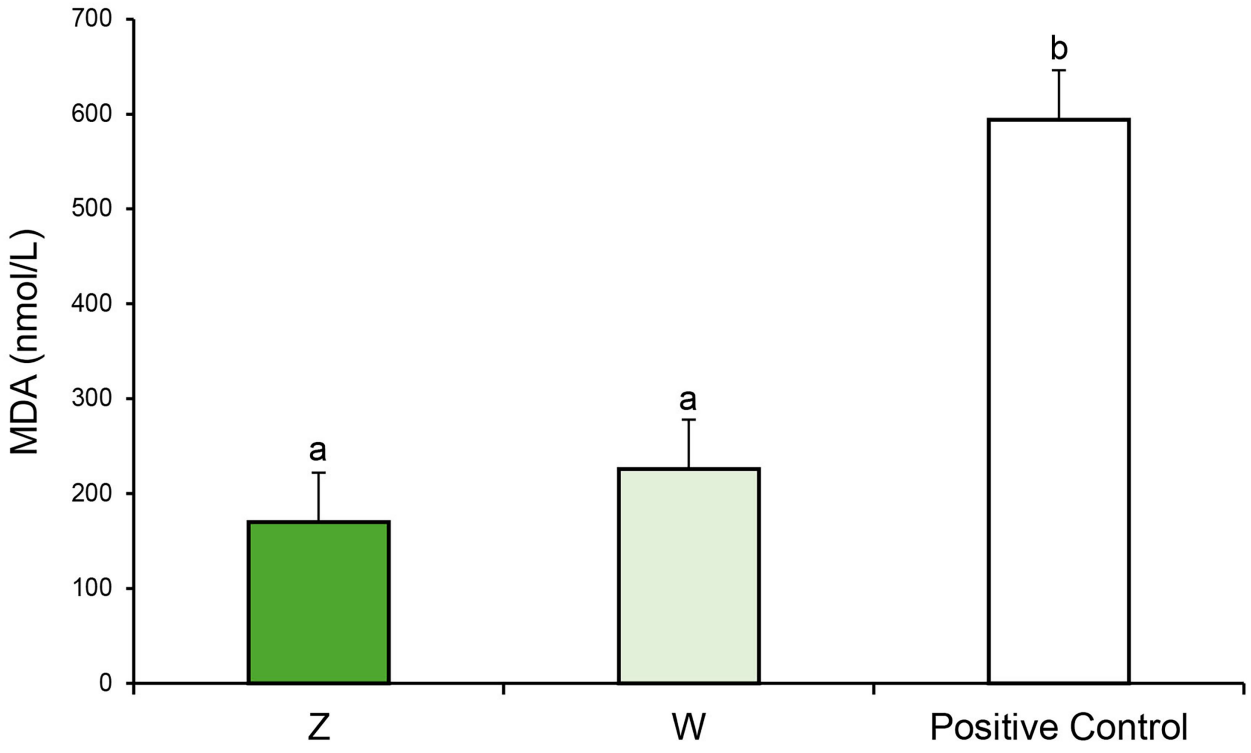

Fig. 4. Quantification of thiobarbituric acid reactive species (TBARs) in *Oscillatoria sp.* after 28 days of growth under autotrophic (Z) or mixotrophic (W) conditions. The positive control refers to TBARs generated in cyanobacterial cells treated with 1 mM H_2O_2 for 24 h. Different letters indicate statistically significant differences ($p < 0.05$); e.g., "a" and "b" are statistically different from each other. MDA, malondialdehyde.

culture (Fig. 1). This behavior is likely due to the consumption of lactose, which may be converted into lactic acid and further metabolized [4,35]. Lactic acid is then converted into pyruvate, a key substrate for the TCA cycle, playing a crucial role in cellular energy generation [33,34]. From day 9 onwards, the concentration of soluble carbohydrates in culture W reached a limiting value, while whey protein levels began to gradually decline (Fig. 5b). These results suggest that under mixotrophic conditions, *Oscillatoria sp*. primarily derives its metabolic energy (ATP) from carbohydrates consumption. Once this carbon source is depleted, the cells shift to exogenous protein catabolism to sustain growth [36].

Lipids constitute another major metabolic product of cyanobacteria. Although lipid content was not assessed here due to resources and technical limitations, it is well-established that lipid accumulation often increases under certain stress conditions, such as nutrient limitation or changes in environmental factors [37]. Given that acidic whey presents a complex and potentially stressful growth substrate, it is plausible that *Oscillatoria sp*. might also modulate its lipid metabolism in response to these conditions. Future investigations incorporating quantitative and qualitative lipid analyses (e.g., solvent extraction followed by chromatographic profiling) would provide valuable insights into the full metabolic adjustments of *Oscillatoria* during growth on acidic whey.

In parallel, the decrease in the content of soluble carbohydrates and proteins in the mixotrophic culture (W) was strongly correlated with a significant reduction (about 75%) in the chemical oxygen demand (COD) after 28 days of culture, compared with the untreated whey (1:1) (Fig. 6). No COD values were detected in *Oscillatoria sp.* grown under autotrophic conditions (Z), which is consistent with the negligible levels of soluble carbohydrates and proteins quantified (Figs. 5,6). Therefore, the difference in COD between the mixotrophic culture (W) and the untreated whey sample (i.e., "Acid Whey" in Fig. 6) is attributed to the consumption of organic matter (e.g., carbohydrates, organic acids, and proteins) by the cyanobacterium. The COD values in the W culture remain above the permissible limit for wastewater discharge (<250 mg/L) [29], indicating the need for further treatment (e.g., extended culturing times). While not intended to meet final discharge standards on its own, the process achieved a substantial reduction in COD (from 35,250 to 8500 mg/L) under non-optimized conditions and without chemical pre-treatment.

These results highlight the viability of *Oscillatoria* as an effective primary remediation agent in handling high organic loads. In practical industrial applications, such a

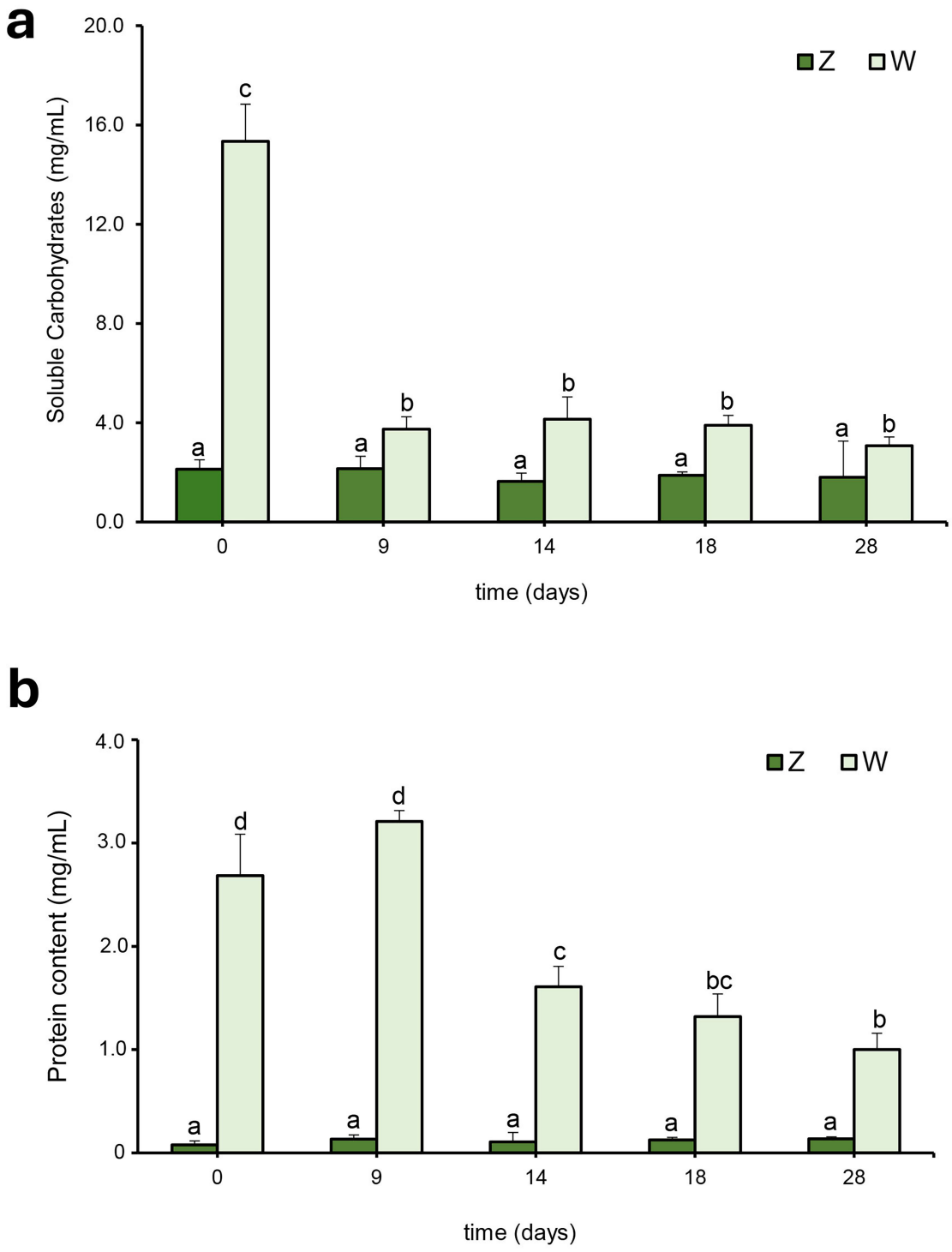

Fig. 5. Changes in the biochemical composition of the culture medium during cyanobacterial growth. (a) Soluble carbohydrate and (b) protein contents in *Oscillatoria sp.* cultures grown under autotrophic (Z) or mixotrophic (W) conditions (the latter using industrial whey as the nutrient source). Different letters indicate statistically significant differences ($p < 0.05$); e.g., "a" and "b" are statistically different from each other; "b" and "c" are statistically different from each other but not from "bc".

biological step would typically be integrated into a multi-stage treatment system, complemented by secondary polishing processes to meet regulatory discharge thresholds. This work therefore serves as a proof-of-concept for the use of cyanobacterial-based treatment in the initial phase of whey effluent management. Furthermore, the mixotrophic behavior demonstrated by *Oscillatoria* underscores its ability to efficiently utilize organic-rich waste streams such as

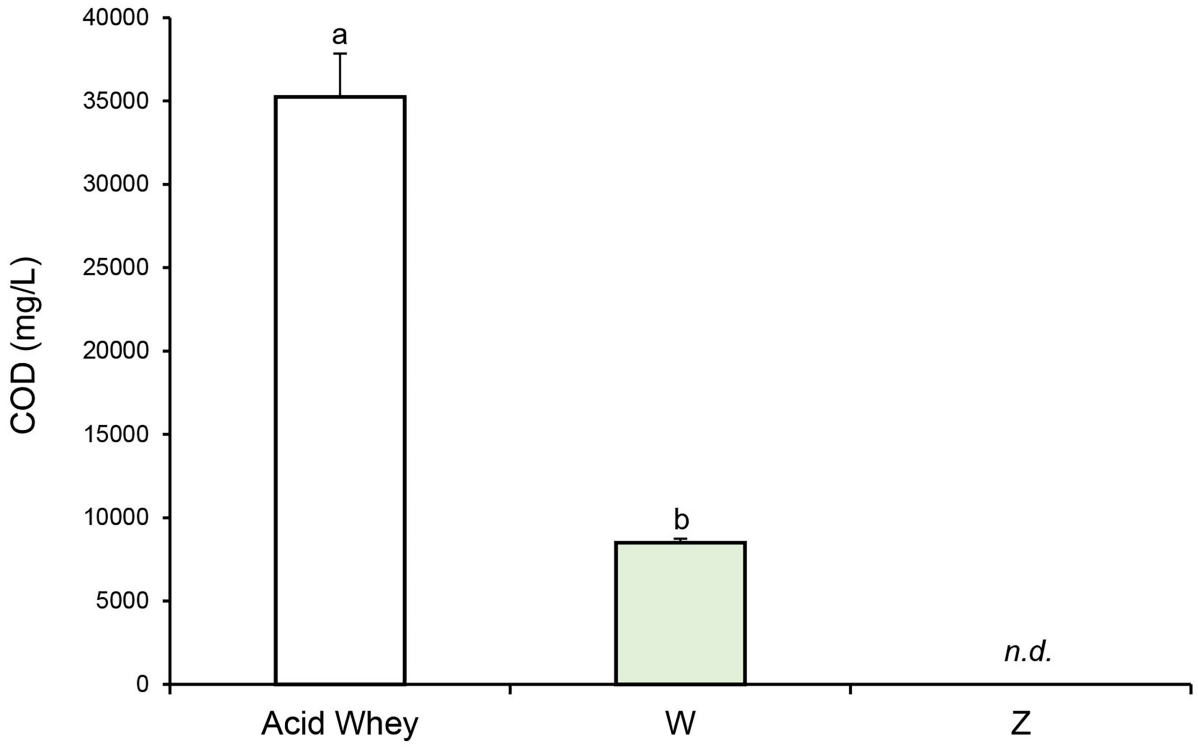

Fig. 6. COD values for the industrial acid whey used in this study (diluted 1:1) and the supernatants of the mixotrophic (W) and autotrophic (Z) cultures of *Oscillatoria sp.* after 28 days. Different letters indicate statistically significant differences ($p < 0.05$); e.g., "a" and "b" are statistically different from each other; *n.d.*, not detected; COD, chemical oxygen demand.

cheese whey as a growth substrate. This capability not only enhances COD removal but also reduces the need for external nutrient inputs in biomass cultivation. As such, the process holds potential for lowering operational costs in dairy wastewater treatment. These features align well with the principles of a circular bioeconomy, wherein industrial waste is repurposed into valuable biological products, promoting both economic and environmental sustainability [38].

3.3 Photopigment Concentration in Oscillatoria sp. Under Autotrophic and Mixotrophic Conditions

The evolution in the concentration of chlorophyll a and total carotenoids during *Oscillatoria sp*. autotrophic or mixotrophic growth is shown in Fig. 7a. In the absence of exogenous carbon sources (i.e., culture Z), cyanobacteria depend entirely on their photosynthetic machinery. Under this condition, the cells initiate the biosynthesis of photopigments to maximize light absorption and CO_2 fixation [39]. Chlorophylls and carotenoids are the most important molecules involved in photosynthesis, with chlorophylls actively participating in capturing and transforming sunlight into chemical energy, and carotenoids playing a key role in neutralizing reactive oxygen species. Carotenoids also play essential roles as accessory light-harvesting molecules, acting as secondary pigments as well as pro-vitamin factors [40].

The results from Fig. 7a also explain the lower dehydrogenase activity detected in culture Z (Fig. 2), as cyanobacteria lacking an external carbon source grow exclusively through photosynthesis. The reduction of tetrazolium compounds, such as TTC, is not inherently linked to cell ATP production [24]. Therefore, the reduced dehydrogenase activity in autotrophic *Oscillatoria sp*. is not necessarily associated with ATP depletion in the photosynthetically growing cells.

On the other hand, a significant reduction in chlorophyll a and total carotenoid content was detected in the cells growing under mixotrophic conditions (culture W) (Fig. 7a), compared to the autotrophic culture, despite the increase in cyanobacterial biomass (Fig. 3). This can be attributed to the high bioavailability of organic carbon provided by the presence of whey, which allows *Oscillatoria sp*. to grow while restricting photosynthesis [33]. During mixotrophy, cyanobacteria can utilize both inorganic (CO_2) and organic carbon sources. The increase in carbon availability can result in faster growth and higher biomass yield, as observed in Fig. 3. However, the presence of organic carbon alters the balance of cellular processes, leading to a decrease in the production of photopigments (Fig. 7). Hence, pigments reduction indicates a shift in metabolic priorities, with cells relying more on heterotrophic metabolism (i.e., organic carbon utilization) and less on photosynthesis. Additionally, the turbidity in culture W as shown in Fig. 7b,

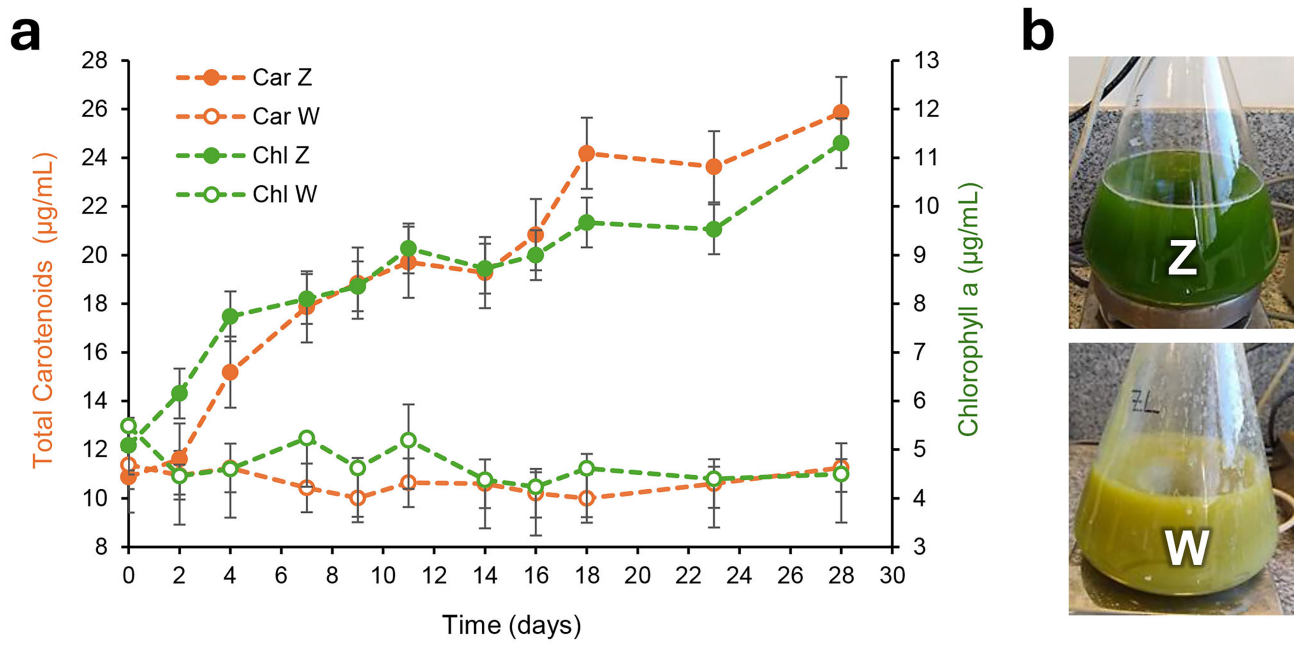

Fig. 7. Evolution of photosynthetic pigments during cyanobacterial growth. (a) Changes in chlorophyll a (Chl) and total carotenoid (Car) content in *Oscillatoria sp.* grown under autotrophic (Z) and mixotrophic (W) conditions over 28 days. (b) Representative photographs of cyanobacterial cultures after 28 days of growth illustrating differences in photopigment content between the two conditions, as described in the text.

caused by the presence of whey, hinders light penetration and its capture by cells, thereby affecting photopigment biosynthesis [39].

While mixotrophic growth of *Oscillatoria sp.* offers the advantage of utilizing organic matter, beneficial for cheese whey bioremediation and boosting biomass, this strategy leads to a decrease in photopigments. *Oscillatoria* shows potential for dairy wastewater treatment, but the trade-off is a reduction in the biosynthesis of valuable feed ingredients. Despite this, the increased cyanobacterial biomass yield offers significant applications, such as biosorbents for heavy metal and dye removal from wastewater, biochar production, and potential use as a biofertilizer in agriculture [22,41,42]. However, before applying such biomass in environmental or agricultural contexts, safety considerations must be addressed. Future studies should therefore assess the potential accumulation of harmful compounds or toxin production by the cyanobacterial biomass. Additionally, post-harvest treatments, such as thermal inactivation, anaerobic digestion, or pyrolysis, may enhance the safe reuse of the biomass as biosorbent material, biofertilizer, or feedstock for bioenergy, in line with circular bioeconomy and waste valorization strategies.

4. Conclusions

In this study, we investigated the potential of the mixotrophic *Oscillatoria sp.* to assimilate organic matter from real cheese whey. Our results demonstrated that the cyanobacterium effectively metabolizes carbohydrates and proteins from whey, transitioning from a predominantly photosynthetic mode to utilizing exogenous carbon sources. This metabolic shift led to a reduction in chlorophyll a and total carotenoid content compared to autotrophic growth. Notably, the high acidity of the cheese whey did not induce oxidative stress or impair cyanobacterial metabolic activity. Furthermore, *Oscillatoria sp.* significantly reduced the chemical oxygen demand of the whey and adjusted the effluent pH to levels suitable for safe disposal. Under mixotrophic conditions, the culture yielded higher biomass after 28 days. These findings underscore the potential of using *Oscillatoria sp.* in milk-processing effluent treatment while simultaneously enhancing cyanobacterial biomass production for further valuable applications. However, considerations around biomass safety and reuse pathways must be addressed through further contaminant profiling and regulatory assessment.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

ICB: methodology; validation; investigation; supervision. DL, SF, and DC: conceptualization; methodology; investigation; formal analysis; visualization. DB: methodology; validation; resources; supervision. LDL: investiga-

tion; formal analysis; writing—original draft. LMP: conceptualization; formal analysis; validation, visualization, writing—review and editing, supervision, project administration, funding acquisition. DL, SF, and DC contribute equally to this work. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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