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Sheyla Santa Isabel Marques, Iracema Andrade Nascimento, Paulo Fernando de Almeida & Fábio Alexandre Chinalia

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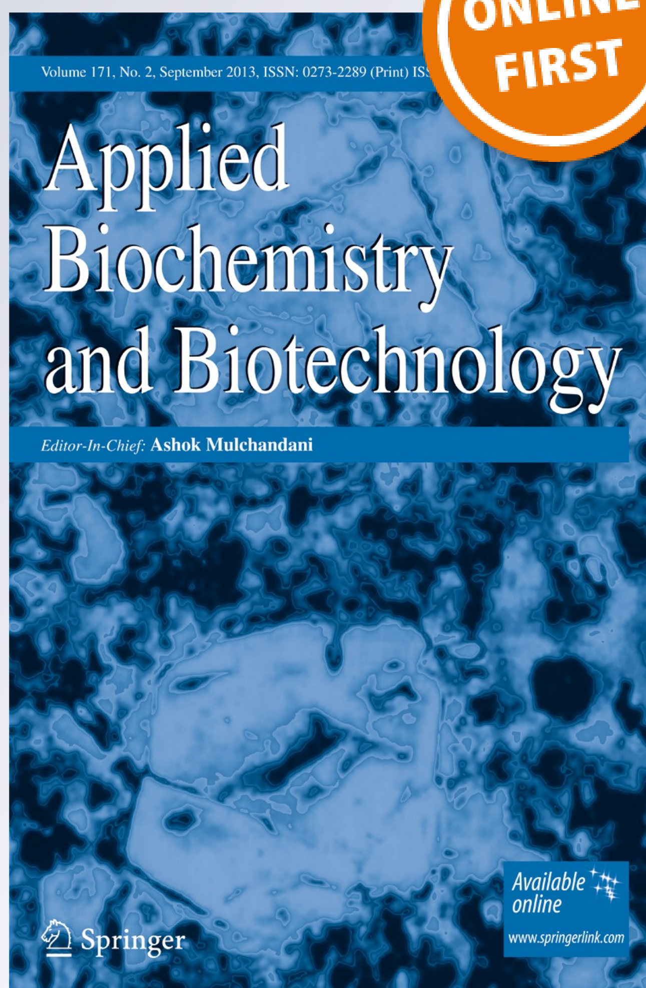

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Abstract Microalgae farming has been identified as the most eco-sustainable solution for producing biodiesel. However, the operation of full-scale plants is still limited by costs and the utilization of industrial and/or domestic wastes can significantly improve economic profits. Several waste effluents are valuable sources of nutrients for the cultivation of microalgae. Ethanol production from sugarcane, for instance, generates significant amounts of organically rich effluent, the vinasse. After anaerobic digestion treatment, nutrient remaining in such an effluent can be used to grow microalgae. This research aimed to testing the potential of the anaerobic treated vinasse as an alternative source of nutrients for culturing microalgae with the goal of supplying the biodiesel industrial chain with algal biomass and oil. The anaerobic process treating vinasse reached a steady state at about 17 batch cycles of 24 h producing about 0.116 $m^3CH_4\ kgCOD_{vinasse}^{-1}$. The highest productivity of *Chlorella vulgaris* biomass (70 mg l^{-1} day^{-1}) was observed when using medium prepared with the anaerobic digester effluent. Lipid productivity varied from 0.5 to 17 mg l^{-1} day^{-1}. Thus, the results show that it is possible to integrate the culturing of microalgae with the sugarcane industry by means of anaerobic digestion of the vinasse. There is also the advantageous possibility of using by-products of the anaerobic digestion such as methane and CO_2 for sustaining the system with energy and carbon source, respectively.

Keywords Vinasse · Anaerobic digestion · Microalgae and biofuels

Introduction

The International Energy Agency has forecasted that the global consumption of oil will rise 30 % by 2035, and that the availability of petrol will reach limiting levels a decade later [1].

S. S. I. Marques · I. A. Nascimento
Laboratório de Biologia Marinha Instituto de Biologia (IB), Universidade Federal da Bahia/Brasil, Salvador, Brazil

P. F. de Almeida · F. A. Chinalia (✉)
Laboratório de Biotecnologia e Ecologia de Microorganismos, Instituto de Ciências da Saúde (ICS), Universidade Federal da Bahia/Brasil, Salvador, Brazil
e-mail: chinalia@hotmail.com

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Transportation was identified as the main consuming sector with about 60 % of the overall consumption. For these reasons, several executive strategies worldwide are favoring the introduction and commercialization of biofuels in the transportation sector [2]. Biodiesel can be readily used by the transportation sector without significant changes in the infrastructure, and because of that, it has been estimated that the next decades will show an 80 % increase on such biofuel production [3]. Currently, land crops are the main source of oil for biodiesel production. Soya beans, sunflower, and rapeseed are the most common. Biodiesel annual production has been estimated at about 166 billion liters with a potential market value of approximately US$139 billion by 2016 [4]. This forecast is due to the fact that several countries are today commercializing blended diesel with biodiesel at ratios ranging from 5 to 20 % mostly [5, 6].

There is also an environmental benefit from the use of biodiesel/diesel blends. It has been observed that the consumption of 20 % biodiesel/diesel blend can reduce CO_2 emissions by 16 % [6]. On the other hand, the use of land crops and arable soil for the production of such a biofuel may in turn offset such benefit [7]. Therefore, a different strategy must be put in place for increasing biodiesel production in order to achieve a more sustainable development for this sector. Wijffels and Barbosa [8] identified microalgae lipids as the main sustainable source for such a goal. Approximately, 46 ton of oil ha^{-1} $year^{-1}$ can be produced from microalgae, which is ten times greater than the amount of oil produced by palm tree, which is the most productive among the conventional land crops [5]. As such, a production would not depend on agricultural land; it would positively mitigate carbon emissions by reducing the use of fossil fuels and fixate atmospheric carbon. Nevertheless, the production of algal oil is still vulnerable to the influence of limiting factors such as the costs with nutrients supply and system operation [9]. At the moment, it is estimated that such costs may represent 50 % of the final algal oil prices [10, 11]. In order to improve such a scenario, it is therefore necessary to find alternative sources of nutrients for growing such algae. A practical suggestion is to associate algal culturing with the treatment of organic wastewater [8, 9, 12, 13]. The integration of algae biomass production with wastewater treatment is today recognized as the most likely solution for economically supporting this industry [14].

Among the distinct systems used for wastewater treatment, the effluent of anaerobic digesters has been already identified as a good medium for algal culturing [15]. This effluent is often rich in ammonia and inorganic phosphate, and it is relatively low in organic matter content. Additionally, this process is also known for producing significant amounts of methane, another renewable energy source which can be directly recycled for the operation of the system. The anaerobic process is also associated to several operational advantages such as comparatively low production of disposable sludge, and it consumes less energy for its operation [16–18]. The anaerobic digestion has also been suggested as the best approach for treating vinasse, which is an organic effluent generated during the production of ethanol [19–21]. For each liter of ethanol, it is also produced about 13 L of vinasse. In Brazil alone, it was estimated a production of approximately 300 billons liters of vinasse between the years 2011 and 2012 [22]. This effluent is currently being used for irrigating agricultural soil, but such practice has also been associated with depletion of fertile soil characteristics [23, 24]. Therefore, associating the production of algal biodiesel with the anaerobic treatment of vinasse seems to be an ideal approach for integrating the production of three renewable biofuels: biodiesel, methane, and ethanol. Therefore, this proposal has clear commercial advantages for it explores the potential for reducing algal biodiesel production costs allied with significant environmental improvements. Therefore, the aim of this research is to evaluate the feasibility of using the effluent of an anaerobic digester treating vinasse for

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growing oil-producing algae. The overall goal is to suggest the integration between distinct processes resulting in the integration of biodiesel, methane, and ethanol production.

Material and Method

Experimental Design

Three distinct conditions, and their respective controls, were used in order to evaluate the feasibility of using the effluent of an anaerobic digester treating ethanol–sugarcane vinasse for growing *Chlorella vulgaris*. Figure 1 shows that ethanol–sugarcane vinasse was diluted with treated portions of domestic wastewater treatment plant (WWTP) effluent for standardizing vinasse chemical oxygen demand (COD) at a final value of 2 g l^{-1}. Chemical characterizations of the distinct waste streams used in this research are shown on Table 1. It is important to highlight that the COD contribution of the WWTP effluent to the final mixture was insignificant compared to the vinasse (Table 1).

After dilution, a fraction was diverted to the anaerobic digesters and another portion was stored at −20 °C to be used as the controls for the *C. vulgaris* growth experiments. The anaerobic digesters were operated until they reached a steady state performance regarding COD removal and methane production. It was only during this period that effluent samples were collected, stored (−20 °C), and later mixed for the growth experiments with *C. vulgaris* (Fig. 1).

Anaerobic Digestion

Ethanol–sugarcane vinasse was obtained at Agrovale S/A (Agroindústrias do Vale do São Francisco S.A., Bahia, Brazil). The treated domestic WWTP effluent and anaerobic sludge were collected at EMBASA S/A (Salvador City, Bahia, Brazil). The anaerobic sludge was obtained from a UASB digester treating domestic effluents of Salvador City. Table 1 shows some chemical variables characterizing the effluents and also the anaerobic sludge used as inoculum. It should be highlighted that the treated domestic effluent showed a COD value 325-fold smaller than what was observed with the vinasse. Treatments were carried out in triplicates using the Automatic Methane Potential Test System device from Bioprocess

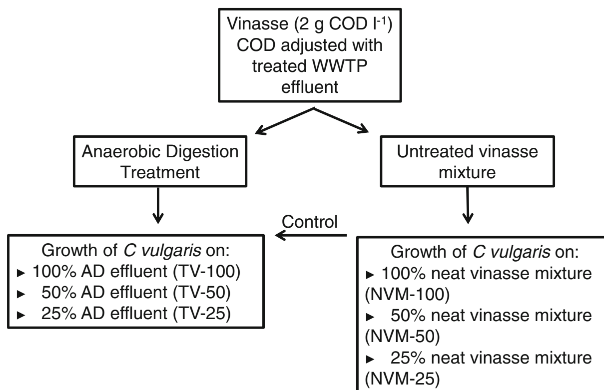

Fig. 1 Diagram showing the experimental setup

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Table 1 Chemical characterization of the waste streams and the anaerobic digester influent and effluent used in this research

	Distinct waste streams used in this research			Operation of AD digester	
($g\ l^{-1}$)	Sludge used as inoculum	Treated sewage effluent	Vinasse	Influent	Effluent
COD	14.583 (±0.729)	0.071 (±0.004)	23.179 (±1.156)	2.06 (±0.040)	0.300 (±0.006)
NO_3	0.028 (±0.002)	0.015 (±0.001)	0.026 (±0.002)	0.009 (±0.000)	0.002 (±0.000)
NO_2	BDL	BDL	BDL	BDL	BDL
NH_4	0.192 (±0.013)	0.005 (±0.000)	0.010 (±0.001)	0.003 (±0.000)	0.020 (±0.004)
PO_4	0.022 (±0.001)	0.010 (±0.000)	0.045 (±0.002)	0.016 (±0.001)	0.014 (±0.001)
K	0.006 (±0.000)	BDL	0.109 (±0.002)	0.034 (±0.005)	0.038 (±0.007)
TS	48.99 (±1.52)	0.466 (±0.03)	14.362 (±0.60)	–[b]	–[b]
FS	28.47 (±0.91)	0.301 (±0.01)	2.968 (±0.72)	–[b]	–[b]
VS	20.52 (±0.60)	0.165 (±0.02)	11.394 (±1.31)	–[b]	–[b]
pH[a]	6.7 (±0.1)	6.5 (±0.3)	3.5 (±0.1)	6.8 (±0.1)	6.7 (±0.1)
Turbidity (NTU)	–[b]	–[b]	–[b]	650 (±5.2)	100 (±6.8)

BDL below detection limit

[a] Index of H^+ concentration

[b] Not assessed

Control®, Netherland, which were operated in batch cycles of 24 h. The reactors were inoculated with 30 % anaerobic sludge and vinasse–WWTP effluent mixture was added to a final volume of 400 ml. After inoculation, reactors were operated with a vinasse volumetric COD loading ratio of 1 kg m^{-3} day^{-1}. Temperature was kept at 35 °C, and mixing was provided within an on/off cycle of 0.5 and 1.0 min, respectively. Biogas was collected and filtered through an alkaline solution (NaOH 3 M) for capturing CO_2 and H_2S. Real-time methane production was measured volumetrically. Anaerobic digestion performance was assessed by comparing methane production kinetics and COD consumption of treatments and controls (Fig. 1).

Growth Kinetics of *C. vulgaris*

Anaerobic digester effluent was used as growth media for culturing *C. vulgaris*. Growth media were identified as TV-100, TV-50, and TV-25 % for anaerobic effluent used neat or diluted with 50 and 75 % with WWTP effluent, respectively. The control experiment was carried out using neat vinasse–WWTP mix (NVM-100, 50, and 25 %) without passing through the anaerobic digester (AD). The former growth media were thus also diluted with 50 and 75 % with WWTP effluent, respectively. Table 2 shows nutrients and COD values present in the influent and effluent of the anaerobic digester. Turbidity was assessed using LaMotte® (Model 2020), and the remaining variables using the Standard Methods for the Examination of Water and Wastewater (2005) [25].

Growth trials were carried out in triplicate using Erlenmeyer flasks containing 600 ml of standardized medium and a 10 % volume of algal inoculums in the exponential growth phase. The flasks were kept under constant temperature and agitation (25±2 °C and 90 rpm, respectively); the aeration rate was maintained at 0.50 vvm (volume gas per volume broth per minute) of atmospheric air enriched with 2 % of CO_2. Cells were incubated at a neutral

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Table 2 Kinetic parameters comparing *C. vulgaris* grown parameters on different concentrations of anaerobically treated vinasse (TV-100, 50 and 25 %), neat vinasse mixtures (NVM-100, 50 and 25 %), and nutrient sufficient media [26]. The table also shows chemical characterization of the biomass and lipid productivity

	TV-100	TV-50	TV-25	NVM-100	NVM-50	NVM-25	Nutrient sufficient media[a]
Biomass productivity (mg l^{-1} day^{-1})	70	62	47	0	0	3	730
μ (d^{-1})	0.76	0.56	0.45	0	0	0.1	0.53
Lipid content (%)	23.68	24.95	26.45	20	20	20.53	28.43
Total lipid (mg l^{-1})	75	64	54	0	0	11	-
Lipid productivity (mg l^{-1} day^{-1})	17	15	12	0	0	0.5	204
Protein content (%)	63	65	64	0	0	63	–[b]
Carbohydrate content (%)	14	10	10	0	0	16	–[b]

[a] Nascimento et al. [26]

[b] Data not available

pH range (6.8±0.8), and light (140 μE m^{-2} s^{-1}) was provided within a photoperiod of 12:12 h light and dark cycles. Growth was monitored every 48 h by hemocytometer cell counting and by optical density (OD). OD was determined at 680 nm using a UNICAM® Spectrophotometer model Helios Epsilon. Counting of cells per milliliter, and/or OD_{680} measurements were plotted against time and used for estimating growth kinetic parameters. Growth kinetic parameters were obtained in triplicates for the distinct strains, and data were compared using ANOVA. Growth curves were adjusted using Boltzman sigmoid model described in Origin software version 7 (Origin Lab Data Analysis and Graphing Software®), which was also tested for the model validity ($p \geq 0.05$). The kinetic parameters were assessed according to Nascimento et al. [26]:

(a) Specific growth rate (micrometer), based on the equation $\mu = \ln (N_y/N_x)/(t_y - t_x)$, where N_y and N_x are the numbers of cells (N) at the start (t_x) and the end (t_y) of the logarithmic growth phase.

(b) Biomass productivity (P_{dwt}), as the dry biomass produced (g l^{-1} day^{-1}) during the exponential growth phase. For P_{dwt} determination, samples were collected at the end of the exponential phase and cells were harvested by centrifugation for 5 min at 5.000 g at 4 °C (Sorvall ultracentrifuge ®, Evolution RC). Supernatant discarded pellets were washed with distilled water, freeze-dried, and the dry weight was determined gravimetrically [26].

(c) Total lipids content (L_c), reported as percentage of the total biomass (dwt%), determined by using the chloroform/methanol approach as previously reported by Nascimento et al. [26].

(d) Volumetric Lipid productivity (L_p) calculated according to the equation $L_p = P_{dwt} \times L_c$ and expressed as mg l^{-1} day^{-1} [27]. The results were compared using ANOVA and multiple range test based on GraphPad Software Inc®.

Results and Discussion

Preliminary tests showed that vinasse was toxic to *C. vulgaris* at a COD concentration above 4 %, and according to the adopted operating design, the anaerobic digestion start-up was unstable at vinasse concentrations above 5 kg COD m^3. Therefore, in order to favor a fast start-up and low

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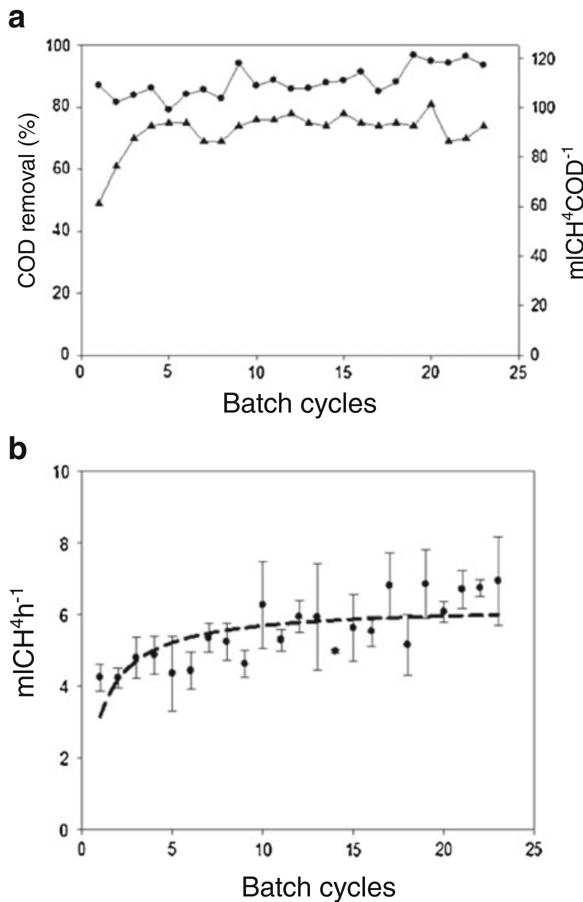

Fig. 2 Monitoring parameters of anaerobic digesters treating vinasse: **a** COD removal efficiencies compared to volumetric methane production rates and **b** specific methane yields of each batch cycle (24 h)

potassium concentration, the experimental set-up was planned to test vinasse at a starting COD concentration of 2 kg COD m^3.

The anaerobic process treating vinasse reached a steady state at about 17 batch cycles of 24 h with a COD removal rate above 80 % (Fig. 2). Methane productivity was of about 0.116 m^3CH_4 $kgCOD_{vinasse}{}^{-1}$. Souza et al. [28] reported a methane productivity of 0.22 m^3CH_4 $kgCOD_{vinasse}{}^{-1}$ when their anaerobic digester was operated at 55–57 °C and with a vinasse volumetric organic load of 25–30 kg COD m^3 day^{-1}. Considering that the COD removal efficiencies were lower than 70 %, the methane productivity reported by the former authors is unique report. Turkdogan-Aydino and Yetilmezsoy [29], on the other hand, reported that methane productivity of vinasse varied from 0.05 to 0.11 m^3CH_4 $kgCOD_{vinasse}{}^{-1}$ when the digester was operated with an organic volumetric loading rate varying from 1.9 to 16 kg $COD_{vinasse}$ m^3 day^{-1}. Nevertheless, it has been observed that anaerobic digesters operated with vinasse at a high organic volumetric loading are often unstable during long-term operation because of accumulation of acids. Mohana et al. [19] and Goodwin et al. [30] thus suggested that for ensuring best performance, anaerobic digesters treating vinasse should commonly be operated during the start-up period with an organic volumetric load between 4.8 and 5.4 kg COD m^3 day^{-1}. According to the former authors,

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increases in organic volumetric loads would therefore be a gradual process and dependent on the production and accumulation of acids within the digester. As stated before, the suggested organic loading interval did not produce a stable start-up phase in this research. Constant decreases in pH were observed even after correction, which strongly suggested accumulation of acids (data not shown). The anaerobic process is directly affected by the type of inoculum, reactor design, and operation. The right balance between the hydrolytic and acetotrophic phases must be reached for ensuring the highest performance and methane yields during the anaerobic process. Considering the conditions and inoculum quality used in this research, the best approach was to use an organic volumetric load of 1 kg m^3 day^{-1} of vinasse. However, it should be highlighted that quality of the effluent should be always compared by means of performance indicators regardless of volumetric organic loading. Thus, independent of high or low organics, the quality of the digester effluents is analogous if the performance indicators are similar. For instance, Manresa et al. [31] reported that fluidized bed reactor can remove 75 % of vinasse COD (79 g l^{-1}) with a hydraulic retention time of 8 h. On the other hand, Cabello et al. [23] observed a COD removal rate below 60 % with a similar digester, but operated with HRT of 24 h and an organic load of 19.5 kg COD m^{-3} day^{-1}. The former authors reported a methane production rate of about 1.25 ml CH4 h^{-1}, which is lower than the observed in this work (6.5 ml CH_4 h^{-1}). Thus, the depuration quality of their effluent is potentially lower when compared to a process with COD removal rate above 80 % and methane yields of 0.116 m^3 CH_4 $kgCOD_{vinasse}{}^{-1}$.

Maximum methane yields during high organic loading of vinasse may be achievable after long periods of gradual adaptation. However, it is very common to observe longer hydraulic retention time as a result of the increase on vinasse organic loading [32, 33]. In order to address these issues, several authors suggest a pretreatment of the vinasse before anaerobic digestion. Sile et al. [34] tested the process of vinasse ozonation in order to reduce the concentration of phenolic and other toxic substances. Rabelo et al. [35] reported 41 % increase in methane productivity after vinasse pretreatment with peroxidases. Therefore, in order to avoid such complications in this research, the anaerobic digesters were operated at an organic loading of 1 kg COD m^3. The main goal was to ensure success of the anaerobic process in order to test its effect on the use of treated and non-treated vinasse for growing *C. vulgaris*. Despite the fact that the vinasse organic loading may be considered as low, the process has achieved significant performance as assessed by COD removal and methane yields (Fig. 2). These variables reached steady state equilibrium at about 17 of the 25 days of operation. This is a clear indication that anaerobic treatment of vinasse was of good quality, and adaptations to the process should make treatment of higher organic loads feasible. Adjustment in the abundance and/or activity of methanogenics may be required for avoiding accumulation of acids.

Ryan et al. [36] reported that vinasse treatment is very expensive for the ethanol industry. Therefore, raw vinasse is commonly used for soil irrigation, but transportation costs and soil management can often be very expensive. In addition, large discharges of vinasse into the soil can also cause a significant environmental impact and fertility loss [36]. Therefore, anaerobic digestion of vinasse prior to irrigation has also been considered as an attractive alternative for reducing organics and toxic compounds [14]. Table 1 not only shows a substantial reduction on organic content but also on turbidity (84 %). However, further work is necessary in order to improve the process for coping with 100 % vinasse in the feed. Apart from the gradual adaptation of the microbial

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community, specific digester's design may yet be required. Nevertheless, the results show that anaerobic digestion is viable and productive biological process.

Growth of *C. vulgaris* on Vinasse

Growth experiments with *C. vulgaris* were prepared with distinct concentrations of anaerobically treated and raw vinasse mixtures as the sole nutrient sources (Fig. 1). Growth kinetic parameters are shown in Table 2, and the highest value was observed with TV-100 (0.76 day^{-1}). Experiments with raw vinasse mixtures showed a negative result for NVM-100 and NVM-50 %, respectively. A modest growth, however, was observed on NVM-25 (0.1 day^{-1}), which corresponds to a final vinasse concentration of 2 %. Valderrama et al. [37] reported a *C. vulgaris* growth of 0.46 day^{-1} on 10 % vinasse medium. However, Valderrama et al. [37] used a mixture of two industrial effluents with unique characteristics. They obtained vinasse from the process of producing citric/acetic acids apart from ethanol. *C. vulgaris* can expressively increase biomass production using acetates mixotrophically [38], and this fact may explain the contrasting algal growth rates at 2 and 10 % vinasse concentrations. In this work, the vinasse was obtained from an ethanol producing industry and it showed to be acutely toxic to *C. vulgaris* at concentrations higher than 4 %. Chemical characteristics are shown in Table 1.

Several authors identified that the presence of phenolic substances and melanoidins are often the cause of algal poor growth performances in the presence of untreated vinasse [19, 20, 39]. Some photosynthetic microorganism (*Oscillatoria boryana*) can tolerate higher concentrations by producing peroxide [40], but growth performance is still negatively affected. The addition of 1 %vinasse in the growth media showed to increase *Chlamydomonas reinhardii* growth rates in about 3.6-fold (from 0.25 to 0.9 day^{-1}), but the mixture was very toxic at ratios above 5 % [41]. A similar effect was observed with *Spirulina maxima* which increased growth rates (from 0.1 to 1.7 day^{-1}) at vinasse concentration of 2 gCOD l^{-1}, but it decreased sharply at higher amendments. In order to address such a toxicity issue, an attempt was made in culturing *C. vulgaris* in conjunction with *Lemma minuscula,* but algal biomass production was not efficient above vinasse concentrations of 10 % [37].

In this research, anaerobic digestion contributed significantly to reducing vinasse toxicity. Algal specific growth rate on TV-100 was 1.4-fold higher (0.76 day^{-1}) than the maximum observed with *C. vulgaris* grown on nutrient sufficient medium (0.53 day^{-1}).On the other hand, biomass and oil productivity was of about tenfold lower compared to nutrient sufficient medium, respectively (Table 2). The growth conditions between treatments and nutrient sufficient medium reported by Nascimento et al. [26] were very similar. The observed difference may be the result of two factors: (a) initial biomass concentration and (b) accumulation of lipid within the cell. Cabanelas et al. [42] showed that distinct initial biomass can generate significant differences in *C. vulgaris* biomass productivity with similar specific growth rates. The distinct accumulation of reserve material in nutrient sufficient conditions is also demonstrated elsewhere [43]. The later, however, can explain only a portion of such a difference, being the former most likely the factor responsible for the observed result. In the trial with nutrient sufficient medium, Nascimento et al. [26] started the experiment with an inoculum of a *C. vulgaris* pre-grown on the same medium. In this research, inoculum of *C. vulgaris* was pre-grown on nutrient sufficient medium and later transferred into the vinasse mixture. It was observed a decrease on OD (10 %) in the first hours, and therefore, it is reasonable to accept that a portion of this inoculum was adversely affected by such a change. In addition, several cells may have lost their capability of adapting and consequently growing in such a new condition. However, after adapting to

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this initial shock, the remaining cells encountered the nutritional condition (nutrients per cell) for growing with high specific growth rates (0.76 day^{-1}), in spite of low biomass productivity (Table 2). This result indicates that growth on treated vinasse mixtures must be improved by nutrient addition and inoculum adaptation. Increases in nutrient concentrations may be achieved by anaerobically treating higher concentrations of vinasse, but further investigations must be carried out in regard to the effect of potassium concentration of algal productivity. Distinct potassium concentrations can directly affect algal cells viability and growth rates [44]. Potassium is used for several functions related to the photosynthesis machinery, but high concentrations are known to be toxic, and they impose some metabolic adaptations [45]. In this research, potassium concentration (Table 3) was comparable to the observed toxic levels for *Microcystis* spp. [44].

The highest concentration and removal of NH_4 and PO_4 were observed in the treatment TV-100. Such initial concentrations were about three times higher than what was reported by Valderrama et al. [37] in comparable trials using vinasse. Working with treated domestic effluent, Cabanelas et al. [42] reported a significant growth rate (0.38 day^{-1}) and nutrient removal rates by *C. vulgaris* at NH_4 and PO_4 starting concentrations of 0.12 and 0.03 g l^{-1}, respectively. The former authors reported that at the cited initial concentrations, NH_4 and PO_4 removal rates were of 0.009 and 0.001 g l^{-1} day^{-1}, respectively. In the experiments with vinasse, the concentrations of the former nutrients were a few times lower than what was reported with domestic effluent (Table 3), but biological removal was efficient. Table 3 also

Table 3 Chemical characterization of the media before and after the growth experiments with *C. vulgaris*, respectively

(gL^{-1})	TV-100	TV-50	TV-25	NVM-25
COD				
Before	0.200 (±0.014)	0.100 (±0.007)	0.050 (±0.004)	0.325 (±0.023)
After	0.119 (±0.008)	0.070 (±0.005)	0.027 (±0.002)	0.083 (±0.006)
PO_4				
Before	0.014 (±0.001)	0.007 (±0.001)	0.004 (±0.001)	0.004 (±0.0002)
After	0.0	0.0	0.0	0.0
K				
Before	0.038 (±0.002)	0.019 (±0.001)	0.010 (±0.001)	0.008 (±0.001)
After	0.030 (±0.002)	0.015 (±0.001)	0.009 (±0.001)	0.012 (±0.001)
NO_3				
Before	0.002 (±0.0)	0.001 (±0.0)	0.000	0.002 (±0.0)
After	0.0	0.0	0.0	0.0
NH_4				
Before	0.020 (±0.0)	0.010 (±0.0)	0.005 (±0.0)	0.000 (±0.0)
After	0.0	0.0	0.0	0.0
pH				
Before	6.7 (±0.1)	6.7 (±0.1)	6.7 (±0.1)	6.7 (±0.1)
After	7.0 (±0.1)	7.5 (±0.1)	7.3 (±0.1)	7.0 (±0.1)
Turbidity (NTU)				
Before	85 (±1.31)	43 (±0.95)	21 (±0.38)	65 (±1.14)
After	0.8 (±0.06)	0.4 (±0.03)	0.2 (±0.02)	0.8 (±0.06)

TV treated vinasse; *NVM* neat vinasse mixture (non-treated)

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shows that *C. vulgaris* have considerably reduced turbidity values, suggesting further complementing action to the treatment obtained with the anaerobic digestion (Tables 1 and 3). It was observed a very small change on COD concentrations (Table 3), suggesting very little contribution of mixotrophic activity to the observed growth rates. As expected, potassium concentrations remain statistically unchanged during vinasse treatment with *C. vulgaris*. Further investigation is needed in order to address this issue. However, the combination of anaerobic digestion and *C. vulgaris* cultivation showed significant reduction of vinasse organics, N and P contents in negative correlation with methane and algal oil production.

Conclusion

Vinasse used in this research was highly toxic to *C. vulgaris* at concentrations above 4 %. Among the treatments, anaerobically treated vinasse (at about 8.6 % of the feed) generated the highest values of specific growth rates (0.76 day^{-1}, respectively). These values are significantly higher than what was observed with nutrient sufficient medium (0.53 day^{-1}) incubated at the same condition. Algal biomass productivity was lower between the vinasse trials and nutrient sufficient media, and such result was associated to lower inoculum viability and cellular accumulation of lipids. A modest growth (0.1 day^{-1}) was also observed with untreated vinasse at about 1.4 %. *C. vulgaris* have significantly reduced N, P, and turbidity values from the media, corroborating the idea of using them as a tertiary process of treatment for effluents. The anaerobic process treating vinasse reached a steady state at about 17 batch cycles of 24 h with a COD removal rate above 80 %. Methane productivity was of about 0.116 $m^3CH_4\ kgCOD_{vinasse}{}^{-1}$. The anaerobic process was not only efficient in removing COD but it also reduced vinasse turbidity in 84 %.

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