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RESEARCH

# Microalgae-based dairy effluent treatment coupled with the production of agricultural biostimulant

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## Abstract

Using effluents as a growth medium for microalgae contributes to the effluent treatment and reduces the costs associated with biomass production. Dairy effluent (DE) is among the most voluminous food industry effluents generated. This study aimed to produce biomass and agricultural biostimulants by cultivating *Chlorella fusca* LEB 111 and *Spirulina* sp. LEB 18 in dairy effluent. The microalgae were grown in pure and diluted DE at 25%, 50%, and 75% in water and, BG 11 and Zarrouk culture media. The treated DE (culture supernatant) was utilized as a biostimulant in the germination of red pear tomato (*Lycopersicon esculentum*) seeds. After the cultivation process, treated effluent was obtained with BOD removal efficiency of 98.5% and 99.1%, COD 96.5% and 97.7%, total phosphorus 98.8% and 85.3%, and ammonia nitrogen 98% and 99% for *C. fusca* LEB 111 and *Spirulina* sp. LEB 18, respectively. Under the condition in which 100% DE was used, the maximum biomass concentrations of 1.03 g $L^{-1}$ and 1.98 g $L^{-1}$ and maximum biomass productivity of 159.70 mg $L^{-1}$ $day^{-1}$ and 187.70 mg $L^{-1}$ $day^{-1}$ were achieved for *Chlorella* and *Spirulina*, respectively. The *C. fusca* LEB 111 supernatant grown with 100% DE increased seed germination, resulting in 100% germinated seeds. Therefore, this study presents an alternative for microalgae cultivation utilizing DE as an alternative medium, resulting in biomass and treated effluent with high potential for application in agriculture.

**Keywords** Sustainable agriculture · Biofertilizers · *Chlorella* · *Lycopersicon esculentum* · *Spirulina* · Wastewater

## Introduction

The global consequences of population growth, industrial activities, climate change, and modern lifestyles have led to the excessive use of fossil fuels, agrochemicals and the high generation of wastewater (Peng et al. 2023). Urban effluents and effluents from food industries, such as dairy products, are among the wastewater generated in larger volumes (Ma et al. 2023). Nitrogen chemical fertilizers, which are heavily used in agriculture, directly contribute to climate change, as the nitrous oxide emitted has a high global warming potential compared to other greenhouse gases produced in agricultural practices (Ammar et al. 2022).

The dairy industry is one of the largest water consumers and producers of effluent among food industries, mainly due to milk treatment, sterilization processes, and equipment cleaning (Kumar et al. 2020). A high organic content with significant organic matter, nitrogen, phosphorus, and micronutrients characterizes dairy effluent (DE). The organic residues in DE are considered environmentally harmful due to their high chemical (COD) and biochemical oxygen demand (BOD) and the associated problems of rapid degradation. If not adequately managed, DE can threaten the ecological balance (Gogoi et al. 2021). By specific legislation in each location, effluents require treatment before being released into water bodies. After conventional wastewater treatment, the generated waste must be sent off-site for further treatment and disposal, increasing operating costs (Ma et al. 2023). However, DE can be an excellent source of nutrients for microalgae cultivation, presenting a potential biological treatment option for this waste (Costa et al. 2021).

Industrial-scale microalgae cultivation demands high nutrient and water concentrations (Kumar et al. 2019).

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Therefore, microalgae can consume high levels of nitrogen and phosphorus found in wastewater, utilizing only solar energy and producing biomass (Acién Fernández et al. 2018). Using effluents as a microalgae cultivation medium has become an alternative to reduce process costs and obtain microalgae biomass with diverse applications (Costa et al. 2019). The microalgal genera *Chlorella* and *Spirulina* (*Arthrospira*) have gained attention in using effluents as an alternative culture medium due to their resistance to cellular stress conditions (Bezerra et al. 2022).

*Chlorella* and *Spirulina* are known to produce biomass rich in protein, with an average content of 50% of their dry weight, along with carbohydrates, lipids, vitamins, minerals, and chlorophyll (Becker 2007; Morais et al. 2015). Consequently, a microalgae biorefinery approach can be employed, where microalgae are cultivated in DE, generating biomass that can be utilized to produce biofuels, animal feed, and in agriculture. Additionally, this process generates treated effluent that can be used as biostimulants for seed germination in agriculture (Costa et al. 2021). Biostimulants are natural substances that, when applied in small quantities, can stimulate plant growth and development, whether under optimal growth conditions or under conditions that cause cellular stress (Kapoore et al. 2021). Microalgae biostimulants pose a low risk of toxicity to the environment and living beings. By utilizing microalgae biostimulants, farmers can reduce their dependence on synthetic chemicals, such as fertilizers and growth regulators, thus mitigating the negative environmental impact associated with the overuse of these products (Braun and Colla 2022).

Therefore, this study aimed to produce biomass and agricultural biostimulants by cultivating *Chlorella fusca* LEB 111 and *Spirulina* sp. LEB 18 in dairy effluent, thus providing products with broad potential for application in agriculture and contributing to the reduction of pollution caused by the disposal of effluents and agrochemicals. This research is aligned with the goal of sustainable agriculture based on microalgae. It contributes to achieving the 17 Sustainable Development Goals (SDGs), particularly SDG-2 "Zero Hunger and Sustainable Agriculture", SDG-6 “Clean Water and Sanitation”, SDG-12 "Responsible Consumption and Production", and SDG-13 "Climate Action".

## Material and methods

### Microalgae and cultivation conditions

The microalgae *Spirulina* sp. LEB 18 and *Chlorella fusca* LEB 111 were obtained from the Biochemical Engineering Laboratory at the Federal University of Rio Grande (FURG) in Rio Grande do Sul, Brazil. The effluent used was obtained from a dairy industry in Timbó, Santa Catarina, Brazil. It was filtered to remove suspended solid particles before being stored at -20 °C until use (Schulze et al. 2017). Zarrouk medium (Zarrouk 1966) and BG 11 medium (Rippka et al. 1979) were used as control media for *Spirulina* sp. LEB 18 and *Chlorella fusca* LEB 111, respectively.

Six experiments were conducted in duplicate for each alga to test the growth of the alga. Various concentrations of dairy effluent diluted in distilled water and BG 11 culture media (for *C. fusca* LEB 111) and Zarrouk (for *Spirulina* sp. LEB 18) were used: 0% (control), 25%, 50%, and 100%. The experiments were carried out in autotrophic growth conditions, with a 12 h light/dark photoperiod, for 20 days in 0.5 L Erlenmeyer flasks with a useful volume of 0.4 L and an initial biomass concentration of 0.2 g $L^{-1}$. The cultures were kept in a thermostated chamber at 30 °C with 70 $\mu mol_{photons}$ $m^{-2}$ $s^{-1}$ of light and agitation was achieved by injecting sterile air.

### Biomass recovery

At the end of the cultivations the biomass was recovered by centrifugation at 9000 ×g for 20 min, resuspending the pellet in distilled water and centrifuging again for 20 min under the same conditions for removing salts from the culture medium. The centrifuged biomass was frozen at – 80 °C for 48 h and then lyophilized. Lyophilized samples were stored at – 20 °C for further characterization. The supernatant of the cultures was stored at – 20 ºC for further characterization and use in seed germination tests.

### Microalgal growth

Biomass concentration was determined daily by measuring the culture optical density (OD) in a spectrophotometer at 670 nm and plotting a standard curve of the OD in relation to dry biomass. The maximum biomass productivity ($P_{max}$, mg $L^{-1}$ $day^{-1}$) was determined according to the equation $P_{max} = (X_t - X_0) / (t - t_0)$, where $X_t$ is the biomass concentration (g $L^{-1}$) at time $t$ (day) and $X_0$ is the biomass concentration (g $L^{-1}$) at time $t_0$ (day) (Bailey and Ollis 1986). The pH of the cultures was also determined daily using direct digital pH meter readings (Mettler Toledo FiveGo, Switzerland).

### Effluent characterization and removal efficiency

The dairy effluent used was characterized both before and after the cultivation of *C. fusca* LEB 111 and *Spirulina* sp. LEB 18 with 100% dairy effluent. The following parameters were determined: pH, sedimentable solids, electrolytic conductivity, hardness, turbidity, nitrate, ammonia, chemical and biochemical oxygen demand (COD and BOD), organic phosphorus, phosphate, total phosphorus, and oils and greases (Baird et al. 2017). The removal efficiency was

calculated as the difference between the parameters evaluated before and after microalgae cultivation in the raw effluent (Ji et al. 2012).

### Characterization of microalgal biomass

Protein and carbohydrate analyses were performed at the end of the experiments using biomass extracts prepared with 5 mg lyophilized biomass and 10 mL distilled water using an ultrasonic probe (COLE PARMER CPX 130, USA) to extract the intracellular compounds for 10 min in cycles of 59 s. The total carbohydrate concentration in the biomass was determined using the phenol–sulfuric acid method with glucose as the standard (Dubois et al. 1956). The protein content in the biomass was determined by the colorimetric method of Lowry et al. (1951), using thermal and alkaline pretreatment with sodium hydroxide to solubilize insoluble proteins and quantification with a standard curve of bovine serum albumin. The lipid concentration in the biomass was determined in 10 mg lyophilized biomass using the colorimetric method of Marsh and Weinsteins (1966). This method is based on the extraction of lipids using a chloroform and methanol mix (1:2 v/v) reaching polar and non-polar lipids and quantification with a standard tripalmitin curve. The moisture content was determined by the methodology described by Official Methods of Analysis—AOAC (2000).

### Seed germination test using supernatants

Supernatants from pure dairy effluent (100%) and dairy effluent diluted in Zarrouk and BG 11 media containing *Spirulina* sp. LEB 18 and *C. fusca* LEB 111 were selected to evaluate their potential as biostimulants in the germination of red pear tomato seeds (*Lycopersicon esculentum*). The seeds were sterilized with a 5% sodium hypochlorite solution for 8 min and then rinsed with distilled water. Subsequently, the seeds were treated with culture supernatants and the control condition (distilled water) for 70 min. The germination test was conducted with 50 seeds per treatment, distributed in plastic boxes (240 × 166 × 101 mm) containing 1 kg of vermiculite moistened with 500 mL of distilled water, with four repetitions per treatment. All experiments were carried out in quadruplicate, in a thermostated chamber, at 30 °C and 100 $\mu mol_{photons}\ m^{-2}\ s^{-1}$, with a photoperiod of 12 h light/dark. The boxes were kept closed and moistened daily with distilled water.

The evaluation of germination was conducted at 5, 7, 9, and 11 days after sowing by counting the number of germinated seedlings. The first count was performed on the 5th day after the initiation of the germination test. The germination speed index was calculated by dividing the number of germinated seeds by the number of days elapsed between sowing and germination. After the germination test, the length of the seedlings and roots were determined. The fresh and dry weight of the seedlings was also determined (Brasil 2009).

### Statistical analysis

All analyses were performed in triplicate and evaluated using analysis of variance, followed by Tukey's post-hoc test, to compare the means with a 95% confidence level.

## Results

### Growth of microalgae in dairy effluent

Based on the biomass concentration curves of microalgae *C. fusca* LEB 111 and *Spirulina* sp. LEB 18 (Fig. 1), it

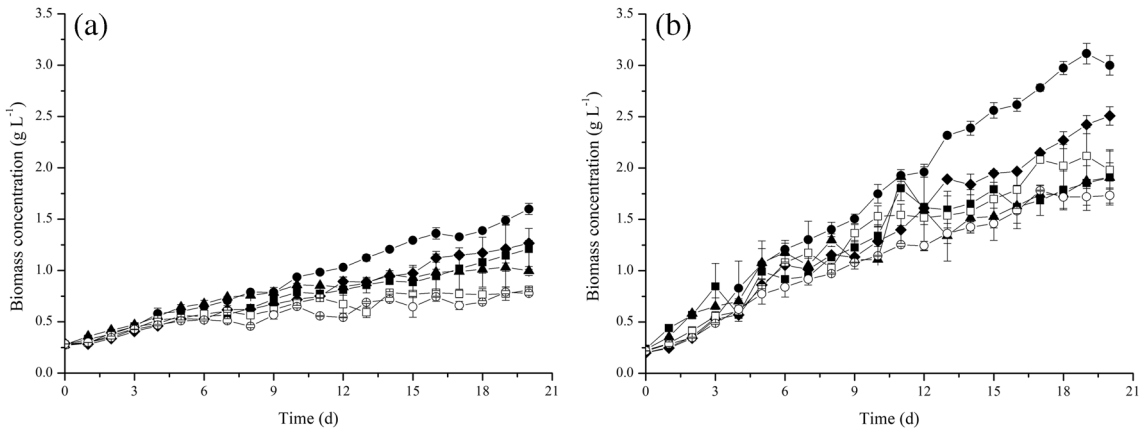


**Fig. 1** Biomass concentration (g $L^{-1}$) during the cultivation of *Chlorella fusca* LEB 111 (**a**) and *Spirulina* sp. LEB 18 (**b**) grown in dairy effluent under the conditions: Control (100% Medium) (♦), 100% Dairy effluent (▲), 50% Dairy effluent/ 50% Medium (■), 25% Dairy effluent / 75% Medium (●), 50% Dairy Effluent/50% Water (□), 25% Dairy Effluent/75% Water (○)

is evident that dairy effluent was conducive to microalgal growth, even when used as the sole culture medium (100% dairy effluent). Furthermore, the microalgae did not display any adaptation phase during the experiments in all tested conditions. In the study by Kumar et al. (2018), raw dairy effluent was also used to cultivate *Ascochloris* sp. ADW007, replacing BG 11 and TAP media, and a significantly higher maximum biomass concentration (2.23 g $L^{-1}$) was obtained compared to the other conditions, indicating the potential of this residue as a microalgae culture medium. However, the microalgae used in their study were already adapted to the effluent as it was isolated from the same. Therefore, this study is noteworthy as the *microalgae Chlorella* and *Spirulina* exhibited $X_{max}$ 1.03 g $L^{-1}$ and 1.98 g $L^{-1}$ and $P_{max}$ 159.70 mg $L^{-1}$ $day^{-1}$ and 187.70 mg $L^{-1}$ $day^{-1}$, respectively, with 100% effluent, without any adaptation phase, even without adaptation to the effluent (Table 1). Moreover, the condition where 25% of effluent and 75% of BG 11 or Zarrouk medium were used proved more favorable for the growth and biomass production of *C. fusca* LEB 111 and *Spirulina* sp. LEB 18, with $X_{max}$ 1.60 g $L^{-1}$ and 3.11 g $L^{-1}$, respectively.

During the cultivation of *Spirulina* sp. LEB 18 in dairy effluent, the pH remained within the range of 10.0 to 11.0, with variations occurring due to microalgal growth. The different concentrations of effluent, culture media, and distilled water did not show a significant change in this parameter. The Zarrouk medium contains sodium bicarbonate as a carbon source, which microalgae use in their carbon assimilation mechanism (Ota et al. 2009). This leads to a pH of around 10 to 10.5. As time progresses, two bicarbonate ions are consumed by the cell, with one internalized in the form of carbon dioxide and the other released in the form of the carbonate, causing the pH of the medium to increase to values greater than 10.5 (Shiraiwa et al. 1993).

The pH of the *C. fusca* LEB 111 cultivation in dairy effluent remained in the range of 8.0 to 11.0, with lower pH values recorded in the first few days and increasing according to the growth of the microalgae, as expected for this species (Duarte et al. 2017). Despite the raw dairy effluent having an alkaline pH of 11.77, it did not affect microalgae growth in any tested conditions. Therefore, adjusting the pH was unnecessary as the effluent's pH did not differ significantly from the media used. Additionally, the experiments were examined daily under an optical microscope, and no contamination was detected until the last day, even though the effluent had not been sterilized before the cultivations.

**Table 1** Maximum biomass concentration ($X_{max}$), maximum biomass productivity ($P_{max}$), maximum specific growth rate ($\mu_{max}$), and generation time (tg) of *Chlorella fusca* LEB 111 and *Spirulina* sp. LEB 18 grown in dairy effluent

| Parameters | *Chlorella fusca* LEB 111 | | | | | |
|---|---|---|---|---|---|---|
| | Control (Medium BG 11) | 100% Effluent | 50% Effluent / 50% Medium | 25% Effluent / 75% Medium | 50% Effluent / 50% Water | 25% Effluent / 75% Water |
| $X_{max}$ (g $L^{-1}$) | $1.27^{b} \pm 0.03$ | $1.03^{c,d} \pm 0.04$ | $1.21^{b,c} \pm 0.27$ | $1.60^{a} \pm 0.05$ | $0.83^{d,e} \leq 0.01$ | $0.79^{e} \pm 0.01$ |
| $P_{max}$ (mg $L^{-1}$ $day^{-1}$) | $83.55^{d} \pm 17.64$ | $159.70^{a} \pm 15.40$ | $127.00^{b} \pm 9.52$ | $106.77^{b,c} \pm 1.95$ | $96.28^{c,d} \pm 11.44$ | $99.34^{c,d} \pm 6.60$ |
| $\mu_{max}$ ($day^{-1}$) | $0.16^{a} \leq 0.01$ | $0.13^{b} \pm 0.01$ | $0.14^{b} \pm 0.02$ | $0.11^{c} \leq 0.01$ | $0.16^{a} \pm 0.01$ | $0.13^{b,c} \leq 0.01$ |
| tg (day) | $4.43^{c} \pm 0.02$ | $5.37^{b} \pm 0.27$ | $5.16^{b} \pm 0.57$ | $6.09^{a} \pm 0.26$ | $4.37^{c} \pm 0.25$ | $5.46^{b} \pm 0.16$ |
| *$R^2$ | $0.99 \leq 0.01$ | $0.98 \leq 0.01$ | $0.99 \pm 0.01$ | $0.98 \leq 0.01$ | $0.98 \leq 0.01$ | $0.98 \leq 0.01$ |
| **Δt (day) | 1–5 | 1–7 | 1–5 | 2–8 | 0–6 | 0–5 |
| Parameters | *Spirulina* sp. LEB 18 | | | | | |
| | Control (Medium BG 11) | 100% Effluent | 50% Effluent / 50% Medium | 25% Effluent / 75% Medium | 50% Effluent / 50% Water | 25% Effluent / 75% Water |
| $X_{max}$ (g $L^{-1}$) | $2.61^{b} \pm 0.24$ | $1.98^{c,d} \pm 0.07$ | $1.97^{c,d} \pm 0.33$ | $3.11^{a} \pm 0.10$ | $2.23^{c} \pm 0.16$ | $1.80^{d} \pm 0.02$ |
| $P_{max}$ (mg $L^{-1}$ $day^{-1}$) | $144.40^{c,d} \pm 15.94$ | $187.70^{a,b,c} \pm 22.52$ | $231.14^{a} \pm 43.04$ | $189.39^{a,b} \pm 30.72$ | $148.01^{b,c,d} \pm 5.22$ | $112.29^{d} \pm 17.98$ |
| $\mu_{max}$ ($day^{-1}$) | $0.29^{b} \pm 0.02$ | $0.29^{a,b} \pm 0.05$ | $0.10^{c} \pm 0.03$ | $0.37^{a} \pm 0.05$ | $0.27^{b} \pm 0.05$ | $0.26^{b} \pm 0.02$ |
| tg (day) | $2.44^{b} \pm 0.21$ | $2.43^{b} \pm 0.41$ | $7.76^{a} \pm 2.33$ | $1.93^{b} \pm 0.29$ | $2.62^{b} \pm 0.37$ | $2.73^{b} \pm 0.26$ |
| *$R^2$ | $0.98 \leq 0.01$ | $0.98 \pm 0.01$ | $0.99 \leq 0.01$ | $0.98 \leq 0.01$ | $0.98 \leq 0.01$ | $0.99 \leq 0.01$ |
| **Δt (day) | 0–6 | 0–5 | 6–10 | 0–5 | 0–7 | 0–7 |

Different lowercase letters superscript on the same line correspond to the significant difference ($p \leq 0.05$) for the same answer. *R2: the coefficient of determination of the linear regression was applied in the logarithmic growth phase. **Δt: initial-end time of the exponential growth phase. Data are presented as the mean ± standard deviation.

### Concentration of macromolecules in *Chlorella fusca* LEB 111 and *Spirulina* sp. LEB 18 grown in dairy effluent

The concentrations of carbohydrates, proteins, and lipids were determined in the biomass of *C. fusca* LEB 111 and *Spirulina* sp. LEB 18 cultured in dairy effluent to verify the variation of these macromolecules when using this alternative culture medium. Carbohydrates (Table 2) were the predominant biomass fraction in *C. fusca* LEB 111 grown with 100% dairy effluent, an increase of 111.5% about the control. In the case of *Spirulina* sp. LEB 18, the condition that most favored the biosynthesis of carbohydrates was with 50% of effluent diluted in 50% of water, resulting in an increase of 455.8% of the control.

Regarding protein concentration, *C. fusca* LEB 111 showed the highest content when cultured with 25% and 50% of effluent diluted in BG 11 medium, with results statistically similar to the control (as shown in Table 2). In the case of *Spirulina* sp. LEB 18, using 25% of effluent and 75% of Zarrouk medium, led to biomass with over 70% protein content, a promising result for agricultural and animal feed applications. Notably, this condition also resulted in a lipid concentration of more than 18%, further supporting the potential use of this biomass as animal feed. In summary, these findings suggest that dairy effluent, when used in appropriate dilutions and media combinations, can promote the biosynthesis of key macromolecules in microalgae, opening up opportunities for sustainable and cost-effective bioproduction.

### Effluent characterization and removal efficiency

The chemical oxygen demand (COD) measurement is used to quantify the amount of organic compounds in wastewater indirectly. Regarding the characterization of the raw effluent used in this study (Table 3), it is evident that this residue has a high organic matter content (BOD 1652.00 mg $L^{-1}$, COD 3840 mg $L^{-1}$), high turbidity (> 1000 NTU), and other characteristics that require treatment to meet the conditions for wastewater release.

### Seed germination test with supernatants of *Spirulina* sp. LEB 18 and *Chlorella fusca* LEB 111 grown in dairy effluent

The germination test conducted on red pear tomato (*L. esculentum*) seeds demonstrated the positive effects of seed treatment with supernatants of *C. fusca* LEB 111 and *Spirulina* sp. LEB 18 grown in dairy effluent. The culture supernatant of *C. fusca* LEB 111 produced with 100% dairy effluent increased seed germination, resulting in 100% germination (Fig. 2, Table 4). Moreover, there was an increase in the count of first germinated seeds, total seed germination, germination speed index, seedling length, root length, fresh and dry matter mass, seedling and root height, and number of leaves in all conditions tested, as compared to the control (water), indicating the potential of these conditions.

## Discussion

All kinetic parameters evaluated in *C. fusca* LEB 111 and *Spirulina* sp. LEB 18 cultivated in dairy effluent under different conditions were higher or statistically equal to those of the controls (Table 1). These findings demonstrate that dairy effluent is a suitable culture medium for the growth of these microalgae, as it provides the necessary nutrients for their growth. The microalga's ability to efficiently absorb nutrients from dairy effluent and produce value-added products makes it a potential and cost-effective method for biomass production (Costa et al. 2021).

Regarding biomass composition, when microalgae are cultured in a medium supplemented with wastewater that

**Table 2** Concentration of carbohydrates, proteins, and lipids in *Spirulina* sp. LEB 18 and *Chlorella fusca* LEB 111 grown in dairy effluent

| Macromolecules | Control | 100% Effluent | 50% Effluent / 50% Medium | 25% Effluent / 75% Medium | 50% Effluent/ 50% Water | 25% Effluent / 75% Water |
|---|---|---|---|---|---|---|
| *Chlorella fusca* LEB 111 | | | | | | |
| Carbohydates (% w/w) | $23.5^{c} \pm 2.1$ | $49.7^{a} \pm 3.1$ | $21.4^{d} \pm 0.7$ | $46.8^{a,b} \pm 5.6$ | $34.6^{b,c} \pm 2.9$ | $35.0^{b,c} \pm 0.5$ |
| Protein (% w/w) | $50.5^{a} \pm 0.3$ | $35.8^{b} \pm 2.8$ | $54.3^{a} \pm 6.3$ | $51.0^{a} \pm 4.1$ | $33.3^{b} \pm 3.6$ | $32.6^{b} \pm 0.7$ |
| Lipids (% w/w) | $26.7^{a} \pm 2.5$ | $12.2^{b} \pm 1.4$ | $16.3^{b} \pm 0.4$ | $5.3^{c} \pm 0.1$ | $13.0^{b} \pm 0.7$ | $12.7^{b} \pm 0.1$ |
| *Spirulina* sp. LEB 18 | | | | | | |
| Carbohydates (% w/w) | $11.1^{e} \pm 0.4$ | $47.7^{d} \pm 0.3$ | $57.6^{b} \pm 0.1$ | $7.5^{f} \pm 0.2$ | $61.7^{a} \pm 0.3$ | $56.1^{c} \pm 0.1$ |
| Protein (% w/w) | $64.4^{a} \pm 3.5$ | $27.2^{c} \pm 1.8$ | $24.1^{c} \pm 0.6$ | $72.6^{a} \pm 3.2$ | $38.6^{b} \pm 2.1$ | $40.2^{b} \pm 4.1$ |
| Lipids (% w/w) | $15.9^{a} \pm 2.2$ | $11.1^{b} \pm 0.1$ | $11.1^{b} \pm 1.2$ | $18.8^{a} \pm 1.3$ | $17.9^{a} \pm 0.3$ | $10.4^{b} \pm 0.4$ |

Different lowercase letters superscript on the same line correspond to the significant difference ($p \leq 0.05$) for the same answer. Results of carbohydrates, proteins, and lipids expressed on a dry mass. Data are presented as the mean ± standard deviation ($n = 3$).

**Table 3** Characterization of raw dairy effluent before and after (culture supernatant with 100% DE) cultivation of *Chlorella fusca* LEB 111 and *Spirulina* sp. LEB 18

| Parameter | Raw effluent | Culture supernatant *C. fusca* LEB 111 | Culture supernatant *Spirulina* sp. LEB 18 | Removal efficiency (%) | |
|---|---|---|---|---|---|
| | | | | *C. fusca* | *Spirulina* |
| Electrolytic conductivity (μS $cm^{-1}$) | 4730.00 ± 3.8 | 5.39 ± 3.8 | 6.54 ± 3.8 | 99.9 | 99.9 |
| BOD (mg $L^{-1}$) | 1652.00 ± 0.20 | 25.6 ± 0.3 | 14.8 ± 0.3 | 98.5 | 99.1 |
| COD (mg $L^{-1}$) | 3840 ± 1 | 133 ± 1 | 87 ± 1 | 96.5 | 97.7 |
| Hardness (mg $L^{-1}$) | 309.360 | 10.931 | 10.055 | 96.5 | 96.7 |
| Phosphate (mg $L^{-1}$) | 13.90 ± 0.01 | 1.42 ± 0.01 | 2.45 ± 0.23 | 89.8 | 82.4 |
| Organic Phosphorus (mg $L^{-1}$) | 52.99 ± 0.06 | 0.60 ± 0.10 | 11.65 ± 0.10 | 98.9 | 78.0 |
| Total Phosphorus (mg P $L^{-1}$) | 66.89 ± 0.001 | 0.82 ± 0.08 | 9.85 ± 0.08 | 98.8 | 85.3 |
| Sedimentable Materials (mL $L^{-1}$) | 23.0 | 0.2 | 0.1 | 99.1 | 99.6 |
| Nitrate (mg $NO_3-$ $L^{-1}$) | 106.40 ± 0.09 | 7.67 ± 1.15 | 7.44 ± 0.09 | 92.8 | 93.0 |
| Ammoniacal Nitrogen (mg $NH_3$ $L^{-1}$) | 5.60 ± 0.08 | 0.10 ± 0.05 | 0.00 ± 0.05 | 98.0 | 99.0 |
| Oils and Greases (mg MSH $L^{-1}$) | 1.092 ± 0.010 | 0.108 ± 0.001 | 0.111 ± 0.001 | 90.1 | 89.8 |
| Turbidity (NTU) | > 1000 ± 0.03 | 4.7 ± 0.003 | 3.8 ± 0.003 | 99.5 | 99.6 |

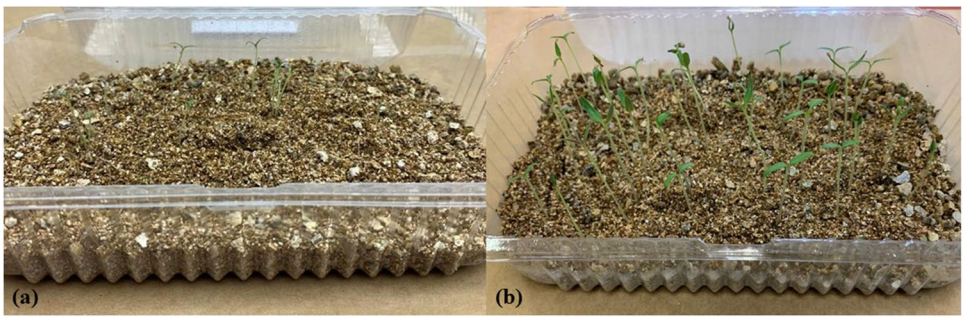


**Fig. 2** Images from the last day of the germination test of *Lycopersicon esculentum* seeds treated with water (**a**—control) and *Chlorella fusca* LEB 111 supernatant grown with 100% raw dairy effluent (**b**)

**Table 4** First count germination (%), Total germination (%), Germination speed index (GSI) (%), Seedling length (SL), Root length (Rl), Fresh plant mass (FM), Dry plant mass (DM), Height of the largest seedling among all repetitions (LS), Length of the longest root among all repeats (LLR), and the number of leaves of the germination test carried out with the supernatants of *Chlorella fusca* LEB 111 and *Spirulina* sp. LEB 18 obtained in cultivation using dairy effluent diluted in BG 11 and Zarrouk media

| Parameters | Supernatant | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control (Water) | *Chlorella fusca* LEB 111 + 25% Effluent + 75% Medium | *Chlorella fusca* LEB 111 + 50% Effluent + 50% Medium | *Chlorella fusca* LEB 111 + 100% Effluent | *Spirulina* sp. LEB 18 + 25% Effluent + 75% Medium | *Spirulina* sp. LEB 18 + 50% Effluent + 50% Medium | *Spirulina* sp. LEB 18 + 100% Effluent |
| First count germination (%) | $21.0^{c} \pm 0.7$ | $28.0^{c} \pm 2.8$ | $46.0^{b} \pm 5.7$ | $60.0^{a} \pm 5.7$ | $21.0^{c} \pm 2.1$ | $52.0^{a,b} \pm 2.8$ | $44.0^{b} \pm 2.8$ |
| Germination (%) | $39.0^{e} \pm 7.8$ | $72.0^{c,d} \pm 5.7$ | $95.0^{a,b} \pm 3.5$ | $100.0^{a} \pm 0.1$ | $60.0^{d} \pm 7.1$ | $78.0^{b,c,d} \pm 1.4$ | $85.0^{a,b,c} \pm 3.5$ |
| GSI (%) | $37.5^{d} \pm 11.9$ | $63.3^{b,c} \pm 13.4$ | $77.1^{a,b} \pm 1.4$ | $94.8^{a} \pm 5.6$ | $51.0^{c,d} \pm 17.2$ | $84.9^{a} \pm 5.3$ | $76.7^{a,b} \pm 3.7$ |
| RL (cm) | $2.88^{b} \pm 0.93$ | $3.38^{b} \pm 0.75$ | $5.25^{a} \pm 0.29$ | $5.00^{a} \pm 0.00$ | $3.88^{b} \pm 0.25$ | $5.00^{a} \pm 0.00$ | $5.50^{a} \pm 0.41$ |
| CR (cm) | $1.63^{b} \pm 0.48$ | $4.50^{a} \pm 1.29$ | $5.75^{a} \pm 0.87$ | $4.50^{a} \pm 1.00$ | $5.75^{a} \pm 0.87$ | $6.00^{a} \pm 0.82$ | $5.38^{a} \pm 1.11$ |
| FM (g) | $0.41^{e} \pm 0.1$ | $0.93^{d} \pm 0.2$ | $1.52^{a} \pm 0.2$ | $1.22^{b} \pm 0.2$ | $1.11^{c} \pm 0.1$ | $1.08^{c} \pm 0.2$ | $1.20^{b,c} \pm 0.2$ |
| DM (g) | $0.06^{d} \pm 0.01$ | $0.10^{c} \pm 0.02$ | $0.11^{b,c} \pm 0.01$ | $0.13^{a,b} \pm 0.01$ | $0.14^{a} \pm 0.01$ | $0.13^{a,b} \pm 0.02$ | $0.11^{b,c} \pm 0.01$ |
| LS (cm) | 4.0 | 4.0 | 5.0 | 6.0 | 4.5 | 5.5 | 5.0 |
| LLR (cm) | 2.0 | 7.0 | 7.0 | 6.5 | 6.0 | 6.5 | 5.0 |
| Number of leaves | $42^{f} \pm 3$ | $74^{d} \pm 2$ | $95^{b} \pm 4$ | $102^{a} \pm 1$ | $60^{e} \pm 3$ | $79^{c,d} \pm 1$ | $87^{b,c} \pm 3$ |

Different lowercase letters superscript on the same line correspond to the significant difference ($p \leq 0.05$) for the same answer. Data are presented as the mean ± standard deviation ($n = 4$).

has a higher nitrogen load, their biomass tends to have higher nitrogen and protein contents. This is because nitrogen is essential for synthesizing amino acids and proteins (Ferreira et al. 2019; Braun and Colla 2022). Using higher proportions of wastewater with alternative nutrient sources can increase the protein percentage in microalgae biomass. Additionally, the increase in nitrogen content may be related to the higher nitrate levels in the medium and the rate of nitrate removal by the microalgae. On the other hand, effluents with higher phosphorus percentages can lead to biomass with higher lipid contents (Baldisserotto et al. 2020). These findings suggest that the nutrient composition of the wastewater used as a culture medium can significantly influence the composition of the microalgae biomass. Therefore, wastewater management and treatment strategies can be crucial in optimizing the production of specific target molecules in microalgae, such as proteins and lipids.

The results regarding the potential use of microalgae biomass in agriculture are once again promising, given that higher protein concentrations in the biomass are necessary for plant growth, as nitrogen is a key component in the process. However, chemical fertilizers can negatively impact the environment due to excess nitrogen lost through denitrification, leaching, or volatilization (Ahmed et al. 2017). In contrast, microalgae-based organic fertilizers can offer several advantages, including the gradual release of nitrogen, phosphorus, and potassium, as well as the presence of plant growth-promoting substances such as phytohormones, vitamins, carotenoids, amino acids, and antifungal compounds (Coppens et al. 2016).

One of the main limitations preventing the incorporation of microalgae into animal feed is the high raw material cost and its limited availability. However, as demonstrated in this study, dairy effluent can provide nutrients for the production of biomass, which can then serve as a raw material for the production of animal feed that is rich in proteins, fatty acids, antioxidants, antimicrobial compounds, and other essential compounds that can prevent diseases and prolong the animals' lifespan. Furthermore, this biomass can be used for extracting compounds such as pigments and antioxidants, which can be purified and utilized in the food and pharmaceutical industries (Dineshbabu et al. 2019; Debeni Devi et al. 2023).

As previously mentioned, there is a need to reduce the production costs of microalgae biomass to enable their biotechnological application, including the *Chlorella* species (Andrade and Andrade 2017). Therefore, the use of *C. fusca* LEB 111 and *Spirulina* sp. LEB 18 could be implemented by dairy industries located near effluent treatment plants. By doing so, in addition to generating additional income, the company would also reap environmental benefits related to water quality improvement and carbon dioxide assimilation.

After cultivating microalgae using raw dairy effluent, the culture supernatant was characterized to determine the removal efficiency of pollutants. Iliopoulou et al. (2022) reported that contaminants in dairy effluent decrease over time during microalgae cultivation, indicating that pollutant consumption is proportional to species growth. This phenomenon was also observed in our study (Table 3), as all pollutants present in the raw effluent were reduced after 20 days of cultivation, confirming the potential of this medium as an alternative for the cultivation of *C. fusca* LEB 111 and *Spirulina* sp. LEB 18.

The cultivation of *C. fusca* LEB 111 and *Spirulina* sp. LEB 18 proved to be an excellent biological treatment for this effluent, exhibiting a removal efficiency greater than 95% for COD and BOD. The reduction in COD by more than 95% demonstrated that the microalgal cells effectively utilized the organic carbon as a substrate for their growth and energy source, producing biomass rich in macromolecules and facilitating the treatment of the raw effluent. The BOD reduction achieved in this study complies with the resolution of the effluent discharge conditions of the 2011 National Environment Council—CONAMA, which mandates a minimum removal of 60% BOD from the effluent.

Microalgae can remove all forms of nitrogen present in wastewater. In microalgae, the assimilation of nitrate involves two transport and two reduction steps: first, nitrate ($NO_3^-$) is transported into the cell, then the cytosolic nitrate reductase enzyme catalyzes the reduction of nitrate to nitrite, which is subsequently transported to the chloroplast, where the nitrite reductase enzyme catalyzes its reduction to ammonium (Lachmann et al. 2019). Finally, ammonium is metabolized (Miflin and Lea 1975). The nitrogen in the effluent, in the form of nitrate, was metabolized by microalgae with a removal efficiency of over 90%. Cultivation led to the total removal of ammoniacal nitrogen (100%). According to Kumar et al. (2010), ammoniacal nitrogen is the preferred source for microalgae as it is directly metabolized, requiring much less energy for its absorption.

Similarly, microalgae efficiently removed phosphorus, an essential nutrient for their growth and several cellular processes, with removal efficiencies of over 98.8% for *C. fusca* LEB 111 and 85.3% for *Spirulina* sp. LEB 18. Yaakob et al. (2021) reported that phosphorus is essential in microalgae cultivation, especially in the phosphate form, which is preferred for microalgal uptake and nucleic acid biosynthesis. In the present study, 89.8% and 82.4% phosphate removal efficiencies were achieved in the cultivation of *C. fusca* LEB 111 and *Spirulina* sp. LEB 18, respectively.

Kumar et al. (2019) conducted a study on treating raw dairy effluent using microalgae *Ascochloris* sp. ADW007. After treating the effluent, the results showed a reduction of 94–96% in COD, 72–80% in nitrate, and 80–97% in total phosphate. In a similar study Hamidian and Zamani (2022)

cultivated *Chlorella sorokiniana* in dairy wastewater and observed a reduction of 59.65% in BOD, 57.17% in COD, 78.82% in nitrate, and 88.17% in phosphate. These results indicate that dairy effluent can be an alternative medium for cultivating several species of microalgae, and the treated effluent and biomass can be utilized in various applications such as animal feed, agriculture, pigments, and biofuels (Costa et al. 2021).

Electrical conductivity is a measurement carried out in effluent to monitor the presence of dissolved substances or impurities in water. Pure water typically exhibits a conductivity of around 5 mS $cm^{-1}$ (Atlas Scientific 2023). The greater the impurities present in the water, the higher the conductivity. In this study, a removal efficiency of 99.9% in electrical conductivity was achieved, indicating the ability of microalgae to remove impurities from the wastewater.

The raw dairy effluent had a sedimentable material concentration of 23.00 mL $L^{-1}$ before cultivation. According to the CONAMA resolution, the release condition should be 1 mL $L^{-1}$ of sedimentable materials in a 1-h test using an Inmhoff cone. After cultivation, a removal efficiency of more than 99% was achieved for this parameter. The treated effluent consisting of a culture supernatant with 100% effluent from the microalgae *C. fusca* LEB 111 and *Spirulina* sp. LEB 18, exhibited sedimentable material concentrations of 0.2 and 0.1 mL $L^{-1}$, respectively.

Based on the characterization of the dairy effluent before and after microalgae cultivation (Table 3), it is evident that although the microalgae *C. fusca* LEB 111 and *Spirulina* sp. LEB 18 exhibit excellent nutrient removal efficiency; nutrients remain in the treated effluent, such as nitrogen and phosphorus, which are essential for plant germination and growth, as demonstrated in this study. Furthermore, microalgae can synthesize phytohormones such as auxins and cytokinins during their growth and release them into the culture medium (Ahmed et al. 2010). Therefore, despite obtaining treated effluent through microalgae cultivation, residual nutrients and essential substances for seed germination, such as nitrogen, phosphorus, and phytohormones such as indoleacetic acid, may remain, depending on the harvesting method used, which justifies the results reported in this study (Table 4).

The results obtained in the germination test of this study were promising since the supernatant of microalgae cultivation is often discarded without any application. The treated dairy effluent was used for tomato seed germination, resulting in excellent germination parameters (Table 4). The term "biostimulant" is used in the literature to refer to agents that promote plant development and growth, including products containing amino acids, microbial inoculants, and microalgae extracts. Biostimulants are known to act on plant physiology, increasing crop yields and improving resistance to abiotic and biotic stresses (Braun and Colla 2022). Therefore, this study developed a new type of biostimulant produced from microalgae grown in effluents with potential use in agriculture to replace synthetic products for seed treatment. In addition to using a residue in the cultivations to replace traditional media, reducing their costs, biomass can be used for other purposes, such as the production of different types of biofertilizers, animal feed, biofuels, or even for the extraction of biocompounds with high added value, such as pigments (Costa et al. 2021).

## Conclusion

The microalgae *Chlorella fusca* LEB 111 and *Spirulina* sp. LEB 18 were able to grow in dairy effluent (DE), showing that it can be used as an alternative source of nutrients in microalgae cultivation. After the cultivations, treated effluent was obtained with BOD removal efficiency of 98.5% and 99.1%, COD 96.5% and 97.7%, total phosphorus 98.8% and 85.3%, ammonia nitrogen 98% and 99%, for *C. fusca* LEB 111 and *Spirulina* sp. LEB 18, respectively. In addition, all parameters of conditions for releasing effluents into water bodies were achieved, showing the potential of this biological treatment for dairy effluents. In the condition in which 100% of effluent was used, maximum biomass concentrations of 1.03 g $L^{-1}$ and 1.98 g $L^{-1}$ and maximum biomass productivity of 159.70 mg $L^{-1}$ $day^{-1}$ and 187.70 mg $L^{-1}$ $day^{-1}$ for *Chlorella* and *Spirulina*, respectively. The condition in which 25% of effluent and 75% of BG 11 or Zarrouk medium were used proved to be more favorable to the growth and biomass production of *C. fusca* LEB 111 and *Spirulina* sp. LEB 18, showing $X_{max}$ 1.60 g $L^{-1}$ and 3.11 g $L^{-1}$. In this condition, the biomass of *Spirulina* sp. LEB 1 with 72.6% protein. Furthermore, the *C. fusca* LEB 111 supernatant grown at 100% DE increased seed germination, contributing to 100% germinated seeds. All supernatants used positively influenced germination, seedling and root length, fresh and dry weight of the plant, and the number of leaves of tomato plants. Thus, this study offers an alternative approach to microalgae cultivation using dairy effluent as a sustainable medium, producing biomass with high potential for use in agriculture and animal feed, as well as treated effluent for sustainable agriculture.

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**Authors' contributions** **Camila Gonzales Cruz:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing—original draft. **Ana Priscila Centeno da Rosa:** Conceptualization, Validation, Formal analysis, Investigation, Writing—review and editing, Supervision. **Brenda Rafaela Strentzle:** Methodology, Validation,

Formal analysis, and Investigation· **Jorge Alberto Vieira Costa:** Conceptualization, Resources, Writing—review and editing, Supervision, Funding acquisition.

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## Declarations

**Competing interests** The authors confirm that there are no conflicts of interest/Competing interests.

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