

## Dual-purpose system for microalgae cultivation reusing dairy industry wastewater

### Sistema de doble propósito para el cultivo de microalgas reusando aguas residuales de la industria láctea

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## Abstract

**Keywords:** Kinetics growth; COD; lipids; proteins.

A dual-purpose system is a technique that increases microalgae biomass production using wastewater as a nutrient source. This work evaluated a dual-purpose system using dairy industry wastewater (DWW) and synthetic mineral medium to increase microalgae biomass yields. Kinetics growth, lipids, proteins, chlorophyll, and nutrient consumption were determined in combinations of bold-basal medium (BBM), and water-wastewater (DWW) medium. The combinations were 40DWW-60TW and 40DWW-60BBM, and the biomass yields were 1.47 g/L and 2.46 g/L, with a COD removal of 85% and 71%, respectively. The tests tried in 4 L flat plate photobioreactors showed that 40DWW-60BBM formulation increased the biomass to 1.65 g/L, with a COD removal of 98%.

## Resumen

**Palabras clave:** Cinética de crecimiento; DQO; lípidos; proteínas.

Un sistema de doble propósito es una técnica que incrementa la producción de biomasa de microalgas utilizando aguas residuales como fuente de nutrientes. Este trabajo evaluó un sistema de doble propósito utilizando aguas residuales de la industria láctea (DWW, por sus siglas en inglés) y un medio mineral sintético para aumentar los rendimientos de biomasa de microalgas. Se determinaron la cinética de crecimiento, los lípidos, las proteínas, la clorofila y el consumo de nutrientes en combinaciones de medio basal bold (BBM, por sus siglas en inglés) y medio de agua-aguas residuales (DWW). Las combinaciones fueron 40DWW-60TW y 40DWW-60BBM, y la producción de biomasa fue de 1.47 g/L y 2.46 g/L, con una eliminación de DQO del 85% y 71%, respectivamente. Las pruebas realizadas en fotobiorreactores de placa plana de 4 L mostraron que la formulación 40DWW-60BBM aumentó la biomasa a 1.65 g/L, con una eliminación de DQO del 98%.

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## Introduction

Environmental pollution is mainly due to industrialization and inadequate management of resources. Hence, the current global trend focuses on the development of sustainable and environmentally friendly processes to reduce the environmental impact (Stichnothe, 2019). One of the alternatives is the integrated management of waste resources for production of biomass using low cost and environmentally friendly strategies. Based on this approach, the concept of dual-purpose systems was developed in 2012 for biomass production and as a bioremediation technology to control the environmental deterioration (Olguín, 2012).

Biomass is a very important source of raw materials for a wide variety of industries (Hemalatha *et al.*, 2019); specifically, microalgal biomass is a source for food supplements, biofuels, chemicals, and fertilizers (Eppink *et al.*, 2019). Microalgae are fast-growing photosynthetic microorganisms that capture carbon dioxide, release oxygen, and consume nutrients from wastewater and water bodies for their biomass production. Moreover, microalgae do not compete against agricultural crops, and all their biomass can be used (Katiyar *et al.*, 2017). According to the dual-purpose approach, the use of agro-industrial, municipal, and high organic content wastewater as culture media for microalgae cultivation not only helps to reduce the production cost but it also allows to recover resources from wastewater by returning nutrients back to the production chain (Olguín, 2012).

The most used wastewater effluents for microalgae cultivation are municipal wastewater, digestates from pig manure, and dairy wastewater, among others. This is due to their high availability, organic content, and nutrients content (Li *et al.*, 2019). The dairy industry generates about 2 L to 10 L of wastewater per 1 L of processed milk, but dual-purpose approach offers a sustainable way of treating these industrial effluents (Hemalatha *et al.*, 2019; Raghunath *et al.*, 2016).

Although there are many reports on the cultivation of isolated stains—native and non-native consortia of microalgae in wastewater (Li *et al.*, 2019; Qin *et al.*, 2016), including dairy industry wastewater (Abraham *et al.*, 2023; Loganathan *et al.*, 2020)—, the reports on biomass yield vary. In some cases, native microalgae consortia showed higher biomass production than isolated microalgae strains when grown on wastewater (Kumar & Singh, 2020; Moreno-García *et al.*, 2019). However, there are few reports on the use of cultivation of native or non-native microalgae consortia in wastewater and standard synthetic media combined medium (Olguín *et al.*, 2015; Wang *et al.*, 2020b).

Hence, it is important to test different combinations to provide the best nutritional conditions for the cultivation of native consortia of microalgae (Gouveia *et al.*, 2017). In addition, most of the studies about cultivation of microalgae using wastewater as a culture medium are focused on obtaining biomass rich in lipids for biofuels. Nevertheless, the accumulation of lipids in microalgae occurs under nutritional stress, which results in low biomass productivity (Barsanti & Gualtieri, 2007). A different approach to improve the nutritional and growth conditions for the microalgae cultivation in medium with residual effluents would be to favor the accumulation of protein and use of microalgal biomass as biofertilizer.

In this work, a dual-purpose systems approach was used, where optimum proportion of dairy industry wastewater and synthetic mineral medium was determined to obtain a high yield of microalgae biomass using a native consortium from the Bajío region of Mexico.

## Materials and methods

### Microalgae consortium (MC) collecting zone and adaptation conditions

The MC was collected from "Neutla Dam" located in Comonfort (20° 42' 33.5" N; 100° 51' 48.5" W), Guanajuato, Mexico. The MC was adapted for three months using bold basal mineral medium (BBM-3N) (Barsanti & Gualtieri, 2007). The following growth conditions were maintained: 25 °C, an initial pH of 7.0, aeration (0.03 vvm), and a photoperiod of 16 h with LED lights (Osram Tube Led Superstar, Múnich, Germany) with 3000 lux (346  $\mu\text{mol photon/m}^2\cdot\text{s}$ ) and 8 h in darkness. To improve the growth of MC, the BBM was modified by adding 0.075 g of  $\text{NaNO}_3/\text{L}$ .

### Morphological identification

The adapted MC was observed using an optical microscope (Carl Zeiss, Microimaging GbmH 37081, Germany), and the observed microalgae were compared with the culture collection of the University of Texas (UTEX) and identified as *Chlorella* sp., *Chlamydomonas* sp., *Pediastrum duplex*, *Scenedesmus* sp., *Closteriosis* sp., *Schroederia* sp., and *Spirulina* sp. (University of Texas [UTEX], 2025).

### Wastewater collection and analyses

The dairy wastewater (DWW) was collected from the dairy industry GRUPO CUADRITOS<sup>®</sup>, located nearby Celaya (20° 37' 56.5" N; 100° 47' 04.8" W), Guanajuato, Mexico. The DWW was filtered using 25  $\mu\text{m}$  membranes to remove larger suspended solids. The pH, chemical oxygen demand (COD, mg/L) (HACH, 2014), phosphate ( $\text{PO}_4\text{-P}$ , mg/L) (HACH, 2017), nitrate ( $\text{NO}_3\text{-N}$ , mg/L) (Alef, 1995), and ammoniacal nitrogen ( $\text{NH}_4\text{-N}$ , mg/L) were determined in DWW by distillation-titration method (Table1) (Saha *et al.*, 2012). Since the main nitrogen source in DWW and in BBM-3N was  $\text{NO}_3\text{-N}$ , the effect of the concentration of  $\text{NO}_3\text{-N}$  as a nitrogen source was evaluated only in the second experimental phase.

Table 1. Characteristics of dairy wastewater (DWW) used.

Parameter	Characteristics
pH	10.9
COD (mg/L)	2441.6
$\text{PO}_4\text{-P}$ (mg/L)	40.0
$\text{NO}_3\text{-N}$ (mg/L)	287.0
$\text{NH}_4\text{-N}$ (mg/L)	7.2

Source: Author's own elaboration.

### Experimental phases

The experimental process was divided into two phases. In the first phase, consumption of nutrients and cell density were analyzed, whereas in the second phase lipids and protein production were studied. This method helps to understand the behavior of the system on a larger scale (Gouveia *et al.*, 2017; Hena *et al.*, 2018).

## Experimental phase I

During the first experimental phase, two combinations of culture media were evaluated: DWW-Tap Water (TW) and DWW-BBM-3N. Each one of these combinations were tested in four treatments, varying the ratios of DWW-TW and DWW-BBM-3N, but under a constant inoculum concentration. The growth conditions of MC were those used during the adaptation period: 25 °C, 7.0 initial pH, aeration (0.03 vvm), photoperiod of 16 h light (346  $\mu\text{mol photon/m}^2\cdot\text{s}$ ) and 8 h darkness. Schott bottles (1 L) with 500 ml of culture medium was used as reactor and constant stirring was maintained. The growth time of 10 d was based on the behavior of MC observed in the adaptation phase. During the experiment, the growth kinetics (Álvarez-Díaz *et al.*, 2015), pH, and chlorophyll contents were determined daily (Ritchie, 2006). The morphological characters of the MC were observed on every third day as mentioned before. Moreover, nitrate (HACH, 2015), COD (HACH, 2014), and TKN (HACH, 2019) consumption were determined at the beginning and end of each batch. A schematic diagram of experimental phase I and the identification code of the treatments are presented in Figure 1.

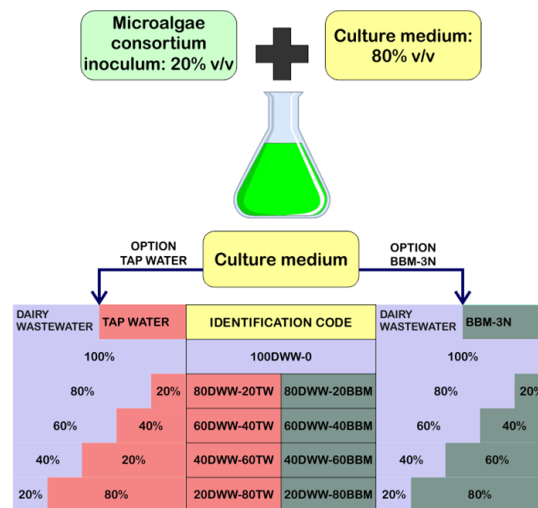


Figure 1. Treatments evaluated in phase I and identification code.  
Source: Author's own elaboration.

## Experimental phase II

In this phase, the treatments, which recorded the highest cell density, and the maximum removal of nutrients and organic load in phase I were selected: viz., 40DWW-60TW, and 40DWW-60BBM. The operational conditions employed in phase I was maintained, but flat-plate photobioreactors (4 L) were used and inoculated with consortia at the rate of 20% (v/v). As a control treatment, BBM-3N (100%) was used. The main features of the transparent acrylic flat-plate photobioreactors are shown in Figure 2. Growth kinetics, chlorophyll content, pH, COD removal, and nitrate consumption were measured. Additionally, the lipids and proteins accumulated at the end of growth phase were estimated.

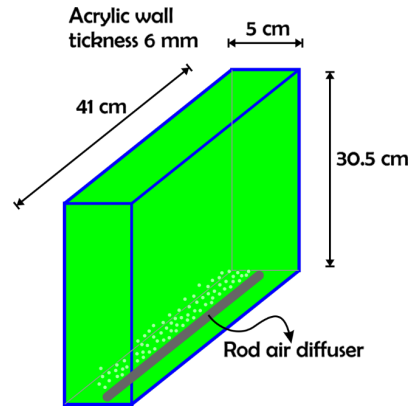


Figure 2. Dimensions and configuration of flat-plate photobioreactor used during the experimental phase II.  
Source: Author's own elaboration.

## Growth kinetics of MC

The growth kinetics of MC was studied by correlating the dry weight (g/L) with the cell density to a linear regression ( $R^2 = 0.9508$ ) (Sarrafzadeh *et al.*, 2015). The cell density was measured at 680 nm, as reported by Álvarez-Díaz *et al.* (2015) using a spectrophotometer (HACH, DR 3900, USA).

## Chlorophyll determination

The total chlorophyll (Chl-a + Chl-b) in green microalgae was determined according to Ritchie (2006). The sample of 1.5 ml was taken in an eppendorf tube and centrifuged (Thermo SCIENTIFIC®, MYSPIN12) at 8860 g for 12 min. The pellet was resuspended with distilled water and centrifuged again at 4430 g for 6 min. The obtained pellet was resuspended in 0.75 ml of methanol for 24 h at 4° C. After incubation, the solution was centrifuged at 8860 g for 12 min, and the supernatant was used for the determination of Chl-a and Chl-b ( $\mu\text{g/ml}$ ) at 652 nm and 665 nm, respectively (equations 1 and 2).

$$\text{Chl a} \approx -8.0962 \times A_{652} + 16.5169 \times A_{665} (\pm 0.04696 \mu\text{g/ml}) \quad (1)$$

$$\text{Chl b} \approx 27.4405 \times A_{652} - 12.1688 \times A_{665} (\pm 0.05776 \mu\text{g/ml}) \quad (2)$$

## Nitrate consumption and COD concentration

Nitrate ( $\text{mg NO}_3\text{-N/ml}$ ) was determined using the commercial kit (NitraVerX Nitrogen-Nitrate Reagent Set, HR, HACH 2605345) in a UV-Vis spectrophotometer HACH (DR3900) at 410 nm (HACH, 2015). Nitrate consumption (%) was calculated by following equation 3:

$$\text{Nitrate removal \%} = \frac{\text{Initial nitrate concentration} - \text{Nitrate concentration in time (t)}}{\text{Initial nitrate concentration}} \times 100 \quad (3)$$

The COD was determined using the kit COD HR (20-1500 mg/L, HACH, method 8000) and was measured at 435 nm (HACH, 2017) using a UV-Vis spectrophotometer HACH (DR3900).

## Lipid content determination

The lipids concentration of the MC was calculated by following the Bligh-Dyer method, according to Feng *et al.* (2011). A stock solution of chloroform:methanol (1:2, v/v) mixture was used as solvent. 50 mg of sample and 3.75 ml of stock solution were added into a tube and mixed for 1 min (vortex). Subsequently, 1.25 ml of chloroform was added and stirred for 30 sec. The samples were centrifuged for 5 min at 7383 g. Then, the chloroform phase located at the bottom was transferred to a constant weight tube. Finally, the solvent of the tube was evaporated in an oven (FELISA, FE-293, Mexico) at 60 °C for 30 min, and finally the weight of the tube was recorded. The lipid percentage was calculated using equation 4:

$$\text{Lipids content (g/100g)} = \frac{V_t m_{dl}}{V_C m_i} \quad (4)$$

where  $m_i$  is the initial sample weight,  $m_{dl}$  is dry lipids weight,  $V_t$  is total volume of chloroform added to the sample, and  $V_C$  is chloroform volume transferred.

## Estimation of total nitrogen (TKN) and protein content

Dried biomass was used for determination of TKN and protein. MC biomass was centrifuged at 4430 g for 10 min in Falcon tubes (15 ml), and the sedimented biomass was washed three times with distilled water and finally dried in an oven (FELISA, FE-293, Mexico) at 80 °C for 24 h. The total nitrogen (TKN) was carried out by Kjeldahl method with Nessler reagent using an UV-Vis spectrophotometer HACH (DR3900) at 460 nm (HACH, 2019). The TKN was calculated according to equation 5, and protein in percentage was determined by multiplying TKN (%) with a factor of 6.25 (Association of Official Analytical Chemist [AOAC], 1980):

$$\frac{A*75}{B*C} = \frac{mg}{Kg} TKN \quad (5)$$

where:  $A$  is spectrophotometer reading in mg/L, according to method 8075 for TKN available in the software of the spectrophotometer HACH (DR3900);  $B$  is digested sample weight; and  $C$  is total volume in the digestion tube in ml.

## Results and discussion

The increase in biomass was determined by linear correlation between biomass concentration (g/L) and optical density (Sarrafzadeh *et al.*, 2015), and the results obtained agreed with those previously reported by Santos-Ballardo *et al.* (2015). The linear correlation was:

$$y = 0.6805x + 0.000095 \quad (6)$$

## Experimental phase I

In experimental phase I, four variables mentioned in the methods section were determined to select the most favorable nutritional conditions for growth and nutrient intake (Figure 3). In a typical composition of DWW,  $\text{NH}_4\text{-N}$  is the main nitrogen source instead of  $\text{NO}_3\text{-N}$  (HACH, 2019); however, it could be observed that  $\text{NO}_3\text{-N}$  is the main nitrogen source in DWW (Table 2) and BBM-3N (Barsanti & Gualtieri, 2007); hence, only  $\text{NO}_3\text{-N}$  was measured in our experiment. Further, the concentration of  $\text{NO}_3\text{-N}$  was almost 40 times bigger than  $\text{NH}_4\text{-N}$  in this work. There are some previous reports where content of  $\text{NO}_3\text{-N}$  is bigger than  $\text{NH}_4\text{-N}$ , especially in wastewater from milk production and processing (Carvalho *et al.*, 2013; Tricolici *et al.*, 2014).

Table 2. Equations used to determine relative growth percentage and relative chlorophyll production; variables analyzed in the radial graphs.

<b>Relative growth</b>	
$x = \frac{\text{Biomass concentration} * 100}{2.46}$	% $\text{NO}_3\text{-N}$ consumption
<b>Relative chlorophyll production</b>	
$x = \frac{\text{Chlorophyll concentration} * 100}{14.81}$	% COD consumption

Source: Author's own elaboration.

The results obtained for the treatments 40DWW-60TW and 40DWW-60BBM are shown in Figures 3a and 3b. The maximum values recorded for relative growth (%) and relative chlorophyll production (%) were used as indicators to choose the best growth conditions. It was also observed that the selected treatments resulted in significant decrease of organic load, which is important for any treatment/bioremediation process. Interestingly, the nitrogen consumption of 40DWW-60BBM did not show a significant decrease, but the growth and photosynthetic activity was superior to all other treatments.

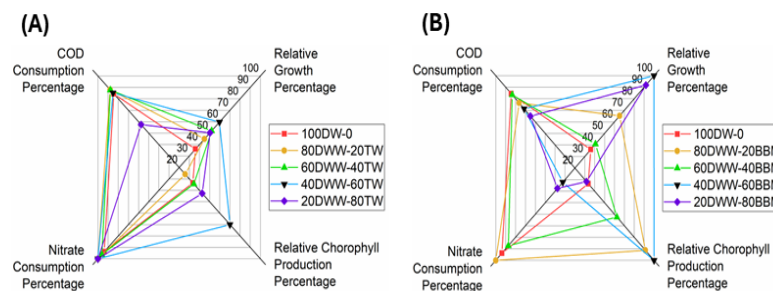


Figure 3. Results of the experimental phase I with dairy wastewater-tap water combined treatments (A) and with dairy wastewater-synthetic medium combined treatments (B).

Note. Black triangles and blue solid line represent the best results. Square contour line corresponds to an increase of 10% for specific variable.

Source: Author's own elaboration.

This indicated that the microalgae consortium found an optimum condition favoring their growth, from which can be inferred that it would result in higher protein yield. Based on the results of this experimental phase I (Table 3 and 4), 40DWW-60TW and 40DWW-60BBM were chosen for the experimental phase II, and a similar approach was used in previous reports (Gouveia *et al.*, 2017; Hena *et al.*, 2018).

It can be observed that the 40DWW-60BBM treatment recorded the highest concentration of biomass and chlorophyll (Table 3 and 4). In the case of the consumption of nitrates and COD removal, the tap water treatments recorded the best results (Table 3 and 4). It was also observed that there was no total nitrate consumption at the end of the study in 40DWW-60BBM, which indicated nutritional sufficiency (Table 3 and 4).

Table 3. Results of the treatment DWW-TW in phase I.

Parameters	Percentual Volumetric Proportion (DWW-TW)				
	100-0	80-20	60-40	40-60	20-80
Biomass (g/L)	0.89	1.11	1.28	1.47	1.24
Chlorophyll ( $\mu\text{g/mL}$ )	4.97	3.85	5.11	10.37	6.32
Initial $\text{NO}_3\text{-N}$ (mg/L)	27.2	25.2	20.9	20.4	17.7
Final $\text{NO}_3\text{-N}$ (mg/L)	1.7	0	0.8	0	0
Initial COD (mg/L)	2111	2455	1915	1321	944
Final COD (mg/L)	329	293	235	196	402

Source: Author's own elaboration.

Table 4. Results of the treatment DWW-BBM-3N in phase I.

Parameters	Percentual Volumetric Proportion DWW-BBM				
	100-0	80-20	60-40	40-60	20-80
Biomass (g/L)	0.89	1.60	1.00	2.46	2.26
Chlorophyll ( $\mu\text{g/mL}$ )	4.97	13.52	9.25	14.81	4.70
Initial $\text{NO}_3\text{-N}$ (mg/L)	27.2	27.5	40.2	68.9	64.8
Final $\text{NO}_3\text{-N}$ (mg/L)	1.7	0	5.1	46.7	40.4
Initial COD (mg/L)	2111	2180	1676	1330	667
Final COD (mg/L)	329	515	279	382	234

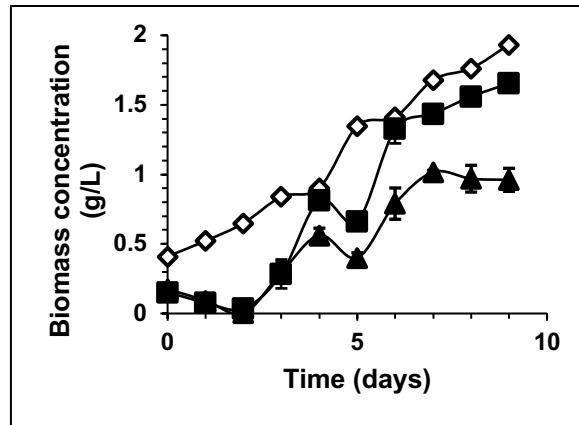
Source: Author's own elaboration.

In previous reports on the combination of different wastewaters—municipal, anaerobic digestates, industrial and agro-industrial (Ling *et al.*, 2014; Lu *et al.*, 2016; Luo *et al.*, 2019; Wang *et al.*, 2020a; Yu *et al.*, 2019)—, DWW is considered poor in their nitrogen content and is supplemented with effluents with high nitrogen concentration such as anaerobic digestates (Lu *et al.*, 2016). This option was not considered in this study since the combination of residual effluents increases turbidity and could affect the growth conditions and analytical process. Further, there are few reports on the use of wastewater-mineral synthetic medium combinations for biomass production, therefore we chose to supplement the DWW with BBM-3N for this phase of study.

## Experimental phase II

### Consortium growth

The algal growth rate is related to the consumption of nutrients, removal of the organic load of the DWW, and operating conditions (Choi, 2016). The maximum cell biomass concentration was recorded in the control treatment (100% BBM 3N), followed by treatment 40DWW-60BBM (DWW/medium BBM-3N) (Figure 4). On the other hand, the 40DWW-60TW treatment reached its maximum concentration between day 8 and 9, and then it declined, which could be due to the limitation of nutrients caused by dilution with tap water (Figure 4).



**Figure 4.** Growth kinetics of the algal consortia (MC) during experimental phase II.

Note. Control (diamonds), treatment 40DWW-60TW (solid triangles), treatment 40DWW-60BBM (solid squares).

Source: Author's own elaboration.

In the present study, based on the monitoring of biomass through microscopical study, it can be inferred that the consortium, in total, recorded better results than monocultures with DWW as culture medium. Through microscopy, it was possible to see the dynamics of the different species and was able to appreciate the dominance of some species over others. Qin *et al.* (2016) reported that algae consortium reached 5.41 g/L of biomass, while *Chlorella* sp. as monoculture showed a growth of 4.72 g/L. According to Pires *et al.* (2013), the high biomass production from MC is caused by the dynamics within the species of the consortium; a decrease in some species is compensated by an increase in growth of some other microalgae species. Further, the demand of nutrients is equilibrated because of a complementation between the different microalgae species of the consortium.

In the adaptation stage, the prevalent population were *Chlorella* sp., *Chlamydomonas* sp., *Closteriosis* sp., *Pediastrum duplex*, *Scenedesmus* sp., and *Schroederia* sp. When these were grown in DWW, *Pediastrum duplex* disappeared completely and the dominant species were *Chlamydomonas* sp. and *Chlorella* sp., while *Scenedesmus* sp., *Schroederia* sp., and *Closteriosis* sp. decreased their population considerably. Moreover, the DWW used was not sterilized, so the existence of microbiota of DWW, which are in cooperation and/or competition with microalgae, cannot be denied. However, the nutritional and operational conditions are defined to favor the growth of the MC. Chinnasamy *et al.* (2010) reported the use of a native MC with a biomass production of 1.47 g/L on day 9, cultivated in DWW with an increased CO<sub>2</sub> (6%). Besides this, Daneshvar *et al.* (2018b) reported a maximum concentration of 0.47 g/L of *Scenedesmus quadricauda* monoculture in DWW. It can be implied from these results that the biomass concentration obtained in this study is similar to the previous reports.

In the 40DWW-60BBM treatment, the addition of BBM-3N enriched the medium with micro and macronutrients and provided optimum conditions to achieve the maximum microalgae growth recorded in this study.

## Total chlorophyll concentration

The total chlorophyll concentration (chlorophyll a and b, for green microalgae) is related to the photosynthetic activity (Ritchie, 2006). The lowest concentration was obtained in 40DWW-60TW treatment (Figure 5), while 40DWW-60BBM presented the highest chlorophyll concentration on day 7 and then declined. These results demonstrated that the MC employed in this study efficiently used both the organic and inorganic carbon sources for their growth (Chen *et al.*, 2010).

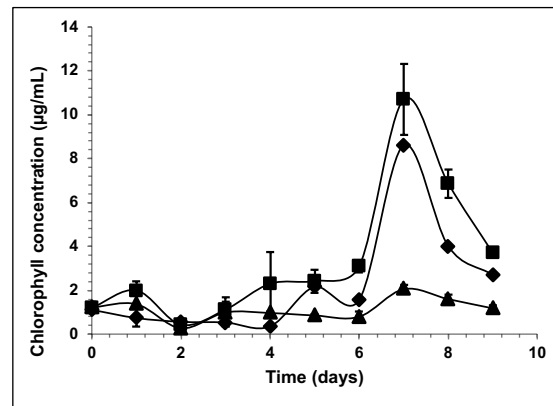


Figure 5. Total chlorophyll concentration during experimental phase II.

Note. Control (diamonds), treatment 40DWW-60TW (solid triangles), treatment 40DWW-60BBM (solid squares).

Source: Author's own elaboration.

Ritchie (2006) reported that the maximum concentration of chlorophyll ranged from 20 µg/ml to 30 µg/ml; and based on these values, the chlorophyll concentrations obtained in the present study were low (Figure 5). This could be attributed to the limited passage of light due to the addition of DWW, which increased turbidity and negatively impacted the photosynthetic process. The results of study on chlorophyll production (Figure 5) were similar to those reported by Chen *et al.* (2010).

## Consumption of nitrate and COD

Consumption of nitrate and COD in experimental phase II is presented in Figure 6. In the case of 40DWW-60TW treatment, the maximum consumption of nitrate was observed on the fifth day of growth. But, the 40DWW-60BBM treatment also showed a high consumption of  $\text{NO}_3\text{-N}$  during the first 4 d of study, which later increased to almost 100%. Consumption of COD was similar to nitrates for treatments (40DWW-60TW and 40DWW-60BBM). The highest percentage of COD consumption was reached on day 6 for both 40DWW-60TW and 40DWW-60BBM. The high biomass obtained in the present study could be related to the consumption of nitrate and COD of DWW. Earlier, Choi (2016) pointed out a linear correlation between nutrient consumption and biomass production for *Chlorella vulgaris* grown in DWW.

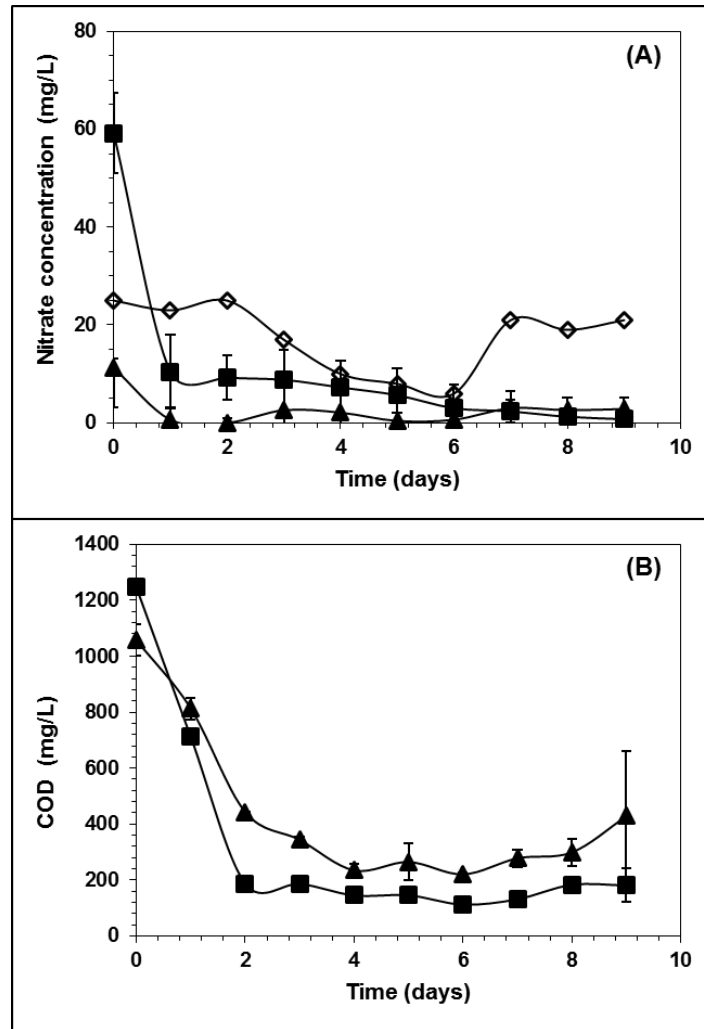


Figure 6. Changes in the (A)  $\text{NO}_3\text{-N}$  concentration (B) COD concentration during the experimental phase II.  
 Note. Treatment 40DWW-60TW (solid triangles), treatment 40DWW-60BBM (solid squares).  
 Source: Author's own elaboration.

However, it must be mentioned that DWW and MC used for this study were neither sterilized nor added with antibiotics to control or inhibit bacterial population. Therefore, it can be assumed that a certain percentage of nutrients were consumed by the present bacteria. However, the operational and nutritional conditions of this study were aimed at promoting microalgae growth as proposed by various authors (Barsanti & Gualtieri, 2007; Camacho-Rodríguez *et al.*, 2015).

In comparison with the results of this study, the efficiency of nutrient consumption by microalgae with DWW as medium was lower in other photobioreactors, such as fed-batch reactor (Göblös *et al.*, 2008), aerobic/anoxic sludge bed (UAASB) (Amini *et al.*, 2013), electrochemical treatment (Güven *et al.*, 2008), and membrane bioreactor (MBR) (Andrade *et al.*, 2014). Finally, it can be observed from the results on almost total consumption of nitrate and 80% removal of COD that dual-purpose systems are promising technology for treatment of agro-industrial wastewaters (Choi, 2016; Olguín, 2012).

## Lipid accumulation

The results on lipid accumulation during phase II is presented in Table 5. Treatment 40DWW-60BBM accumulated the highest lipids percentage. Previously, Loganathan *et al.* (2020) reported 21% of lipid accumulation in a consortium grown on DWW. Recently, Shu *et al.* (2018) mentioned a lipid accumulation of 27.7% of *Chlorella zofigiensis* monoculture using DWW. The lipids concentration in microalgae grown under nutritional sufficiency are estimated to be between 20% and 30%, which is dependent on the type of microalgae species (Chisti, 2007). The low content of nutrients in culture medium, mainly nitrogen, causes the lipids accumulation in oleaginous microorganisms (Cheirsilp *et al.*, 2011). Therefore, the low lipid content reported in this study could be due to the optimum nutrient availability for microalgal species of MC (Loganathan *et al.*, 2020), which can be validated from the results on biomass obtained.

Table 5. Yield of proteins and lipids in experimental phase II.

%	Proteins	Lipids
Control	68.9063	7.03 ± 0.24
40DWW-60TW	24.14 ± 2.58	8.71 ± 1.97
40DWW-60BBM	43.82 ± 3.05	14.63 ± 0.78

Source: Author's own elaboration.

Previous works using DWW for lipid production (Pandey *et al.*, 2019; Sun *et al.*, 2017; Woertz *et al.*, 2009) have reported that lipid accumulation occurs under stress conditions and is inversely proportional to biomass productivity. It can be observed that DWW offers an advantage with respect to biomass production due to its nutrient's composition, and the resultant biomass can be exploited to obtain various value-added products (Daneshvar *et al.*, 2018b; Sreekanth *et al.*, 2014). This study aimed to find suitable conditions for growth of microalgae with high cell density and high protein content. The results obtained showed that DWW enriched with synthetic medium recorded promising results in these regards. The stress that induced the lipid accumulation in this study could be related to the culture conditions (consortium in photoautotrophic conditions) and the source of organic carbon in the culture medium (Chisti, 2007).

## Protein production

The results on the protein production by MC are presented in Table 5. The control treatment recorded the highest protein content, while it was low in the 40DWW-60BBM treatment, which could be due to low adaptation time of the MC to the nutrients and their levels in DWW. On the other hand, it was observed that the treatment with the highest protein production recorded the lowest accumulation of lipids (Velea *et al.*, 2017). The protein concentration obtained from microalgae biomass has potential use in the food industry, livestock feed, food for human, and nutritional supplements, even the biomass can be used directly as a biofertilizer (Becker, 2007; Renuka *et al.*, 2018).

## Conclusions

The supplementation of BBM-3N with 40% volume of DWW favored the growth of the native MC studied and reached a maximum removal of nitrate and organic load.

This work demonstrates that a dual-purpose system represents a viable alternative for the reuse of dairy industry waste.

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## Conflict of interest

The authors declare that they have no conflict of interest.

## References

- Abraham, J., Abimbola, T., Braidia, W. J., Terracciano, A., Su, T. L., Christodoulatos, C., Koutsospyros, A., RoyChowdhury, A., Smolinski, B., & Lawal, A. (2023). On-site pilot-scale microalgae cultivation using industrial wastewater for bioenergy production: a case study towards circular bioeconomy. *Bioengineering*, 10(12), 1339. <https://doi.org/10.3390/bioengineering10121339>
- Alef, K. (1995). Enrichment, isolation and counting of soil microorganisms. In K. Alef & P. Nannipieri (eds.), *Methods in applied soil microbiology and biochemistry* (pp. 123-191). Elsevier Science. <https://doi.org/10.1016/B978-0-12-513840-6.X5014-9>
- Álvarez-Díaz, P. D., Ruiz, J., Arbib, Z., Barragán, J., Garrido-Pérez, M. C., & Perales, J. A. (2015). Wastewater treatment and biodiesel production by *Scenedesmus obliquus* in a two-stage cultivation process. *Bioresource Technology*, 181, 90–96. <https://doi.org/10.1016/j.biortech.2015.01.018>
- Amini, M., Younesi, H., Lorestani, A. A. Z., & Najafpour, G. (2013). Determination of optimum conditions for dairy wastewater treatment in UAASB reactor for removal of nutrients. *Bioresource Technology*, 145, 71–79. <https://doi.org/10.1016/j.biortech.2013.01.111>
- Andrade, L. H., Mendes, F. D. S., Espindola, J. C., & Amaral, M. C. S. (2014). Nanofiltration as tertiary treatment for the reuse of dairy wastewater treated by membrane bioreactor. *Separation and Purification Technology*, 126, 21–29. <https://doi.org/10.1016/j.seppur.2014.01.056>
- Association of Official Analytical Chemist. (1980). (W. Horwitz, Ed.; 13th ed.). AOAC.
- Barsanti, L., & Gualtieri, P. (2007). Algae: anatomy, biochemistry and biotechnology. *Journal of Phycology*, 43, 412-414. <https://doi.org/10.1111/j.1529-8817.2007.00335.x>
- Becker, E. W. (2007). Micro-algae as a source of protein. *Biotechnology Advances*, 25(2), 207–210. <https://doi.org/10.1016/j.biotechadv.2006.11.002>
- Camacho-Rodríguez, J., Cerón-García, M. C., Fernández-Sevilla, J. M., & Molina-Grima, E. (2015). The influence of culture conditions on biomass and high value product generation by *Nannochloropsis gaditana* in aquaculture. *Algal Research*, 11, 63–73. <https://doi.org/10.1016/j.algal.2015.05.017>
- Carvalho, F., Prazeres, A. R., & Rivas, J. (2013). Cheese whey wastewater: characterization and treatment. *Science of the Total Environment*, 445–446, 385–396. <https://doi.org/10.1016/j.scitotenv.2012.12.038>
- Cheirsilp, B., Kitcha, S., & Torpee, S. (2011). Co-culture of an oleaginous yeast *Rhodotorula glutinis* and a microalga *Chlorella vulgaris* for biomass and lipid production using pure and crude glycerol as a sole carbon source. *Annals of Microbiology*, 62, 987–993. <https://doi.org/10.1007/s13213-011-0338-y>
- Chen, H. B., Wu, J. Y., Wang, C. F., Fu, C. C., Shieh, C. J., Chen, C. I., Wang, C. Y., & Liu, Y. C. (2010). Modeling on chlorophyll  $\alpha$  and phycocyanin production by *Spirulina platensis* under various light-emitting diodes. *Biochemical Engineering Journal*, 53, 52–56. <https://doi.org/10.1016/j.bej.2010.09.004>
- Chinnasamy, S., Bhatnagar, A., Hunt, R. W., & Das, K. C. (2010). Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresource Technology*, 101(9), 3097–3105. <https://doi.org/10.1016/j.biortech.2009.12.026>
- Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology Advances*, 25(3), 294–306. <https://doi.org/10.1016/j.biotechadv.2007.02.001>
- Choi, H. J. (2016). Dairy wastewater treatment using microalgae for potential biodiesel application. *Environmental Engineering Research*, 21(4), 393–400. <https://doi.org/10.4491/eer.2015.151>

- Daneshvar, E., Antikainen, L., Koutra, E., Kornaros, M., & Bhatnagar, A. (2018a). Investigation on the feasibility of *Chlorella vulgaris* cultivation in a mixture of pulp and aquaculture effluents: treatment of wastewater and lipid extraction. *Bioresource Technology*, 255, 104–110. <https://doi.org/10.1016/j.biortech.2018.01.101>
- Daneshvar, E., Zarrinmehr, M. J., Hashtjin, A. M., Farhadian, O., & Bhatnagar, A. (2018b). Versatile applications of freshwater and marine water microalgae in dairy wastewater treatment, lipid extraction and tetracycline biosorption. *Bioresource Technology*, 268, 523–530. <https://doi.org/10.1016/j.biortech.2018.08.032>
- Eppink, M. H. M., Olivieri, G., Reith, H., van den Berg, C., Barbosa, M. J., & Wijffels, R. H. (2019). From current algae products to future biorefinery practices: a Review. In K. Wagemann, & N. Tippkötter (eds.), *Biorefineries. Advances in Biochemical Engineering/Biotechnology*, vol. 166 (pp. 99–124). Springer. [https://doi.org/10.1007/10\\_2016\\_64](https://doi.org/10.1007/10_2016_64)
- Feng, D., Chen, Z., Xue, S., & Zhang, W. (2011). Increased lipid production of the marine oleaginous microalgae *Isochrysis zhangjiangensis* (Chrysophyta) by nitrogen supplement. *Bioresource Technology*, 102(12), 6710–6716. <https://doi.org/10.1016/j.biortech.2011.04.006>
- Göblös, S., Portörő, P., Bordás, D., Kálmán, M., & Kiss, I. (2008). Comparison of the effectivities of two-phase and single-phase anaerobic sequencing batch reactors during dairy wastewater treatment. *Renewable Energy*, 33(5), 960–965. <https://doi.org/10.1016/j.renene.2007.06.006>
- Gouveia, L., Oliveira, A. C., Congestri, R., Bruno, L., Soares, A. T., Menezes, R. S., Filho, N. R. A., & Tzovenis, I. (2017). Biodiesel from microalgae. In C. Gonzalez-Fernandez, & R. Muñoz (eds.), *Microalgae-based biofuels and bioproducts* (pp. 235-258). Elsevier. <https://doi.org/10.1016/B978-0-08-101023-5.00010-8>
- Güven, G., Perendeci, A., & Tanyolaç, A. (2008). Electrochemical treatment of deproteinated whey wastewater and optimization of treatment conditions with response surface methodology. *Journal of Hazardous Materials*, 157(1), 69–78. <https://doi.org/10.1016/j.jhazmat.2007.12.082>
- HACH (2014). *Oxygen Demand, Chemical, Dichromate Method*. <https://latam.hach.com/asset-get.download.jsa?id=7639983816>
- HACH (2015). *Nitrate, Chromotropic Acid TNT Method*. <https://latam.hach.com/asset-get.download.jsa?id=7639983738>
- HACH (2017). *Reactive Phosphorus, PhosVer 3 Method*. <https://latam.hach.com/asset-get.download.jsa?id=7639983836>
- HACH (2019). *Total Kjeldahl Nitrogen, Nessler Method*. <https://latam.hach.com/asset-get.download.jsa?id=7639983809>
- Hemalatha, M., Sravan, J. S., Min, B., & Mohan, S. V. (2019). Microalgae-biorefinery with cascading resource recovery design associated to dairy wastewater treatment. *Bioresource Technology*, 284, 424–429. <https://doi.org/10.1016/j.biortech.2019.03.106>
- Hena, S., Znad, H., Heong, K. T., & Judd, S. (2018). Dairy farm wastewater treatment and lipid accumulation by *Arthrospira platensis*. *Water Research*, 128, 267–277. <https://doi.org/10.1016/j.watres.2017.10.057>
- Katiyar, R., Gurjar, B. R., Biswas, S., Pruthi, V., Kumar, N., & Kumar, P. (2017). Microalgae: an emerging source of energy based bio-products and a solution for environmental issues. *Renewable and Sustainable Energy Reviews*, 72, 1083–1093. <https://doi.org/10.1016/j.rser.2016.10.028>
- Kumar, A., & Singh, J. S. (2020). Microalgal bio-fertilizers. In E. Jacob-Lopes, M. Manzoni, M. I. Queiroz, & L. Queiroz (eds.), *Handbook of microalgae-based processes and products* (pp. 445-464). Academic Press. <https://doi.org/10.1016/B978-0-12-818536-0.00017-8>
- Li, K., Liu, Q., Fang, F., Luo, R., Lu, Q., Zhou, W., Huo, S., Cheng, P., Liu, J., Addy, M., Chen, P., Chen, D., & Ruan, R. (2019). Microalgae-based wastewater treatment for nutrients recovery: a review. *Bioresource Technology*, 291, 121934. <https://doi.org/10.1016/j.biortech.2019.12.1934>
- Ling, J., Nip, S., Cheok, W. L., Alves, R., & Shim, H. (2014). Lipid production by a mixed culture of oleaginous yeast and microalga from distillery and domestic mixed wastewater. *Bioresource Technology*, 173, 132–139. <https://doi.org/10.1016/j.biortech.2014.09.047>
- Loganathan, B. G., Orsat, V., & Lefsrud, M. (2020). Phycoremediation and valorization of synthetic dairy wastewater using microalgal consortia of *Chlorella variabilis* and *Scenedesmus obliquus*. *Environmental Technology*, 42(20), 3231–3234. <https://doi.org/10.1080/09593330.2020.1725143>

- Lu, Q., Zhou, W., Min, M., Ma, X., Ma, Y., Chen, P., Zheng, H., Doan, Y. T. T., Liu, H., Chen, C., Urriola, P. E., Shurson, G. C., & Ruan, R. (2016). Mitigating ammonia nitrogen deficiency in dairy wastewaters for algae cultivation. *Bioresource Technology*, 201, 33–40. <https://doi.org/10.1016/j.biortech.2015.11.029>
- Luo, L., Ren, H., Pei, X., Xie, G., Xing, D., Dai, Y., Ren, N., & Liu, B. (2019). Simultaneous nutrition removal and high-efficiency biomass and lipid accumulation by microalgae using anaerobic digested effluent from cattle manure combined with municipal wastewater. *Biotechnology for Biofuels and Bioproducts*, 12(218). <https://doi.org/10.1186/s13068-019-1553-1>
- Moreno-García, L., Gariépy, Y., Bourdeau, N., Barnabé, S., & Raghavan, G. S. V. (2019). Optimization of the proportions of four wastewaters in a blend for the cultivation of microalgae using a mixture design. *Bioresource Technology*, 283, 168–173. <https://doi.org/10.1016/j.biortech.2019.03.067>
- Olguín, E. J. (2012). Dual purpose microalgae–bacteria-based systems that treat wastewater and produce biodiesel and chemical products within a biorefinery. *Biotechnology Advances*, 30(5), 1031–1046. <https://doi.org/10.1016/j.biotechadv.2012.05.001>
- Olguín, E. J., Dorantes, E., Castillo, O. S., & Hernández-Landa, V. J. (2015). Anaerobic digestates from vinasse promote growth and lipid enrichment in *Neochloris oleoabundans* cultures. *Journal of Applied Phycology*, 27, 1813–1822. <https://doi.org/10.1007/s10811-015-0540-6>
- Pandey, A., Srivastava, S., & Kumar, S. (2019). Isolation, screening and comprehensive characterization of candidate microalgae for biofuel feedstock production and dairy effluent treatment: a sustainable approach. *Bioresource Technology*, 293, 121998. <https://doi.org/10.1016/j.biortech.2019.12.1998>
- Pires, J. C. M., Alvim-Ferraz, M. C. M., Martins, F. G., & Simões, M. (2013). Wastewater treatment to enhance the economic viability of microalgae culture. *Environmental Science and Pollution Research*, 20, 5096–5105. <https://doi.org/10.1007/s11356-013-1791-x>
- Qin, L., Wang, Z., Sun, Y., Shu, Q., Feng, P., Zhu, L., Xu, J., & Yuan, Z. (2016). Microalgae consortia cultivation in dairy wastewater to improve the potential of nutrient removal and biodiesel feedstock production. *Environmental Science and Pollution Research*, 23, 8379–8387. <https://doi.org/10.1007/s11356-015-6004-3>
- Raghunath, B. V., Punnagaiarasi, A., Rajarajan, G., Irshad, A., & Elango, A. (2016). Impact of dairy effluent on environment—a review. In M. Prashanthi & R. Sundaram (eds.), *Integrated waste management in India* (pp. 239–249). Springer. [https://doi.org/10.1007/978-3-319-27228-3\\_22](https://doi.org/10.1007/978-3-319-27228-3_22)
- Renuka, N., Guldhe, A., Prasanna, R., Singh, P., & Bux, F. (2018). Microalgae as multi-functional options in modern agriculture: current trends, prospects and challenges. *Biotechnology Advances*, 36(4), 1255–1273. <https://doi.org/10.1016/j.biotechadv.2018.04.004>
- Ritchie, R. J. (2006). Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. *Photosynthesis Research*, 89, 27–41. <https://doi.org/10.1007/s11120-006-9065-9>
- Saha, U. K., Sonon, L., & Kissel, D. E. (2012). Comparison of conductimetric and colorimetric methods with distillation–titration method of analyzing ammonium nitrogen in total Kjeldahl digests. *Communications in Soil Science and Plant Analysis*, 43(18), 2323–2341. <https://doi.org/10.1080/00103624.2012.708081>
- Santos-Ballardo, D. U., Rossi, S., Hernández, V., Vázquez, R., Rendón-Unceta, M. C., Caro-Corrales, J., & Valdez-Ortiz, A. (2015). A simple spectrophotometric method for biomass measurement of important microalgae species in aquaculture. *Aquaculture*, 448, 87–92. <https://doi.org/10.1016/j.aquaculture.2015.05.044>
- Sarrafzadeh, M. H., La, H., Seo, S., Asgharnejad, H., & Oh, H. M. (2015). Evaluation of various techniques for microalgal biomass quantification. *Journal of Biotechnology*, 216, 90–97. <https://doi.org/10.1016/j.jbiotec.2015.10.010>
- Shu, Q., Qin, L., Yuan, Z., Zhu, S., Xu, J., Xu, Z., Feng, P., & Wang, Z. (2018). Comparison of dairy wastewater and synthetic medium for biofuels production by microalgae cultivation. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, 40(7), 751–758. <https://doi.org/10.1080/15567036.2014.907847>
- Sreekanth, D., Pooja, K., Seeta, Y., Himabindu, V., & Manikya R. P. (2014). Bioremediation of dairy wastewater using microalgae for the production of biodiesel. *International Journal of Science Engineering and Advance*, 2, 783–791.
- Stichnothe, H. (2019). Sustainability evaluation. In K. Wagemann & N. Tippkötter (eds.), *Advances in Biochemical Engineering/Biotechnology*, Vol. 166 (pp. 519–540). Springer. [https://doi.org/10.1007/10\\_2016\\_71](https://doi.org/10.1007/10_2016_71)

- Sun, Z., Fang, X., Li, X., & Zhou, Z. (2017). Oleaginous microalgae from dairy farm wastewater for biodiesel production: isolation, characterization and mass cultivation. *Applied Biochemistry and Biotechnology*, 184, 524–537. <https://doi.org/10.1007/s12010-017-2564-7>
- Tricolici, O., Bumbac, C., & Postolache, C. (2014). Microalgae–Bacteria system for biological wastewater treatment. *Journal of Environmental Protection and Ecology*, 15, 268–276.
- University of Texas (UTEX). (2025). *Culture collection of algae*. <https://utex.org/collections/living-algal-strains>
- Velea, S., Oancea, F., & Fischer, F. (2017). Heterotrophic and mixotrophic microalgae cultivation. In C. Gonzalez-Fernandez & R. Muñoz (eds.), *Microalgae-based biofuels and bioproducts* (pp. 45–65). Woodhead Publishing. <https://doi.org/10.1016/b978-0-08-101023-5.00002-9>
- Wang, L., Chen, L., & Wu, S. (2020a). Microalgae cultivation using screened liquid dairy manure applying different folds of dilution: nutrient reduction analysis with emphasis on phosphorus removal. *Applied Biochemistry and Biotechnology*, 192, 381–391. <https://doi.org/10.1007/s12010-020-03316-8>
- Wang, Y., Wang, S., Sun, L., Sun, Z., & Li, D. (2020b). Screening of a Chlorella-bacteria consortium and research on piggery wastewater purification. *Algal Research*, 47, 101840. <https://doi.org/10.1016/j.algal.2020.101840>
- Woertz, I., Feffer, A., Lundquist, T., & Nelson, Y. (2009). Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. *Journal of Environmental Chemical Engineering*, 135(11), 15–1122. [https://doi.org/10.1061/\(asce\)ee.1943-7870.0000129](https://doi.org/10.1061/(asce)ee.1943-7870.0000129)
- Yu, H., Kim, J., & Lee, C. (2019). Potential of mixed-culture microalgae enriched from aerobic and anaerobic sludges for nutrient removal and biomass production from anaerobic effluents. *Bioresource Technology*, 280, 325–336. <https://doi.org/10.1016/j.biortech.2019.02.054>