

# ***Chlorella vulgaris* growth in different biodigested vinasse concentrations: biomass, pigments and final composition**

Elias Trevisan, Rodrigo Felipe Bedim Godoy, Fernando Aparecido Dias Radomski, Enzo Luigi Crisigiovanni, Kemely Bruna Zandonadi Ferriani Branco and Pedro Augusto Arroyo

## **ABSTRACT**

Vinasse, an effluent generated during sugar and alcohol production, has great potential for soil and water pollution; however, it can be treated, used in biomass production and reused in sugarcane plantations. Thus, this work uses different types of biodigested vinasse to produce more biomass. The effect is the removal of ammonia nitrogen quickly and the end of the exponential growth phase of microalgae at different levels from the sixth day of cultivation. Among the concentrations used, the use of 50% biodigested vinasse showed the highest biomass concentration ( $255 \text{ mg L}^{-1}$ ) after 10 days of growth, coinciding with the end of ammoniacal nitrogen availability and stabilization of effluent color removal. The addition of biodigested vinasse also provides an increase in Chlorophyll a ( $5.33 \text{ mg L}^{-1}$ ) and b ( $4.66 \text{ mg L}^{-1}$ ) levels, obtained on the sixth day with 40% of vinasse, as well as protein (40.50%) with 50% effluent. Therefore, with the obtained results we noticed the variation of the biomass composition according to the vinasse concentration and increase of the pigment concentration in the presence of the effluent with higher nutrient concentration. Thus, the higher concentration of vinasse was more productive of the cultivation of *Chlorella vulgaris*.

**Key words** | algal biotechnology, color removal, effluent treatment, microalgae cultivation, nutrients remediation, sugarcane

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## **INTRODUCTION**

Brazil is one of the world's biggest ethanol producers and, just in the 2018/2019 crop, produced over 33 million  $\text{m}^3$  of it (UNICA 2019). The ethanol industries generate, depending on the distillery equipment, 10–15 L of vinasse per liter of produced ethanol (Christofoletti *et al.* 2013). Vinasse is a dark brown wastewater with high turbidity, high levels of organic matter and mineral nutrients, low pH and high biochemical oxygen demand (Brasil *et al.* 2017). This residue disposal in aquatic environments is harmful to microorganisms and wildlife and its use as fertilizers in the sugar cane crops can result in soil salinization due to its high content of potassium (Brasil *et al.* 2017). Thus, vinasse treatment and disposal are relevant environmental issues to refineries.

Microalgae can be used to treat some industrial effluents and sewage, therefore, several authors investigated its potential to treat vinasses (Francisca Kalavathi *et al.* 2001; Valderrama *et al.* 2002; Ramirez *et al.* 2014; de Mattos & Bastos 2016). Although this use represents an ecological approach to the vinasse problem, microalgae production nowadays also represents an alternative source for several bioproducts, such as biofuels, special oils, pigments, polymers and others (Perez-Garcia *et al.* 2011). In fact, microalgae lipids were identified as the main sustainable source for biodiesel production (Wijffels & Barbosa 2010); however, the production of microalgal biomass is still not economically viable due to high costs of cultivation, harvesting and processing (Quinn & Davis 2015). In order to improve this scenario, the use of organic wastewater treatment as a low cost source of nutrients for algal culturing were evaluated and today they are recognized as the most

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likely solution for economically supporting this industry (Brasil et al. 2017).

Many authors tried to produce microalgae with diluted non-treated vinasse (Altenhofen da Silva et al. 2017; Santana et al. 2017), however, Barrocal et al. (2010) and España-Gamboa et al. (2011) showed that vinasse pre-treated by an anaerobic process can provide better conditions for microalgae growth. In this context, Marques et al. (2013) evaluated the feasibility of using the effluent of an anaerobic digester treating vinasse for growing *Chlorella vulgaris*, an oil-producing algae. The authors observed that the non-treated vinasse obtained from an ethanol plant showed high toxicity to *C. vulgaris* at concentrations higher than 4% and the anaerobic digestion contributed significantly to reduce vinasse toxicity.

In fact, the algal specific growth rate observed on non-diluted treated vinasse ( $0.76 \text{ day}^{-1}$ ) was higher than the maximum observed with *C. vulgaris* on nutrient sufficient medium ( $0.53 \text{ day}^{-1}$ ). On the other hand, biomass and oil productivity was about tenfold lower compared to nutrient sufficient medium. Thus, Marques et al. (2013) concluded that microalgae growth on treated vinasse mixtures may be improved by nutrient addition and inoculum adaptation. Besides that, the authors highlighted the necessity of further investigations regarding the effect of potassium concentration in this culture, because, although it is known that potassium is used for several functions related to the photosynthesis machinery, high concentrations are toxic and the potassium concentrations remained statistically unchanged during vinasse treatment with *C. vulgaris* in this study.

Santana et al. (2017) studied 40 microalgae strains for growth in sugarcane vinasse at different concentrations. Although two microalgae strains (*Micractinium* sp. Embrapa|LBA32 and *C. biconvexa* Embrapa|LBA40) presented vigorous growth, the authors pointed the existence of issues relating the low light transmittance of vinasse, which is directly related to its turbidity. On the other hand, Candido & Lombardi (2016) evaluated growth and biomass yield of *C. vulgaris*, same algae as Marques et al. (2013), in conventional and in biodigested vinasses that have been treated by filtration or centrifugation before their use as microalgae culture medium.

Specific growth rates results showed that in 60% filtered conventional and 80% biodigested vinasses, *C. vulgaris* performed as well as the controls in nutrient rich synthetic culture media, with growth rates of up to  $1.2 \text{ day}^{-1}$ . Moreover, the authors also found that a potassium reduction up to 50% was promoted by the filtering processing and this result could solve the soil salinization problem of the vinasse application on sugar cane crops. Turbidity reduction was

also significantly obtained by filtration. Thus, the use of treated vinasse as culture medium can lower the costs of microalgae production with the advantage of increasing the residue value.

Seeing that the use of biodigested vinasse to produce microalgae presents several promising applications, this study aims to evaluate the growth of *Chlorella vulgaris* using different concentrations of vinasse pre-treated with anaerobic digestion, focusing in the analyses of nutrients, removal of color of biodigested vinasse and composition of biomass produced, since those parameters appear to be crucial in this process.

## METHODS

### Wastewater samples

Biodigested vinasse samples were obtained in a power generation unit from the digestion of residues from sugar and alcohol production ( $-23.26589$ ,  $-52.49256$ ). Located approximately 450 m above sea level in the state of Paraná, Brazil, the region has a climate classified as Aw/As according to the Köppen-Geiger classification. After the digestion process, 50 L of biodigested vinasse were collected. Samples were evaluated for characterization (Table 1) according to the standard methods for the examination of water and wastewater of the American Public Health Association (APHA) (APHA 2005).

The total dissolved solids content was employed drying at  $180^\circ\text{C}$  (Method 2540 C) and total suspended solids dried at  $103\text{--}105^\circ\text{C}$  (2540 D) (APHA 2005). The color was determined using the cobalt platinum scale according to Hach's DR/2010 Spectrophotometer Method 8025 (Hach

**Table 1** | Physicochemical characteristics of biodigested vinasse

Parameter	Biodigested vinasse
Total suspended solids, mg/L	$2,102 \pm 5.09$
Ammonia nitrogen, mg/L	$109.0 \pm 1.08$
Nitrate, mg/L	UA
Nitrite, mg/L	UA
Phosphate, mg/L	$418.0 \pm 1.01$
Potassium, mg/L	$1258.0 \pm 1.03$
Chemical oxygen demand, mg/L	$5,440 \pm 2.23$
Color, mg PtCo/L	$1,812 \pm 0.98$
pH	$8.05 \pm 0.01$

UA indicates undetected amount by tests.

1996). Chemical oxygen demand (COD) was determined by the colorimetric method according to APHA (Method 5220D) (APHA 2005). Ammonia was determined following the 4500-NH<sub>3</sub> D method, using an ion selective electrode (Orion™ ISE Filling Solutions, CAT 951202, Thermo Fischer Scientific, Waltham, MA, USA) with a gas permeable hydrophobic membrane (APHA 2005). Phosphate, nitrate and nitrite were determined by ion chromatography according to the EPA 300.1 method (Hautman & Munch 1997) using a Metrohm model 850 Professional IC ion chromatograph with a Metrosep A Supp 5 column (ion exchange). Potassium was determined by atomic absorption spectroscopy (AAS) (VARIAN – SpectrAA 50B) according to APHA (method 3111B) (APHA 2005).

After characterization, the biodigested vinasse was sedimented and then filtered with quantitative filter paper (blue strip, D = 125 mm, Unifil) to remove solids. To the crops were added 10, 20, 30, 40 and 50% of biodigested vinasse to supply nutrients and removal (Table 2).

### Microalgae strain and growing environment

Microalgae strain *C. vulgaris* was kindly provided by the PhD Armando Augusto H. Vieira, of the Botany Department of the Federal University of São Carlos. The experiments were conducted in the Laboratory of Heterogeneous Catalysis and Biodiesel (LCHBio), of the State University of Maringá, Brazil. Strain maintenance and inoculum production was used in the culture medium (Dertmer's medium for green algae) proposed by Watanabe (1960).

For the experiment, microalgae were cultivated in 2 L Erlenmeyers flasks containing approximately 19 mg of inoculum and 1,990 mL of culture medium composed of a solution containing 10, 20, 30, 40 and 50% biodigested vinasse ( $n = 15$ ). The conical flasks were kept under

constant illumination of 5,000 lux (24 h photoperiod), provided by fluorescent lamps for 16 days. The culture room was maintained at  $25 \pm 2.0$  °C, measured by a thermo-hygrometer (Thermo-hygrometer 7666, Incoterm, Porto Alegre, Brazil).

### Monitoring

The following variables were monitored during cultivation: pH (Orion Star 4, Thermo Fisher Scientific, Waltham, MA, USA), color (DR/2010, Hach), phosphate by vanadomolibdophosphoric acid (4500-PC) colorimetric method (APHA 2005), and ammonia nitrogen (method 4500 NH<sub>3</sub> D). Biomass concentration was carried out by total suspended solids dried at 103–105 °C (2540 D) (APHA 2005), Chlorophyll and carotenoids as proposed by He (He et al. 2015).

For microalgae recovery, 50 mg L<sup>-1</sup> of natural tannin-based flocculant (Tanfloc SL, Tanac SA, Montenegro, RS, Brazil) was used with agitation of 700 rpm for 30 s (Mechanical shaker, IKA 20 RW digital). After 25 min of sedimentation it was filtered at 20 mesh. The biomass was oven dried (MA033/1, Marconi, Piracicaba, Brazil) at 60 °C for 24 h, homogenized at 20 mesh, and stored at -8 °C for further characterization.

### Microalgae composition

Total lipids were determined according to Hosseini et al.'s (2015) adaptation of the methodology proposed by Folch et al. (1957). Test tubes with a known mass of microalgae and 1.2 mL of a chloroform: methanol solution at the volumetric proportion of 2:1 (v/v) were kept in ultrasonic bath at 42 Hz (Cristófoli) for 15 min. The samples are centrifuged for 20 min at 4,500 rpm and the supernatant arranged in a tube of known mass, the procedure was repeated three

**Table 2** | Physicochemical characteristics of cultivation solutions containing 10%, 20%, 30%, 40% and 50% of biodigested vinasse

Concentration	10%	20%	30%	40%	50%
Total suspended solids, mg/L	211.1 ± 1.50	430 ± 2.61	599 ± 9.01	843 ± 1.03	999.0 ± 0.4
Ammonia nitrogen, mg/L	9.16 ± 0.28	13.51 ± 0.31	19.37 ± 0.01	22.15 ± 0.09	24.35 ± 0.12
Nitrate, mg/L	UA	UA	UA	UA	UA
Nitrite, mg/L	UA	UA	UA	UA	UA
Phosphate, mg/L	6.40 ± 0.06	18.73 ± 1.88	33.45 ± 1.50	54.34 ± 0.01	76.09 ± 0.54
Potassium, mg/L	103.4	168	359.2	503.1	629.9
Color, mg PtCo/L	102.3 ± 1.56	378.7 ± 2.44	542.7 ± 0.89	745.0 ± 2.00	982.3 ± 5.11
pH	6.97	6.99	6.95	6.94	6.95

UA indicates undetected amount by tests.

times. The solvent was evaporated at 60 °C to constant mass and determined by gravimetry.

The protein was extracted by alkaline hydrolysis (Meijer & Wijffels 1998). Test tubes with 5 mg microalgae and 0.8 mL NaOH (0.1 M) solution were heated in a water bath for 30 min at 95 °C. Then, it was cooled in an ice bath and neutralized with 0.2 mL of HCl (0.4 M). For quantification, bovine serum albumin (BSA) was used as a standard to estimate protein content (Bradford 1976). The extracted protein (0.5 mL) was added in Bradford reagent solution (2.5 mL) and then incubated for 10 min. The absorbance intensity at 595 nm by spectroscopy (DR/2800, Hach Company, Loveland, CO, USA).

Ash was determined by the method 2540 E (APHA 2005). Carbohydrates were determined using the formula carbohydrates (%) = 100 – (total lipids + protein + ash) adapted from Gong et al. (2014).

### Statistical analysis

All the experiments were performed in three independent replicates ( $n = 3$ ). In the graphs are presented the means and  $\pm$  error bars. Box-plot graph was also plotted to verify the data variation. Analysis of variance (ANOVA) and

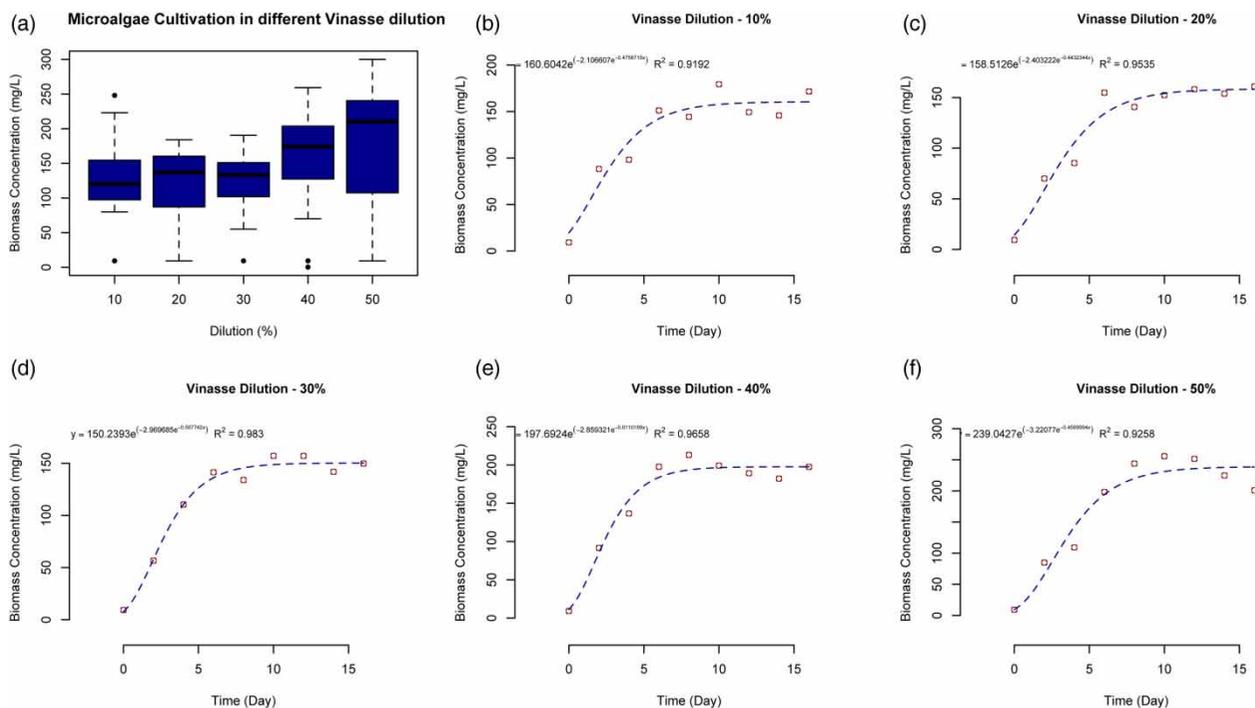
Tukey tests were used to determine statistically significant differences ( $p < 0.05$ ) among averages, after the Shapiro-Wilk homogeneity test. All the statistical tests were conducted using Software R.

## RESULTS AND DISCUSSION

### Growth curve and biomass concentration

Figure 1 shows the Box-plot graph (Figure 1(a)) and Gompertz growth curve model (Figure 1(b)–(f)) applied for the results of dry biomass concentration over 16 days of cultivation under different vinasse dilution. The highest value seen during the cultivation was with 50% dilution of vinasse in the 10th day, with approximately 300 mg/L. It could probably be associated with the higher nutrients concentrations and availability at this vinasse concentration for these photosynthetic organisms when compared to other dilutions. In addition, as ammoniacal nitrogen was just totally consumed at this day, it means that during the previous days of cultivation there was a source of nitrogen for enzymatic processes of these organisms.

It is also seen that in the dilution of 20% and 30% the highest biomass concentration was lower than the



**Figure 1** | Biomass concentration over 16 days of cultivation. (a) Box-plot of biomass concentration and percentage of Vinasse dilution. (b) Gompertz growth curve model applied for vinasse dilution in 10%. (c) Gompertz growth curve model applied for vinasse dilution in 20%. (d) Gompertz growth curve model applied for vinasse dilution in 30%. (e) Gompertz growth curve model applied for vinasse dilution in 40%. (f) Gompertz growth curve model applied for vinasse dilution in 50%.

maximum found on dilution of 10%. This situation could be associated with a stress generated at this cultivation due to lack of nitrogen (ammoniacal nitrogen consumed at the second day of cultivation for 10%). However, the cultivation with 40 and 50% presents high concentrations of ammoniacal concentration that contributes for the growth.

At the first day of cultivation, all samples and their respective replicas ( $n = 3$ ) started approximately with 9.3 mg/L of dry biomass. At the last day of cultivation, the mean concentrations were 171.7 mg/L, 160.9 mg/L, 149.8 mg/L, 197.5 mg/L, 200.9 mg/L for 10%, 20%, 30%, 40% and 50% of Vinasse concentration, respectively. Candido & Lombardi (2016) also found highest final biomass yield (in number of cell) with higher concentration of biodigested vinasse when compared to lower dilution of vinasse. The authors suggested that the best growth conditions for *Chlorella vulgaris* were by the 80–100%.

According to these results, higher vinasse concentrations higher dry biomass concentration for *C. vulgaris*. However, the vinasse characteristics could affect the growth of other species. Ramirez et al. (2014) verified the growth of *Scenedesmus* sp. using different vinasse concentrations. The highest biomass concentration was with 10% of vinasse added to Guillard Modified Medium.

Gompertz growth curve model was applied with experimental data to verify the  $R^2$  value to these cultivation (Figure 1(b)–1(f)). The best value was found for dilution of

30% ( $R^2 = 0.983$ ). In addition, it is still notable that the box-plot at this dilution did not show high variation compared to other treatments. For dilutions of 10, 30 and 50% the highest biomass concentration mean was found on tenth day, while for dilutions of 20 and 40% were at 6th and 8th days, respectively. On other words, the dry biomass concentration started to stabilize after the 10th day when the ammoniacal nitrogen is totally consumed. Lavín & Lourenço (2005) verified in their experiment that ammoniacal nitrogen as the most important source of nitrogen for some microalgae species. Thus, this nutrient can affect the growth and consequently the dry biomass concentration for lower dilution.

### Color removal

Figure 2 shows the box-plot graph and scatter plot graph for color removal from cultivation with different dilutions of vinasse. Figure 2(a) shows the color concentration variation between the days of cultivation for each treatment. The results show outliers in 20% (1), 30% (1), 40% (3) and 50% (3). In fact, vinasse effluent presents high color concentration, therefore lower dilution (10%) presented low variation over the cultivation. The reduction of color treatment in lower dilution could also be associated with lower concentration of nutrients such as ammoniacal nitrogen.

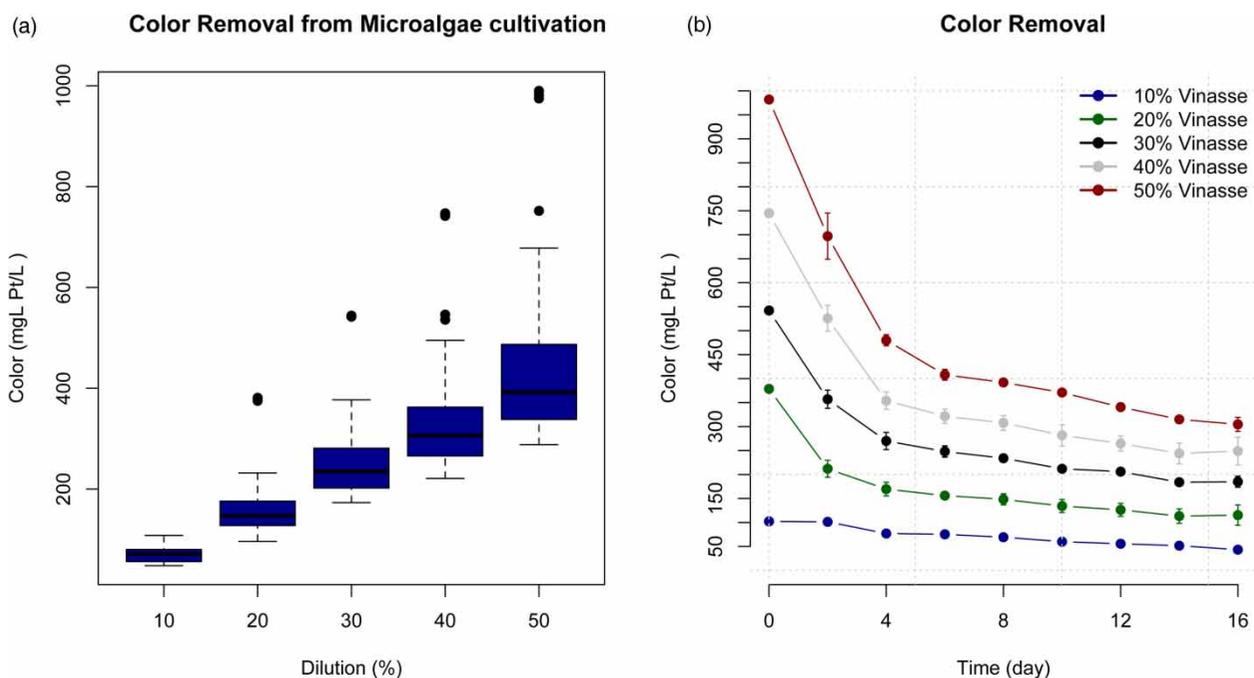


Figure 2 | Color removal from microalgae cultivation under different vinasse concentration as source of nutrients.

In addition, the presence of outliers could indicate there were values well above compared the other values along the cultivation days for the others percentage of dilution. The possible explanation for it is that during the first days there were a higher color removal from cultivation while the final days the removal is not too intensive due to the growth phase of the microorganisms and less availability of nutrients.

Figure 2(b) shows the color removal from microalgae cultivation from day 0 until the last day of cultivation (day 16). The percentage of color removal for each treatment was 47.89% (10% of vinasse), 69.57% (20% of vinasse), 65.86% (30% of vinasse), 66.57% (40% of vinasse) and 69.04% (50% of vinasse). The removal of color is most intensive during the first five days, although the removal keeps happening until last day of cultivation.

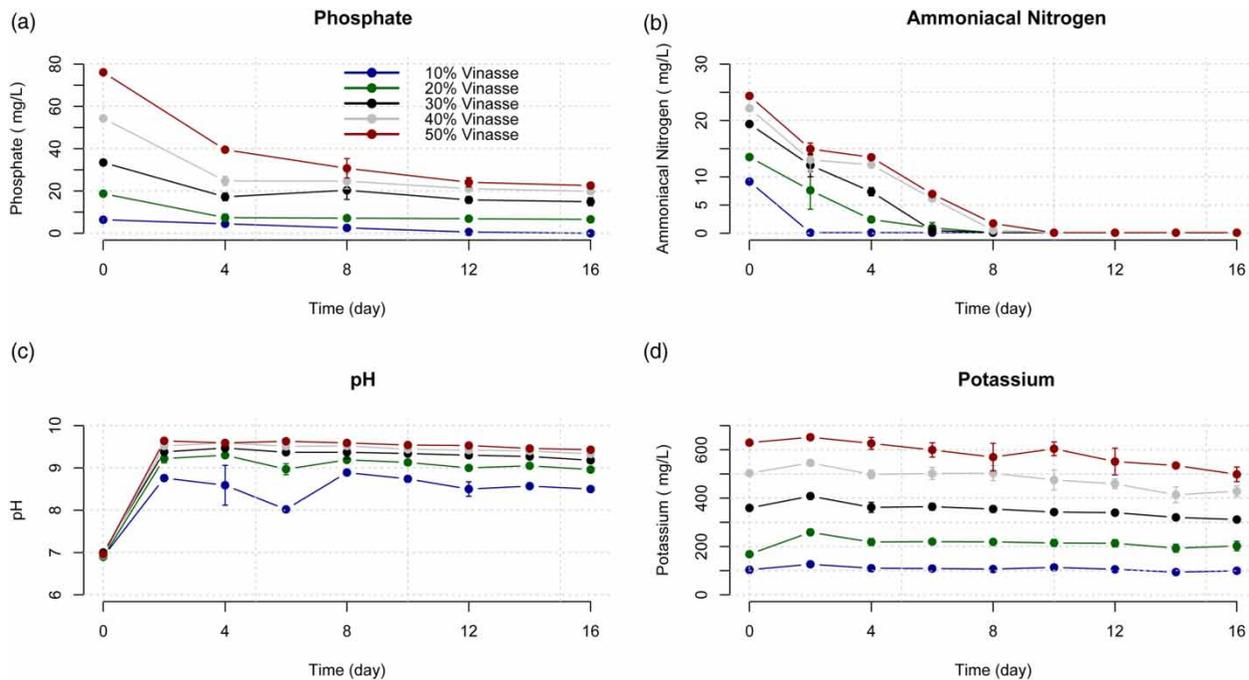
### Nutrients concentration variation

Figure 3 shows the daily phosphate (Figure 3(a)), ammoniacal nitrogen (Figure 3(b)), pH (Figure 3(c)) and potassium (Figure 3(d)) variation. The highest phosphate variation was at treatment with 50% of vinasse varying from 76 mg/L until 22.55 mg/L. Although the initial values for treatment with 30 and 40% were not high (33.45 and 54.33 mg/L, respectively) they did not present high variation as the last

treatment at the last day of cultivation (14.90 and 19.85 mg/L, respectively). In the treatment of 10% of vinasse, the phosphate was removed by microorganisms after the 12th day.

Ammoniacal nitrogen did not present high concentrations such as Phosphate. The highest mean concentration was 24.35 mg/L at 1st day of cultivation at treatment of 50% (Figure 2(b)). In addition, the ammoniacal nitrogen concentration turned less than 0.1 mg/L at the 10th day for the treatment of 50%. For other treatments, the values got lower than 0.1 mg/L at the 2nd day for treatment with 10%, at the 8th day for 20 and 30% dilution of vinasse and at the 10th day for 40% of vinasse.

After complete consumption of ammoniacal nitrogen is notable the decrease of phosphate removal and dry biomass growth. According to Kim et al. (2016), each nitrogen source is first reduced to ammonium form for some microalgae species growth, where this form will be assimilated into amino acids. Thus, decrease of ammoniacal nitrogen concentration could have affected the production of dry biomass. For pH results, the values stayed between 6.9 and 9.67. The results were a little bit similar with Candido & Lombardi (2016), results for filtered biodigested vinasse and centrifuged biodigested vinasse (until day 6). In addition, it is notable that pH means were higher with higher dilution of vinasse (50%).



**Figure 3** | Nutrients (phosphate, ammoniacal nitrogen and potassium) concentration and pH variation over 16 days of cultivation of *Chlorella vulgaris*. (a) Phosphate variation over 16 days of cultivation. (b) Ammoniacal nitrogen variation over 16 days of cultivation. (c) pH variation over 16 days of cultivation. (d) Potassium variation over 16 days of cultivation.

Potassium mean concentrations presented an increase from the day 0 to the second day of cultivation for all treatment. Despite that, at the end of cultivation there were a decrease in the concentration of potassium for almost all treatment. For treatment with 20% of vinasse potassium, mean concentration varied from 168 mg/L at day 1 to 202 mg/L at the last day of cultivation.

### Pigments concentration variation and final biomass composition

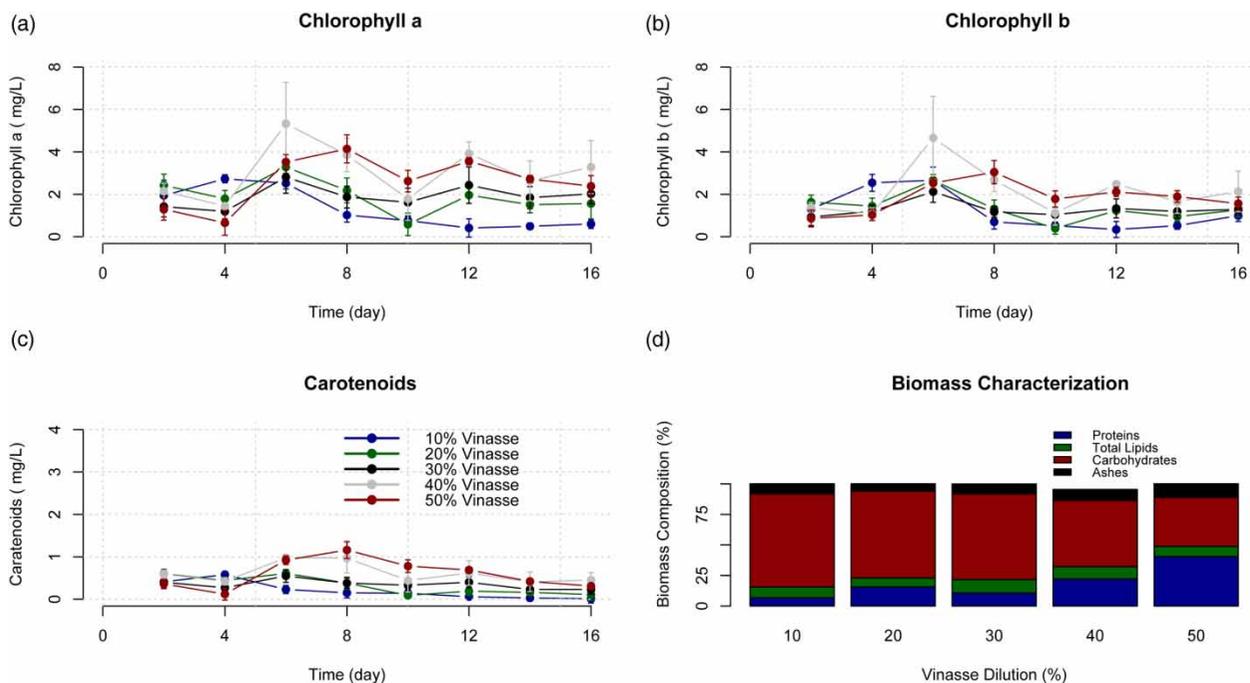
Figure 4(a) shows the variation of Chlorophyll a concentration for all treatments. The highest value was found at the 6th day at treatment with 40% of vinasse (5.33 mg/L). This result was also seen by He et al. (2015), when the authors found the highest concentration of Chlorophyll a for *Chlorella* sp. at 6th day of cultivation with the light condition of 200  $\mu\text{mol photon m}^{-2}/\text{s}$ . In the end of cultivation, Chlorophyll a mean concentrations varied from 1.31 mg/L (10%) to 3.05 mg/L (40%). Although the highest value was found with 40% of vinasse, the value is next to 50% according to the error bars. Thus, with increase of vinasse there was increase of production of Chlorophyll a.

Figure 4(b) shows the variation of Chlorophyll b concentration for all treatments. The highest value was found at the

6th day at treatment with 40% of vinasse (4.66 mg/L). In addition, at the end of cultivation Chlorophyll b mean concentrations varied from 0.99 mg/L (10%) to 2.13 mg/L (40%). The values followed the same pattern from Chlorophyll a.

The results of carotenoids were not too different from Chlorophyll a and Chlorophyll b. The highest value was found on the 6th day at treatment with 50% of vinasse (1.16 mg/L). In addition, at the end of cultivation carotenoids mean concentrations varied from 0.015 mg/L (10%) to 0.45 mg/L (40%). Thus, with increase of vinasse there was increase of production of carotenoids. The carotenoid curve showed similarity with results of He et al. (2015) with low luminosity (40  $\mu\text{mol photon m}^{-2}/\text{s}$ ).

Figure 4(d) shows the biomass characterization after 16 days of cultivation. It is visible that increasing concentration of vinasse increases the percentage of proteins and decrease of carbohydrates. Santana et al. (2017) also found this trend; however, in their experiment they compared 50% of vinasse to 100% of vinasse. This fact could be explained in that higher concentration of vinasse increased concentration of nitrogen, which is an element essential for amino acids constitution and consequently protein formation (Lavín & Lourenço 2005).



**Figure 4** | Concentration of Chlorophyll a, Chlorophyll b and carotenoids in the 16 days of cultivation and final biomass composition. (a) Chlorophyll a concentration variation along 16 days of cultivation. (b) Chlorophyll b concentration variation along 16 days of cultivation. (c) Carotenoids concentration variation along 16 days of cultivation. (d) Biomass characterization.

**Table 3** | Analysis of variance of biomass, Chlorophyll a and Chlorophyll b, for the different concentrations of vinasse in the culture medium

Concentration mg/L	Df	Sum Sq	Mean Sq	F value	p value
Biomass	4	7873	1968.1	6.058	0.00966**
	10	3249	324.9		
Chlorophyll a	4	5.532	13.830	9.533	0.00192**
	10	1.451	0.1451		
Chlorophyll b	4	19.600	0.4900	7.046	0.00578**
	10	0.6955	0.0695		

\*\*Significance  $p < 0.01$ .

Proteins in the biomass composition varied from 6.74% at treatment with 10% to 40.50% at treatment with 50% of vinasse. Total lipids presented highest value (around 10.90%) with treatment of 30% of vinasse. Carbohydrates followed the opposite results from proteins. At treatment with 10% of vinasse, the composition of biomass was 76.40% of carbohydrates and with 50% of vinasse this value was 40.10%. And, ashes did not present values much different from each treatment. The lower value (6%) at treatment with 20% of vinasse and the highest value was found (11.20%) at treatment of 50%.

### Statistical analysis

The heteroscedasticity of the sampled values of biomass, Chlorophyll a and Chlorophyll b was attested by Shapiro-Wilk with a 95% confidence interval. When submitted to analysis of variance, a significant difference ( $p < 0.05$ ) between the vinasse concentrations was identified for the three parameters analyzed (Table 3). Tukey's test showed that differences can be felt, in short, with an increase of 20% in the concentration of vinasse. For example, in the ranges of 10–30%, or 30–50% there is a difference between the sampled parameters for the treatments employed.

The analysis of variance confirms and accepts the *priori* hypothesis, that increasing concentration of vinasse in the culture medium, the growth of the algae population and photosynthetic activity would increase proportionally until equilibrium was reached.

### CONCLUSION

The results demonstrated that the cultivation of microalgae with vinasse is suitable; however, to work with different concentrations could affect the biomass composition and consequently change its use. Biomass with more percentage

of proteins need to have more concentration of vinasse in the cultivation medium, while more carbohydrates need less concentration of vinasse. In addition, the advantage to use vinasse effluent to cultivate these photosynthetic microorganisms serve to remove color (at this experiment approximately 70%), phosphate, ammoniacal nitrogen and potassium. Increase of vinasse concentration as a cultivation medium could increase the concentration of pigments. In this study, the percentage of vinasse most interesting to pigments was with 40% of vinasse.

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