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Biomass productivity of *Chlorella vulgaris* cultivated in fish and dairy cattle wastewaters

Produtividade da biomassa de *Chlorella vulgaris* cultivada em águas residuais de peixes e gado leiteiro

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Highlights

Microalgal biomass productivity depends on cultivation conditions.
Farm wastewater is an interesting cultivation media for microalgae.
Chlorella vulgaris was successfully grown in fish and dairy cattle wastewaters.
The wastewaters differed in chemical and nutrient composition.
C. vulgaris biomass productivity was higher in dairy cattle wastewater.

Abstract

The biomass productivity and nutrient composition of microalgae, such as *Chlorella vulgaris,* depend on the cultivation process and the nutrient content of growth media. Thus, in this study aimed to investigate the biomass productivity of *C. vulgaris* cultivated in fish and dairy cattle wastewaters. Thirty wastewater samples (2.5 L) were collected from system of production. Microalgae were cultivated in Erlenmeyer flasks containing 10 mL of microalgae and 1,790 mL of wastewater under constant light of 5,000 lux for 16 days at 25 ± 2.0 °C. Wastewater samples differed in composition. Biomass productivity was 47 % higher ($P < 0.0001$) in dairy cattle than in fish wastewater, reaching 67.61 g m^{-3} day^{-1} (dry matter basis, DM) when compared by Student's t-test ($P < 0.05$). Cultivation media also had an effect on biomass chemical composition. The *C. vulgaris* grown in dairy cattle wastewater was higher in crude protein and ash contents (359.6 g kg^{-1} DM and 230.4 g kg^{-1} DM, respectively), whereas microalgae grown in fish wastewater had higher nitrogen-free extract content (347.8 g kg^{-1} DM). Crude fat content did not vary greatly (mean of 313.15 g kg^{-1} DM). The pH (8.0 – 8.7) and ammonia concentration (0.07 to 0.4 mg L^{-1}) in fish wastewater was stable throughout the 16-day experimental period. In dairy wastewater, pH increased up (6.3 to 8.9) to the fourth day and remained constant thereafter, and ammonia concentration increased up (24.3 to 28.7 mg L^{-1}) to the eighth day and

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then it declined (2.1 mg L^{-1}). The *C. vulgaris* was successfully grown in both wastewaters, but productivity was higher in dairy cattle wastewater.

Key words: Chemical composition. Runoff. Microalgae.

Resumo

A produtividade e a composição de nutrientes da biomassa oriunda de microalgas, como *Chlorella vulgaris,* dependem do processo de cultivo e do teor de nutrientes dos meios de crescimento. Assim, este estudo teve como objetivo investigar a produtividade de biomassa de *C. vulgaris* cultivada em águas residuais da criação de peixes e de gado leiteiro. Trinta amostras (2.5 L) foram coletadas dos sistemas de produção. As microalgas foram cultivadas em frascos de Erlenmeyer contendo 10 mL de microalgas e 1,790 mL de águas residuais sob luz constante de 5,000 lux por 16 dias a 25 ± 2,0 ° C. As amostras de águas residuais diferiram na composição. A produtividade de biomassa foi 47% maior (P < 0,0001) nas águas residuais de produção de bovinos leiteiros do que de peixes, atingindo 67,61 g m^{-3} dia^{-1} (base de matéria seca, MS) quando comparada pelo teste t de Student (P < 0,05). O meio de cultivo também afetou a composição química da biomassa. *C. vulgaris* cultivado em águas residuais de gado leiteiro teve maiores teores de proteína bruta e cinzas (359,6 g kg^{-1} MS e 230,4 g kg^{-1} MS, respectivamente), enquanto microalgas cultivadas em águas residuais de criação de peixes apresentaram maior teor de extrativo não nitrogenado (347,8 g kg^{-1} DM). O teor de extrato etéreo não variou (média de 313,15 g kg^{-1} MS). O pH (8,0 a 8,7) e a concentração de amônia (0,07 a 0,40 mg L^{-1}) as águas residuais dos peixes permaneceram estáveis ao longo do período experimental de 16 dias. Nas águas residuais de laticínios, o pH aumentou (6,3 para 8,9) até o quarto dia e permaneceu constante a partir de então, e a concentração de amônia aumentou até o oitavo dia (24.3 to 28.7 mg L^{-1}) e então declinou (2.1 mg L^{-1}). A *C. vulgaris* foi cultivada com sucesso em ambas as águas residuais, mas a produtividade foi maior nas águas residuais de gado leiteiro.

Palavras-chave: Composição química. Escoamento. Microalgas.

Chlorella vulgaris is an important photosynthetic microalgal species with a variety of application possibilities. It has been used for the production of biofuel biomass, fertilizer, feed, and food (Sukačová et al., 2019; Wild et al., 2019).

Research on microalgae has grown considerably in recent decades because, in addition to providing valuable products, microalgae can be used in wastewater treatment systems. Microalgal growth and nutrient accumulation are highly dependent on the cultivation process and the nutrient content of growth media (Wild et al., 2019). Industrial and agricultural wastewater show potential as nutrient-rich cultivation media and can be used in microalgae production units as a strategy to avoid the discharge of untreated wastewater into the environment (Sukačová et al., 2019).

Large volumes of wastewater are generated in animal production. In fish farms, the volume of wastewater depends on the water exchange rate. For instance, in tilapia (*Oreochromis niloticus*) tanks, water must be exchanged at 0.10 m^3 h^{-1} (Cyrino, Bicudo, Sado, Borghesi, & Dairiki, 2010). Dairy farm runoff is estimated at 0.045–0.060 m^3 $animal^{-1}$ day^{-1}, but this value may vary depending on the volume of water used to clean stalls and equipment (Campos et al., 2002). The reuse of agricultural wastewaters in microalgae cultivation can provide economic and environmental advantages, as they are a low-cost source of nutrients and can help to reduce water consumption (Moreno-Garcia, Gariépy, Barnabé, & Raghavan, 2019). Algae require an adequate supply of carbon, nitrogen, and phosphorus (Moreno-Garcia et al., 2019). Nutrient and CO_2 levels of fish and dairy cattle wastewater differ greatly. Thus, the objective of this study was to investigate and compare the biomass productivity of *C. vulgaris* cultivated in fish farm and dairy farm wastewaters.

For this, wastewater samples were obtained from a fish farm (23°52′ S, 52°04′W, 550 m) and a dairy farm (23°36′ S, 52°07′W, 550 m) both located in the state of Paraná, Brazil. The sites are classified as Aw/As according to the Köppen–Geiger climate classification. Thirty wastewater samples (2.5 L) were collected from system of production. Fish farm wastewater consisted of drainage water from fish tanks. Dairy farm runoff contained cattle urine and feces. Firstly, samples were characterized according to the standard methods for the examination of water and wastewater of the American Public Health Association [APHA] (2005). Total solids concentration was determined after oven drying the samples at 105 °C by American Public Health Association (APHA, 2005). Total nitrogen was determined by the Kjeldahl method (method 984.13), according to Association of Official Analytical Chemists [AOAC] (2005). Ammonia nitrogen was determined according to method 4500-NH_3 of the APHA (2005) using an ion-selective electrode (Orion™ ISE Filling Solutions, CAT 951202, Thermo Fisher Scientific, Waltham, USA) and a gas permeable hydrophobic membrane (Orion™ Gas-Sensing ISE membrane 951214, Thermo Fisher Scientific, Waltham, USA). Chemical oxygen demand was measured by the colorimetric method 5220D, according to APHA (2005). Wastewater color was measured using the platinum-cobalt scale, as described method 8025 according to Hach (1996). Turbidity was measured by absorptiometry using a portable turbidimeter (DR/2100, Hach Company, Loveland, USA), as described by method 8237 according to Hach (1996). Following characterization, samples were filtered through a 7 µm filter and autoclaved at 135 °C for 30 min.

C. vulgaris strains were obtained from the Laboratory of Heterogeneous Catalysis for Biodiesel Production (LCHBio) of the State University of Maringá, Brazil. *C. vulgaris* was cultivated (pH 6.0), at laboratory scale and multiplied in Detmer's medium according to Watanabe (1960). For this, was used $Ca(NO_3)_2 \cdot 4H_2O$ (1.00 g L^{-1}), KCl (0.25 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.55 g L^{-1}), H_2PO_4 (0.26 g L^{-1}), $FeSO_4 \cdot 7H_2O$ (0.02 g L^{-1}), and A5 solution1 (mL L^{-1}) Composed of 2.90 g L^{-1} H_3BO_3, 1.81 g L^{-1} $MnCl_2 \cdot 4H_2O$, 0.11g L^{-1} $ZnCl_2$, 0.08 g L^{-1} $CuSO_4 \cdot 5H_2O$, 0.018 g L^{-1} $3(NH_3)_2O \cdot 7MoO_3 \cdot 4H_2O$.

For the experiment, microalgae were cultivated in 2 L Erlenmeyer flasks containing 10 mL of inoculum and 1,790 mL of fish wastewater (n = 15) or dairy cattle wastewater (n = 15). Flasks were kept under constant light of 5,000 lux (24 h photoperiod) for 16 days at 25 ± 2.0 °C, as measured by a digital thermo-hygrometer (Thermo-hygrometer 7666, Incoterm, Porto Alegre, Brazil). The experiments were aerated with 2 L min^{-1} atmospheric air, supplied by an air compressor (Jet Master 1/3 HP, Schulz, Joinville, Brazil).

During cultivation, pH was monitored at 2-day intervals (Orion Star 4, Thermo Fisher Scientific, Waltham, USA), ammonia nitrogen was measured at 4-day intervals using an ion-selective electrode, and growth rate were measured at 4-day intervals. Biomass concentration was determined by comparing sample absorbance at 670 nm (UV/Vis 1203 spectrophotometer, Shimadzu do Brasil, Barueri, Brazil) to a standard curve of microalgal dry mass (Yeh & Chang, 2012). Biomass productivity was calculated by using the following equation:

$$P = \frac{X_t - X_0}{t - t_0}$$

where P is the biomass productivity, expressed as g L^{-1} day^{-1} cell dry mass (CDM); Xt is the biomass concentration (g L^{-1} CDM) at time t (day), and X0 is the biomass concentration (g L^{-1} CDM) at time t0 (day).

Dry biomass (cell concentration) was obtained by flocculation with a tannin-based flocculant (Tanfloc SL, Tanac S.A., Montenegro, Brazil). Following this procedure, samples were decanted and filtered through a 20 mesh filter. Algal biomass was oven dried (MA033/1, Marconi, Piracicaba, Brazil) at 60 °C for 24 h, homogenized to 1 mm, and stored at −8 °C.

Because the volume of biomass in each Erlenmeyer flask was small, a composite sample was prepared for chemical analyses. Moisture (method 930.15), crude protein (method 984.13), crude fat (method 954.02), crude fiber (method 962.09), and ash (method 942.05) determinations were carried out according to AOAC (2005). Crude protein was determined on the basis of total nitrogen (%N) using the formula CP = N × 6.25. The generally accepted nitrogen-to-protein conversion factors for microalgae (4.78) Lourenço, Barbarino, Lavín, Lanfer Marquez and Aidar (2004) and C. vulgaris (5.14) Tibbetts et al. (2015) were also used to calculate protein concentration. Nitrogen-free extract (NFE) was calculated using the formula NFE = 100 − (moisture + crude protein + crude fat + crude fiber + ash), as established by AOAC (2005).

Biomass productivity data were subjected to analysis of variance (ANOVA) followed by Student's t-test ($P<0.05$) for comparison of means. Analyses were carried out using SAS version 9 (SAS Institute, North Carolina, USA). Results of the chemical composition analysis are presented as descriptive statistics.

The biomass productivity of *C. vulgaris* differed ($P<0.0001$) between wastewaters (Table 1). Dairy cattle wastewater resulted in 47 % higher productivity than fish wastewater. Microalgae grown in cattle wastewater had higher crude protein (43 %) and ash (78 %) contents (Table 1), whereas cells cultivated in fish wastewater contained a higher percentage of nitrogen-free extract (29 %). Both media resulted in similar crude fat content (313.15 g kg^{-1} DM).

Table 1
Biomass productivity and chemical composition (dry matter basis) of Chlorella vulgaris grown in fish and dairy cattle wastewaters

Variable	Fish wastewater[1] (*n* = 30)	Dairy cattle wastewater[2] (*n* = 30)
Physicochemical characteristics		
Total solids, mg L^{-1}	97	11,119
Total nitrogen, mg L^{-1}	980	3,200
Ammonia nitrogen, mg L^{-1}	0.13	133.95
Nitrate, mg L^{-1}	5.34	15.25
Nitrite, mg L^{-1}	0.14	1.46
Total phosphorus, mg L^{-1}	0.02	252.00
Chemical oxygen demand, mg L^{-1}	3.50	7,198.3
Color, uC	54	4,775
Turbidity, FAU[3]	13	900
Productivity		
C. vulgaris (g m^{-3} day^{-1})	31.94^{b}	67.61^{a}
C. vulgaris (g $flask^{-1}$ day^{-1})	0.057^{b}	0.122^{a}
Proximate nutrients		
Crude protein, g kg^{-1} (×6.25)	156.1	359.6
Crude protein, g kg^{-1} (×5.14)	128.4	295.7
Crude protein, g kg^{-1} (×4.78)	119.4	275.0
Crude fat, g kg^{-1}	316.8	309.5
Ash, g kg^{-1}	179.4	230.4
Nitrogen-free extract, g kg^{-1}	347.8	100.5

[1] Drainage water from fish tanks. [2] Dairy farm runoff containing cattle manure and urine. [3] Formazin attenuation unit. Standard error of mean to *C. vulgaris* (g m^{-3} day^{-1}) = 2.82 and Standard error of mean to *C. vulgaris* (g $flask^{-1}$ day^{-1}) = 0.005. $_{a,b}$ Means in a row followed by different letters are significantly different by Student's *t*-test ($P<0.05$).

The pH (Figure 1) of fish wastewater remained stable throughout the 16 days of cultivation. The pH of cattle wastewater increased in the first four days and remained stable thereafter. Ammonia nitrogen concentration (Figure 1) was low and stable throughout the 16 days of microalgal cultivation in fish wastewater. In dairy cattle wastewater, ammonia nitrogen concentration increased up to the eighth day of cultivation and then gradually declined.

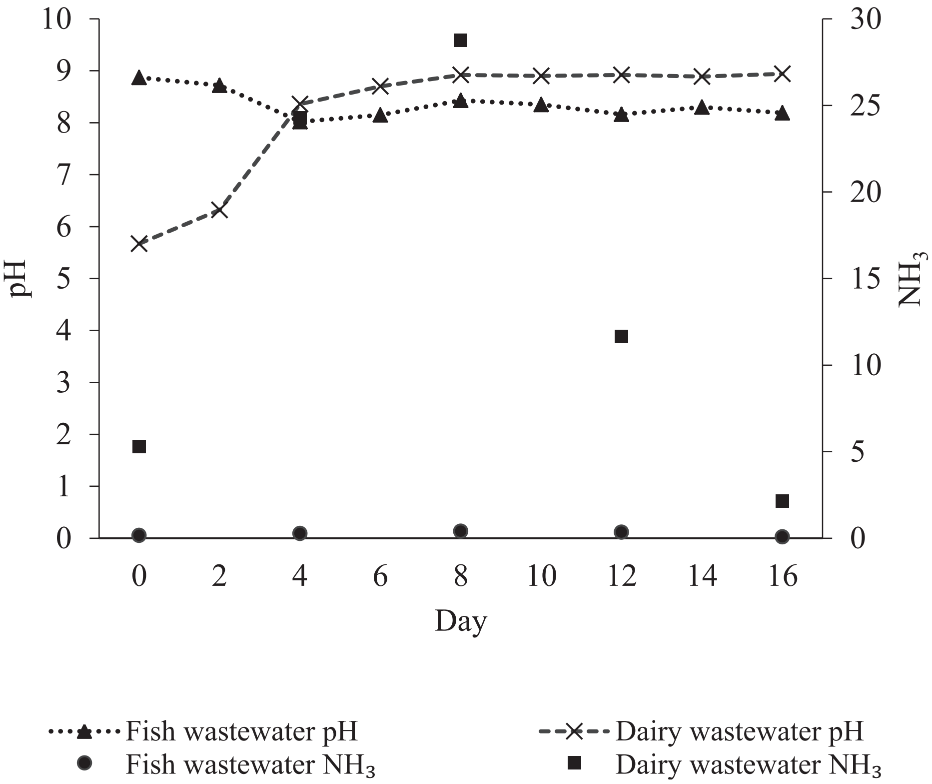

Figure 1. Ammonia nitrogen concentration (NH_3, mg L^{-1}) and pH of fish and dairy cattle wastewaters during 16 days of *Chlorella vulgaris* cultivation.

In this study, we investigated the feasibility of cultivating *C. vulgaris* in fish and cattle wastewater. Microalgae grown in these media differed in biomass productivity and nutrient composition, which was expected, as the two wastewaters differed greatly in composition (Table 1) and microalgal production is directly influenced by the concentration of substrates in the medium (Wild et al., 2019). Previous studies reported similar findings (Moreno-Garcia et al., 2019; Wild et al., 2019).

Wild et al. (2019) and Sukačová et al. (2019) reported biomass productivities of 0.22 and 0.23 g L^{-1} day^{-1}, respectively, for *Chlorella* sp. cultivated in different concentrations of nutrients. In the present study, values were much lower (0.032 and 0.068 g L^{-1} day^{-1} for microalgae grown in fish and cattle wastewater, respectively). Other studies also obtained higher biomass productivities (Sukačová et al., 2019; Wild et al., 2019). Our productivity values were close to those obtained by Wild et al. (2019) under CO_2-deficient conditions and by Moreno-Garcia et al. (2019) in a blend of wastewaters. It is likely that the cause of such low productivity was the lack of essential nutrients, such as nitrogen and CO_2 (Moreno-Garcia et al., 2019; Wild et al., 2019). Gouveia et al. (2016), studying the treatment of urban wastewater with C. vulgaris, obtained mean biomass productivity of 0.05 g L^{-1} day^{-1}.

The proximate nutrient composition of *C. vulgaris* biomass was in agreement with values previously reported in the literature for the species (Gouveia et al., 2016; Wild et al., 2019). Halfhide, Åkerstrøm, Lekang, Gislerød and Ergas (2014), studying aquaculture wastewater treatment, noted that microalgae should be harvested at the peak production of the desired product. The authors observed mean biomass productivity of 4.89 mg L^{-1} h^{-1} for *C. vulgaris* and expressive nitrogen removal (18 %).

Microalgal growth and biomass productivity can be influenced by several factors, including light (quality and quantity), temperature, pH, salinity, qualitative and quantitative nutrient profiles, dissolved oxygen concentration, and presence of toxic compounds in the medium (Gonçalves, Pires, & Simões, 2017). The optimum pH range for most microalgal species is between 7.0 and 9.0 (Gonçalves et al., 2017). Because pH changes can negatively impact microalgal metabolism, it is crucial to maintain the growth medium at a stable pH within the optimum range to avoid culture loss (Gonçalves et al., 2017). Fish wastewater pH and ammonia nitrogen concentration (Figure 1) were stable over the days. Wastewater from dairy cattle showed a marked increase in pH until the fourth day and ammonia nitrogen concentration until the eighth day of cultivation, followed by a gradual reduction (Figure 1).

In microalgal production, it is common to observe an increase in pH resulting from CO_2 uptake (Halfhide et al., 2014; Gonçalves et al., 2017), but this may have limited microalgal growth and productivity. Moreno-Garcia et al. (2019) concluded that the exponential growth phase of microalgae lasted until the sixth day of cultivation when increasing concentrations of organic carbon and CO_2 led to reduced growth.

Increased ammonia nitrogen may be associated with the leaching processes of organic (protein, sugars, organic acids) and inorganic (K, Ca, Mg, and Mn) compounds (Li et al., 2016). These compounds come from animal feed and may not have been fully leached, as this process can last from a few days to weeks. While the fall of ammonia nitrogen is related to the consumption and depletion of compounds that are nitrogen sources for the formation of microalgae. Nitrogen deprivation is the most widely used strategy to redirect protein synthesis to lipid synthesis and enhance the biomass value of microalgae for biodiesel production. According to Li et al. (2016), nitrogen is the most critical single nutrient affecting lipid accumulation in microalgae. The most common forms of inorganic nitrogen are NO_3-N, NH_4-N, and urea (Halfhide et al., 2014; Gonçalves et al., 2017).

In this study, the effects of nitrogen deprivation on nutrient accumulation were clear. Microalgae grown in dairy cattle wastewater had higher crude protein content than those cultivated in fish wastewater (Halfhide et al., 2014; Wild et al., 2019). Li et al. (2016) noted that it is essential to maintain a balance between low nitrogen stress and high photosynthetic capacity by regulating the initial nitrogen supply to maximize lipid yield.

Phosphorus is required in the form of soluble phosphates and should be supplied in large quantities because not all phosphorus compounds are bioavailable for microalgae (Halfhide et al., 2014; Gonçalves et al., 2017). The higher proportion of nitrogen and phosphorus in dairy cattle wastewater resulted in higher protein synthesis (Table 1). These findings are consistent with the literature (Wild et al., 2019). In general, microalgae have high nitrogen and phosphorus removal efficiencies, ranging from

60–99 % and 54–95 %, respectively (Gonçalves et al., 2017).

Fish and dairy cattle wastewaters proved to be suitable media for *C. vulgaris* cultivation, but cattle wastewater led to higher biomass productivity. The results indicate that it is technically feasible to cultivate microalgae in dairy cattle wastewater. This environmentally friendly technique obviates the need for freshwater and expensive substrates. Future studies should evaluate the existence of possible contaminants such, as undesirable microorganisms.

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