

Optimization and qualitative comparison of two vinasse pre-treatments aiming at microalgae cultivation

Otimização e comparação qualitativa de dois pré-tratamentos da vinhaça visando ao cultivo de microalgas

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ABSTRACT

The cultivation of microalgae is a possible destination for vinasse, a residue from the sugar and alcohol industry. This use can help reduce the costs of microalgae production and remediate this residue rich in nutrients. However, the physicochemical characteristics of vinasse limit its use for microalgae growth at low concentrations, except when the residue is pretreated. This work aimed at optimizing the vinasse pretreatments of centrifugation and adsorption by smectite clay and activated charcoal on laboratory scale in terms of amounts of materials used and time spent, making them more viable on larger scales. The optimized processes were then compared in productive, economic, and environmental terms. The dilution of treated vinasse with distilled water resulted in similar growth of *Chlorella vulgaris* to those obtained with the dilution in BG11 medium, indicating that the addition of nutrients in culture media is not necessary. Although microalgae growth occurs in higher concentrations of vinasse treated by adsorption, the results show that centrifugation required less processing time, has cheaper processing costs, and generated much less residue. Centrifugation treatment has greater economic and environmental viabilities and was more sustainable than the adsorption, even though the algae did not grow in the centrifuged residue in concentrations as high as it did after the adsorption treatment. Therefore, this article brings a new view about the economic and environmental aspects on the use of pretreated vinasse for microalgal growth, giving a lucrative destination for a highly polluting waste.

Keywords: waste use; physicochemical treatments; sustainable production; algae biomass.

RESUMO

O cultivo de microalgas é um possível destino para a vinhaça, um resíduo da indústria sucroalcooleira. Esse uso pode ajudar a reduzir custos da produção de microalgas, além de remediar esse resíduo rico em nutrientes, entretanto as características físico-químicas da vinhaça limitam seu uso para o crescimento de microalgas a baixas concentrações, exceto quando o resíduo é pré-tratado. Este trabalho objetivou otimizar os pré-tratamentos da vinhaça de centrifugação e de adsorção por argila esmectita e carvão ativado em escala laboratorial quanto à quantidade de materiais utilizados e tempo gasto, tornando-os mais viáveis em escalas ampliadas. Os processos otimizados foram então comparados em termos produtivos, econômicos e ambientais. A diluição das vinhaças tratadas com água destilada resultou em crescimento de *Chlorella vulgaris* semelhante ao obtido com diluições com meio BG11, indicando que a adição de nutrientes nos meios de cultura não é necessária. Embora o crescimento de microalgas ocorra em concentrações mais altas de vinhaça tratada por adsorção, os resultados mostram que a centrifugação exigiu menos tempo de processamento, tem custos mais baratos e gerou menos resíduos. De maneira comparativa, o tratamento por centrifugação possui maior viabilidade econômica e ambiental e foi mais sustentável do que a adsorção, embora as algas não tenham crescido no resíduo centrifugado em concentrações tão altas quanto após o tratamento de adsorção. Portanto, este artigo traz uma nova visão sobre os aspectos econômicos e ambientais do uso da vinhaça pré-tratada para o crescimento de microalgas, proporcionando um destino lucrativo para um resíduo altamente poluente.

Palavras-chave: uso de resíduos; tratamentos físico-químicos; produção sustentável; biomassa de algas.

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INTRODUCTION

The production of ethanol from sugarcane releases high volumes of vinasse, whereas 1 L of ethanol generates about 12 L of vinasse (UNICA, 2017). As a world leader in ethanol production from sugarcane, Brazil generates about 3×10^8 m³ of vinasse yearly (UNICA, 2017). According to Silva, Griebeler and Borges (2007), sugarcane vinasse carries high mineral and organic nutrients, which has led to its application as a soil fertilizer. However, the excessive application of the residue in the soil can generate its salinization, and such vinasse use is controlled by governmental agencies (SILVA; GRIEBELER; BORGES, 2007). The need to improve vinasse quality, so reducing fertirrigation-related problems, has been investigated by Rocha, Lora and Venturini (2008). They analyzed the life cycle and feasibility of vinasse biodigestion, dewatering and combustion, concluding that such techniques are necessary for alternative uses of vinasas, likewise the search for other destinations for the residue, that are welcome and can help mitigate the related environmental problems.

The nutritive value of vinasse, that allows its use in the soil, also permits its use as a culture media for microalgae. However, vinasse characteristics such as dark coloring, high turbidity and light absorption, osmolarity, toxicity and yeasts competition can be problematic for microalgae, impairing their viabilities and reproductive capacities (KADIOGLU; ALGUR, 1992; RAVEN; EVERT; EICHHORN, 2007). The development of alternative technologies for vinasse use, as microalgae cultivation, could help ethanol-producing plants to bioremediate the waste, generate new sources of energy, create ways to produce biomass for animal supplementation and save water with their productive processes (REIS; HU, 2017).

A pioneer study by Oliveira (1988) about the use of vinasse in microalgae culture medium showed that high dilutions of the residue are necessary to microalgae growth. The author used vinasse at concentrations of 0.1 and 0.5% in *Chlorella vulgaris* cultures with addition of urea to complement the nitrogen source. Since then, other authors have used raw vinasse to grow microalgae. Kadioglu and Algur (1992) succeeded in increasing the growth of *Chlamydomonas reinhardtii* in vinasse at concentrations up to 2.5%, in relation to the control. Barrocal et al. (2010) supplemented Schlösser medium (SCHLÖSSER, 1994) with 5 g L⁻¹ of vinasse for the growth of *Spirulina maxima*. Budiyo, Sumardiono and Sasongko (2014) used biodigested vinasse up to 0.8% to grow *Spirulina platensis*. Coca et al. (2015) cultivated this same alga in vinasse up to 2 g L⁻¹, what promoted increased protein production. More recently, Santos et al. (2016) cultivated *Spirulina maxima* under heterotrophic and mixotrophic conditions at 0.1 and 1% of vinasse. Gollo et al. (2016) cultivated the vegetative form of *Haematococcus pluvialis* in 3% of vinasse, the highest concentration found in the literature for raw vinasse diluted. It is clear from the cited literature that just highly diluted raw vinasse can support microalgae growth. Thus, raw vinasse is not a good substrate for microalgae cultivation, hence the need for pretreatments that result in better algal development.

Mitra, Van Leeuwen and Lamsal (2012) investigated a vinasse treatment prior to microalgal cultivation and grew *C. vulgaris* in previously sedimented and siphoned corn vinasse. They used 100% of the supernatant and obtained high lipids in the algal biomass. Marques et al. (2013) cultivated *C. vulgaris* in anaerobically treated vinasse and obtained growth rates of 0.76 d⁻¹. Using commercial bold basal medium (BBM) supplemented with up to 5% of centrifuged vinasse, Engin et al. (2018) obtained 0.17 g L⁻¹ day⁻¹ of *Micractinium sp.* in mixotrophic cultures, with increase in lipid content. Candido and Lombardi (2017) obtained

growth rates as high as 1.2 d⁻¹ for *C. vulgaris* cultivated in vinasse that was treated by centrifugation or adsorption in smectite clay and activated charcoal. The authors evaluated the use of the vinasse treated by the two processes, diluted from 10 to 100% with water, for *C. vulgaris* and obtained the concentrations that allowed optimum algal growth, which represented a balance between nutrient availability and reduction in turbidity, in contamination and in toxicity of the residue. While higher algal growth rate was obtained in 20% of centrifuged vinasse, for the adsorption in smectite clay and active charcoal treated vinasse, similar growth parameters were obtained at 60% of vinasse (CANDIDO; LOMBARDI, 2017).

The studies that analyze microalgae culture in pretreated vinasse always deal with the parameters of algal growth in the residue. There are no authors who considered and discussed procedural aspects and viabilities of the investigated pretreatments, regarding the use of time, equipment, materials and energy and the generation of waste. In the present research, the objectives were to optimize the two different pretreatments of vinasse proposed in Candido and Lombardi (2017): centrifugation, a physical process, and adsorption, a physicochemical process, on smectite clay and activated charcoal. The optimization consisted of using as little as possible of materials and time for treatments. Different from most reports on the subject, we compared and discussed the economic and environmental viabilities of both treatments.

METHOD

The vinasse used was collected at the São João Plant, located in Araras, São Paulo, Brazil, in 20 L thermoresistant plastic gallons previously washed. The vinasse was collected at approximately 80°C and cooled to room temperature (~ 25°C). Subsequently, the residue was passed through a sieve with 0.8 mm pore to remove large materials such as vegetable debris and soil particles. It was dispensed in 1-L plastic bottles previously washed and frozen at $-5 \pm 2^\circ\text{C}$ until use. The vinasse was subjected to two different treatment processes, centrifugation and adsorption, onto smectite clay and activated charcoal, as described ahead.

Centrifugation

Plastic tubes with approximately 45 mL of raw vinasse were centrifuged at the temperatures of 10, 15, 20 or 25°C, with rotations of 2,000, 3,000 or 4,000 rpm, corresponding to around 630, 1,410 or 2,510 g, respectively (Sorvall Legend XTR centrifuge, Thermo Fisher Scientific, United States). Samples were centrifuged under the described conditions at increasing intervals every two minutes, reaching a maximum time of 20 minutes. The supernatants resulting from each condition were analyzed for pH (Logen Scientific, Brazil), absorbance at 570 and 455 nm (Nanocolor spectrophotometer, Macherey-Nagel, United Kingdom) and visualized under optical microscope (Eclipse E200, Nikon, Japan), with magnification of 400 times, for observation of yeasts and particulates.

Neutral pH values are closer to those of natural aquatic environments and, therefore, more amenable to algal development (REYNOLDS, 2006), in contrast to the acidic pH (~ 4.5) of the crude vinasse. The absorbance at 570 nm is related to the amount of particulate material in the medium (COSTA *et al.*, 2003), while the absorbance at 455 nm is relative to the vinasse color (APHA, 1995). Both the particulate material and the vinasse coloring are related to light input into the medium, an essential resource for microalgae, which are photosynthetic organisms (RAVEN; EVERT; EICHHORN, 2007; REYNOLDS, 2006). Besides that, most of the particulates present in vinasse correspond to yeasts,

which can compete for mineral and space resources with microalgae, reducing their growth and increasing the final contamination of the biomass produced (CANDIDO; LOMBARDI, 2018). Already the dark vinasse color is attributed to the presence of phenolic compounds called melanoidines, related to the toxicity of the residue to microorganisms (MOHANA; ACHARYA; MADAMWAR, 2009; RYAN *et al.*, 2008). Therefore, improvements in the physicochemical properties of vinasse can be linked to the possibility of optimal algal growth.

From the collected data, the lowest time, rotation speed and temperature decrease that allowed the best vinasse improvement, with lower values of absorbance and without the presence of yeasts and visible particles in optical microscopy, were selected.

Adsorption

According to Candido and Lombardi (2017), the adsorption of vinasse onto smectite clay and activated charcoal reduced the amount of particulate matter and the excess of mineral nutrients, and increased its pH, making the residue favorable for algal development. The procedures are described in detail in a patent registration (BR 10 2015 024100 3 — Brazilian National Institute of Industrial Property — CANDIDO; LIMA; LOMBARDI, 2014).

Smectite clay and activated charcoal are highly adsorbent materials commonly used in industrial processes (GUPTA; SUHAS, 2009; TENORIO ARVIDE *et al.*, 2008). To make the process faster, instead of adsorbing the vinasse by smectite clay by means of percolation, as described in Candido and Lombardi (2017), we mixed the clay with the vinasse using a rotating motor with a pitched-blade impeller attached. Preliminary tests confirmed that the improvement was the same as that one generated by the process dependent solely on gravity.

For the optimization of the smectite clay and vinasse mixture, amounts of 250, 200, 150, 100, 80 and 50 g of clay per liter of vinasse were tested. The velocity used in the rotor was the lowest as possible, to allow all the clay to be suspended in the vinasse (90 to 130 rpm), which was directly proportional to the mass of clay used. Samples were collected at intervals of 5, 10, 15, 20, 30, 40 and 60 min of mixing. These samples were centrifuged at 1,410 g, at 20°C for 10 min, only to accelerate the separation of the clay from the vinasse, and the supernatant was immediately analyzed for pH and absorbance at 570 and 455 nm.

In a plant wishing to grow algae on a large scale, centrifuging the vinasse and clay mixture would add a further stage to the treatment and make the process more expensive, although accelerating it. A cheaper alternative for this separation would be the decantation of the clay and adsorbed material. Imhoff cones were used to evaluate the decantation time and decanted volume. At times of 10, 20, 30, 40, 50, 60, 90, 120, 150 and 180 min and 4, 5, 6, 9, 12, 18 and 24 h, the samples were analyzed for pH and absorbance at 570 and 455 nm.

As for the adsorption through activated charcoal, previous tests showed that 1-minute contact time of the vinasse with the charcoal in a column is enough to reduce vinasse color before the saturation of the solid. Therefore, in the optimization of this step, we measured the maximum amount of vinasse that could pass through the charcoal before its clogging. Mixing the charcoal with the vinasse did not result in improvement of the process, since the charcoal could not be posteriorly separated from the vinasse nor by decantation, filtration or centrifugation. Therefore, the adsorption of vinasse by charcoal was made by percolation, with only gravity action. For the percolation of the vinasse by this material, the activated charcoal was deposited in a paper porous membrane to prevent the material scape during the process.

Comparison of the treatments

Based on the optimized steps, the processes of centrifugation and adsorption were compared considering the algal yield and the necessary investments and wastes generated.

The vinasse resulted from the treatments of centrifugation, adsorption through smectite clay and adsorption through smectite clay and activated charcoal was analyzed for physicochemical properties, as described in Table 1.

This physicochemical vinasse analyses enabled certification of the modifications promoted by each treatment. The excessive amount of mineral nutrients present in the vinasse generates an osmotic obstacle to the survival of microalgae (KADIOGLU; ALGUR, 1992), hence the importance of reducing these values in the pre-treatments of the residue aiming at algal cultivation.

In order to evaluate the effectiveness of the previously optimized vinasse pre-treatments for algal cultivation, cultures of *C. vulgaris* were performed at the optimal concentrations determined by Candido and Lombardi (2017) of 20% for the centrifuged residue and 60% for the adsorbed one. The vinasse dilutions were made with distilled water, as performed by Candido and Lombardi (2017), and also with BG11 medium (RIPPKA *et al.*, 1979), which is known to be rich in nutrients and widely used industrially. The media had their pH values adjusted to 6.8–7, and cultures were performed in three experimental replicates in tissue culture flasks with a vented lid containing 150 mL of medium. The samples were kept in a culture room with controlled temperature of $25 \pm 2^\circ\text{C}$ and internal luminosity of $130 \mu\text{mol of photons m}^{-2} \text{s}^{-1}$. Over 96 h, cell densities were evaluated daily in the Muse Cell Count & Viability Assay (Merck Millipore, United States) automatic counter. Growth rates were obtained from the linear regressions in the exponential phases of cultivation.

Table 1 - Methods used for the vinasse characterization.

Parameter	Methods
COD	SMEWW 22 ^a Ed 2012 Method 5220 D - POPDAM029 vs.22:2014
BOD	SMEWW 22 ^a Ed 2012 Method 5210 B - POPDAM009 vs.22:2015
Total suspended solids	SMEWW 22 ^a Ed 2012 Method 2540 D - POPDAM024 vs.17:2014
Calculated hardness	MEWW 22 ^o Ed 2012 Method 2340 B - POPDAM011 vs.16:2014
Electrolytic conductivity	SMEWW 22 ^a Ed 2012 Method 2510 B - POPDAM006 vs.14:2014
Anions	USEPA3001 rev 1:1997- POPDAM054 vs. 08:2013
Ammoniacal nitrogen	SMEWW 22 ^a Ed 2012 Method 4500-NH3D - POPDAM016 vs.14:2013
Kjeldahl nitrogen	SMEWW 22 ^a Ed 2012 Method 4500-Norg B - POPDAM107 vs.06:2013
Total metals	SMEWW 22 ^a Ed 2012 - Method 3030E USEPA 6010 C - rev.03:2007 POPDAM060
Malic and trans-aconitic acids	High performance liquid chromatography (HPLC)
Organic, inorganic and total carbon and total nitrogen	Auto Sampler Shimadzu model ASI-L (Japan)

COD: chemical oxygen demand; BOD: biochemical oxygen demand; USEPA: United States Environmental Protection Agency.

Source: elaborated by the authors.

A qualitative overview of the quantity of materials, equipment, procedures and energy required for the execution of each treatment, as well as its mechanisms of action, generation of residues and durability of the machinery involved, was obtained.

All the results were plotted on graphs using the Origin 8.5 program.

RESULTS AND DISCUSSION

Centrifugation

The centrifugation process did not affect vinasse's pH, whose values ranged within 4.52 to 4.61, indicating the need for the basic substances use such as sodium hydroxide (NaOH), to neutralize the pH for good algal development (REYNOLDS, 2006). Figure 1 shows particulate and color reductions. The differences in centrifugal intensities had more influence on the reduction of the absorbance values (570 and 455 nm) than the temperature.

Figure 2 shows the presence or absence of yeasts and other particulates that were evaluated after centrifugation of vinasse samples under optical microscope.

The removal of yeasts and particulates was the best at the lowest temperature and the highest centrifugation intensity. Again, the differences in centrifugal intensities had more influence on the reduction of contaminants than the temperature. Considering that sugar-alcohol plants usually have no refrigerated industrial centrifuges (SALES *et al.*, 1986; SAUZE, 1973), the temperature of 25°C, next to the ambient temperature for most Brazilian regions (INMET, 2017), was adopted for subsequent evaluations. Also, based on the experiments, rotation intensity of 2,510 g and 12-minute centrifugation time were defined as the best.

Adsorption

Adsorption through smectite clay did not affect vinasse's pH, which varied from 4.47 to 4.58, but absorbance at 570 and 455 nm were significantly affected, as

shown in Figure 3. The reductions in absorbance promoted by the concentrations of 250, 200, 150 and 100 g L⁻¹ smectite clay per liter of vinasse were similar, and in 80 and 50 g L⁻¹ they were lower. Thus, 100 g L⁻¹ of smectite clay with minimum contact time of 20 min was the best combination.

Figure 4 reports the smectite clay settling as function of time. After 1 h, about 50% of the clay had settled, and after 6 h 68% was obtained. This led to 73% absorption reduction at 570 nm and 60% at 455 nm in relation to the raw residue.

Therefore, after 6 h for sedimentation, the supernatant followed to subsequent adsorption through activated charcoal. The maximum amount of vinasse previously adsorbed by smectite clay that could be adsorbed by activated charcoal before its saturation was reported in Figure 5.

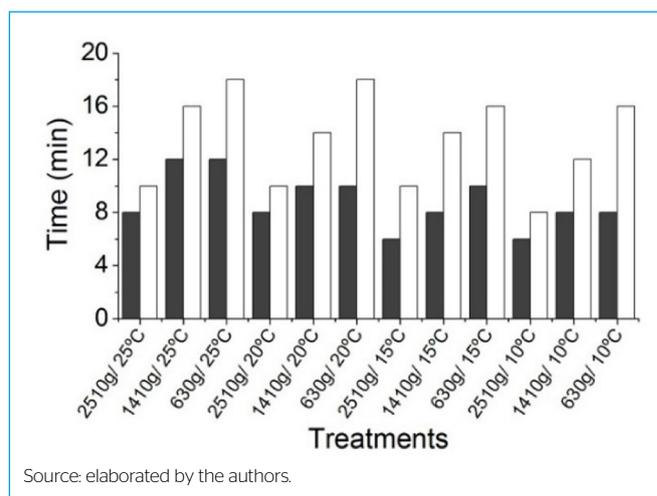


Figure 2 - Minimum times (min) required for the elimination of yeasts (dark bars) and other particulates (light bars), visible under optical microscope, for each centrifugation intensity/temperature condition.

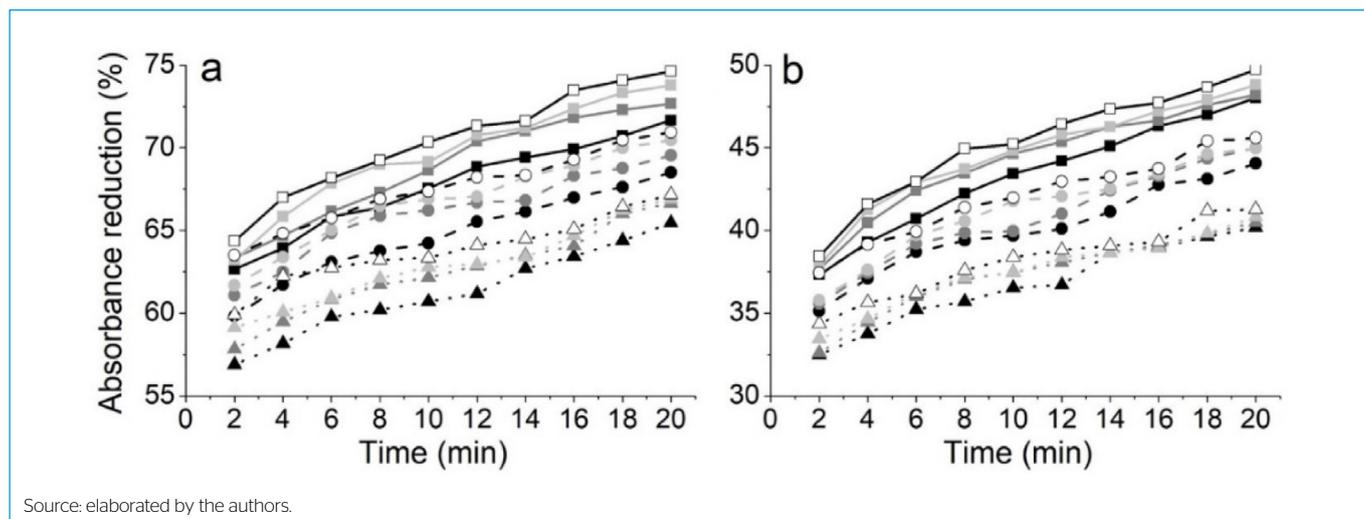
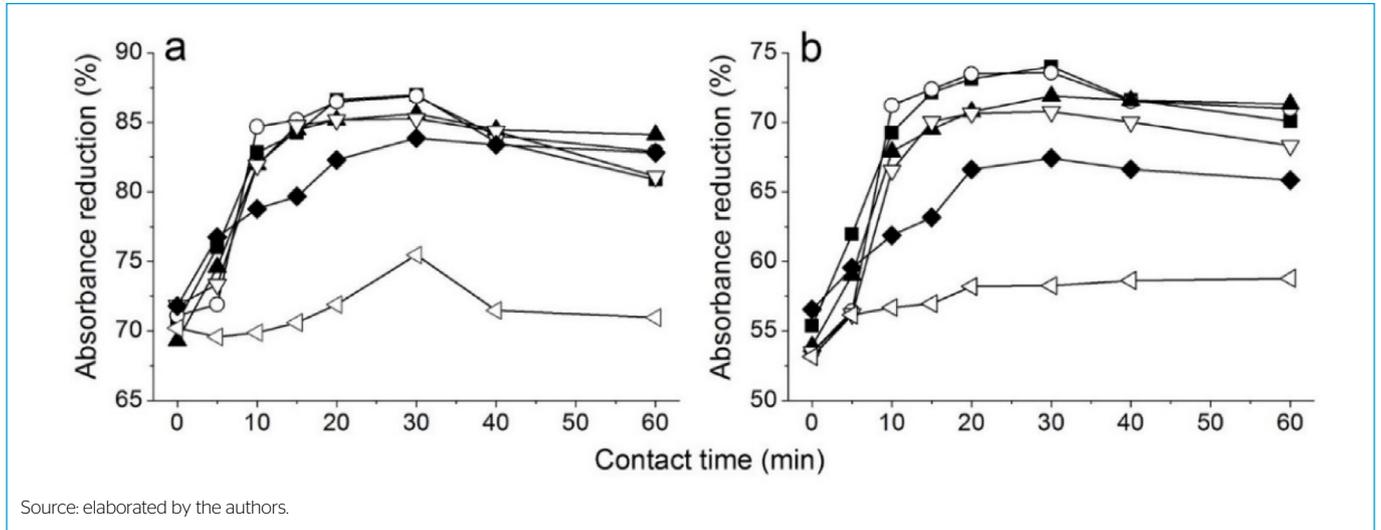
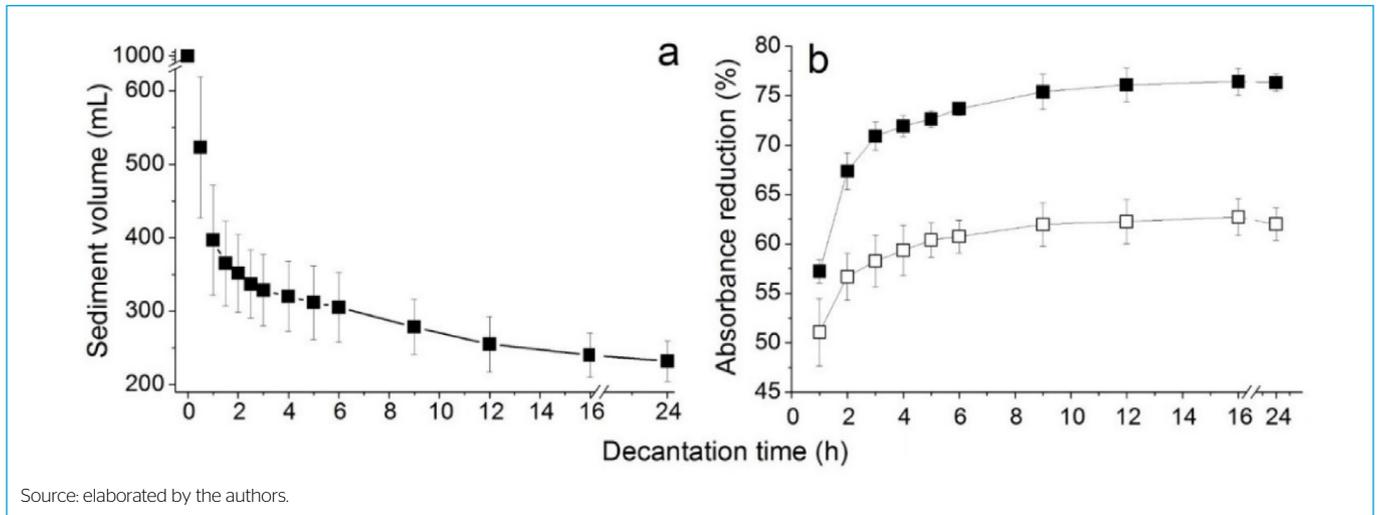


Figure 1 - Percent of absorbance reduction (%) at (A) 570 nm and at (B) 455 nm along the time of centrifugation (min). Conditions: 2,510 g / 25°C, 1,410 g / 25°C, 630 g / 25°C, 2,510 g / 20°C, 1,410 g / 20°C, 630 g / 20°C, 2,510 g / 15°C, 2,510 g / 15°C, 630 g / 15°C, 2,510 g / 10°C, 2,510 g / 10°C and 630 g / 10°C.



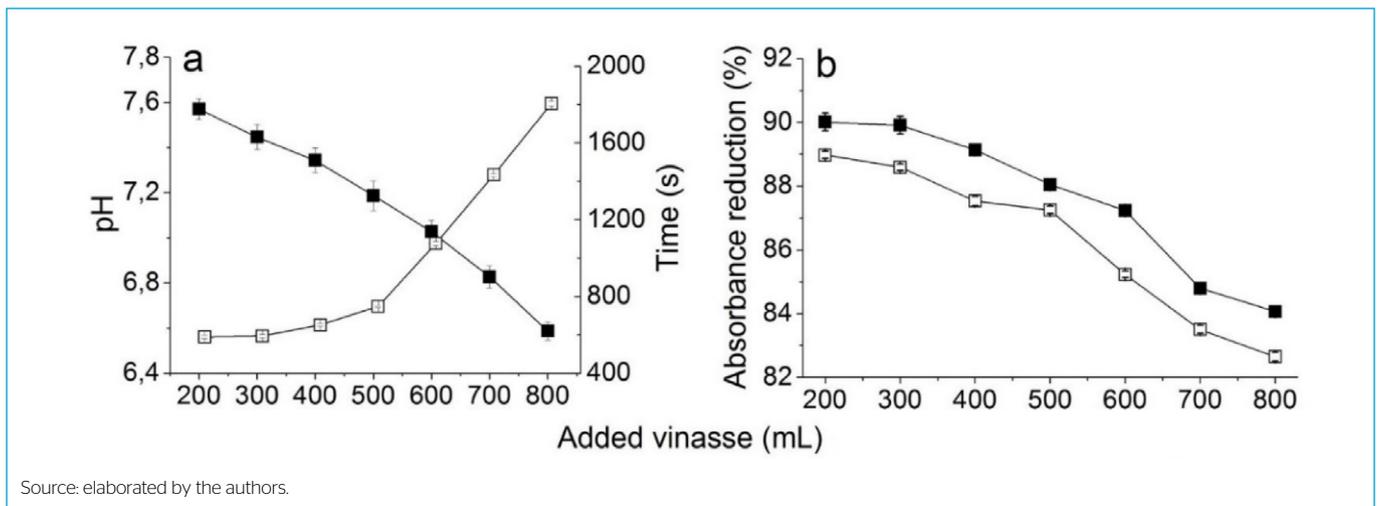
Source: elaborated by the authors.

Figure 3 - Percent of absorbance reduction (%) at (A) 570 nm and (B) 455 nm promoted by mixing 1 L of vinasse with distinct masses of smectite clay (g) as function of contact time (min). Symbols apply for 250 g L⁻¹, 200, 150, 100, 80 and 50 g L⁻¹.



Source: elaborated by the authors.

Figure 4 - (A) Volume of sediment (clay + vinasse particulate; mL) and (B) reduction of absorbances at 570 and 455 nm along the settling time (h) performed on Imhoff cones.



Source: elaborated by the authors.

Figure 5 - (A) Values of pH in the vinasse treated by smectite clay and activated charcoal and percolation time for obtain 100 mL of final treated residue. (B) Percent of absorbance reduction (%) relative to the raw vinasse at 570 nm and at 455 nm as a function of pretreated vinasse volume added in 30 g of activated carbon.

The adsorption of 500-mL vinasse by a fixed bed of charcoal was the optimum considering adsorption rate, pH increase and absorbance reductions, with values closer to those obtained for the first 100 mL. Above this volume, pH values had a smaller increase, and adsorption rate almost stopped. So, the best ratio of vinasse volume (mL): activated charcoal mass (g) was defined as 500 mL: 30 g.

Treatments' comparison

The physicochemical results for the treated vinasses are reported in Table 2. It shows the values of pH, absorbance at 570 and 455 nm, chemical oxygen demand, biochemical oxygen demand and chemical composition of the raw vinasse, the centrifuged one, the one treated only with smectite clay and the one treated with smectite clay and activated charcoal. The results refer to those procedures considered as optimum conditions, as previously determined.

These values show that, for important parameters as pH, color (absorbance at 455 nm) and total organic carbon, the treatment with just smectite clay

Table 2 – Physicochemical analysis of the vinasses. Except for the raw vinasse (Rv), whose values presented are absolute, the other ones are reported as percent in relation to the raw vinasse (%) for the centrifuged vinasse (Cent.), vinasse treated with smectite clay (Sc) and vinasse treated with smectite clay and activated carbon (Sc Ac).

Parameters evaluated	Vinasse samples			
	Rv	Cent.	Sc	Sc Ac
pH ($-\log[H^+]$)	4.58	0	0	+61.1
Absorbance 570 nm (UA)	3.650	-81.3	-83.8	-89.3
Absorbance 455 nm (UA)	5.653	-67	-73.9	-88.5
COD ($\text{mg O}_2 \text{ L}^{-1}$)	51,817	-13.4	-26.8	-37.2
BOD (mg L^{-1})	24,207	-29.7	-30.3	-47.3
Total suspended solids (mg L^{-1})	45,900	-97.3	-95.5	-98.2
Calculated hardness ($\text{mg CaCO}_3 \text{ L}^{-1}$)	3,034	-48.1	-36.5	-44.8
Electrolytic conductivity ($\mu\text{S cm}^{-2}$)	19,852	-41.5	-45	-31.4
Total carbon (mg L^{-1})	17,416	-7.2	-13.5	-40.1
Total organic carbon (mg L^{-1})	17,388	-7.2	-13.6	-40.4
Total inorganic carbon (mg L^{-1})	28.16	-24.2	+20.8	+1676
Total nitrogen (mg L^{-1})	764	-17.2	-28.4	-58.6
Nitric N (mg L^{-1})	< 11.400	ND	ND	ND
Nitrous N (mg L^{-1})	< 0.300	ND	ND	ND
Ammoniacal N (mg L^{-1})	134.7	-74.8	-78.5	-81.6
Kjendahl N (mg L^{-1})	492	-30.8	-43.8	-59.5
Na (mg L^{-1})	36.6	-71.6	-62.3	-65.6
Ca (mg L^{-1})	735	-11.8	+5	+5.7
K (mg L^{-1})	4,340	-1.4	-12	-11.8
Mg (mg L^{-1})	498	0	-2.8	-5
Sulfate (mg L^{-1})	1,327	-0.8	+39.8	+60.5
Fosphate (mg L^{-1})	< 2,000	ND	ND	ND
K_2O ($\text{kg K}_2\text{O m}^{-3}$)	5.21	-0.9	-12.1	-11.9
Malic acid	1,630,61	-19.4	-38.4	-36.4
Trans-acotinic acid	626,88	-19.6	-51.6	-62.5

COD: chemical oxygen demand; BOD: biochemical oxygen demand; ND: values beyond the detection limit of the methods used.

Source: elaborated by the authors.

promoted minor modifications in comparison with the treatment with smectite clay and activated charcoal. However, the changes in vinasse chemical composition promoted by the treatment with smectite clay and activated charcoal are similar to those generated by smectite clay by itself. Considering the reduction in particulate material (absorbance 570 nm), the centrifugation caused improvements similar to those obtained from these two stages of adsorption.

According to the results of the microalgae *C. vulgaris* cultivation, presented in Candido and Lombardi (2017), both the centrifugation and the treatment of the vinasse with smectite clay and activated charcoal promoted changes in the vinasse that made it more suitable for the algal development, with different optimum dilutions. However, the nature of these modifications and the mechanisms of action of each process were different, as demonstrated in this study. The vinasse centrifugation reduced mainly the particulate materials, confirmed by the reduction in absorbance at 570 nm (COSTA *et al.*, 2003) and in total suspended solids. This process also reduced the organic and mineral contents, but this effect was lower than that promoted by the treatment with smectite clay and activated charcoal. Besides the removal of particulate materials, the centrifugation removed the yeasts as well, a major problem for the growth of microalgae in vinasse, as reported in Candido and Lombardi (2017; 2018) The yeasts reduce light penetration necessary to the photosynthetic organisms and compete with the microalgae for mineral and organic nutrients (CANDIDO; LOMBARDI, 2018; REYNOLDS, 2006).

The treatment with just smectite clay promoted particulate reduction similar to the centrifugation, since it also removed yeast from the vinasse, as it can be observed by optical microscopy of the adsorbed residue. However, vinasse adsorption by smectite clay caused important chemical modifications that were not effectuated by the centrifugation alone. In general, a trend towards reduction in the concentration of vinasse components, with the exception of calcium and sulfate. According to Lira Junior *et al.* (2017), this may be due to the solubilization of calcium and sulfate ions from the clay.

The adsorption of vinasse previously treated with smectite clay through activated charcoal was the most efficient step in the removal of mineral content, as well as its color. According to Robles-González *et al.* (2012), the melanoidins are the main components of the vinasse responsible for its dark color. Therefore, activated charcoal may have retained these compounds, confirming the results of Liakos and Lazaridis (2016), that used activated charcoal for discoloration of molasses. Besides that, smectite and charcoal treatment reduced more the concentrations of trans-aconitic acid, the predominant organic acid in sugar-cane leaf juice (ZAPATA, 2007). Its greater reduction may indicate the capacity of these substrates on removing complex organic particles. Thus, vinasse treated with smectite clay and activated charcoal allows for greater penetrance of light, lower nutrient content, osmotic stress and toxic components to an algal production, hence the fact that we can use the treated waste under these conditions in larger concentrations.

Daily cell densities of *C. vulgaris* obtained in media with treated vinasse by means of optimized centrifugation or adsorption are shown in Figure 6.

The average growth rates in controls on BG11 were 1.215 day^{-1} . Table 3 shows the growth rates for the microalga in each culture.

Comparing the algal growths obtained with the treatments optimized with the growth rates achieved by Candido and Lombardi (2017) in the same treatments without the optimization, we noticed that, for the adsorption by smectite clay and activated carbon, the optimization did not interfere in the

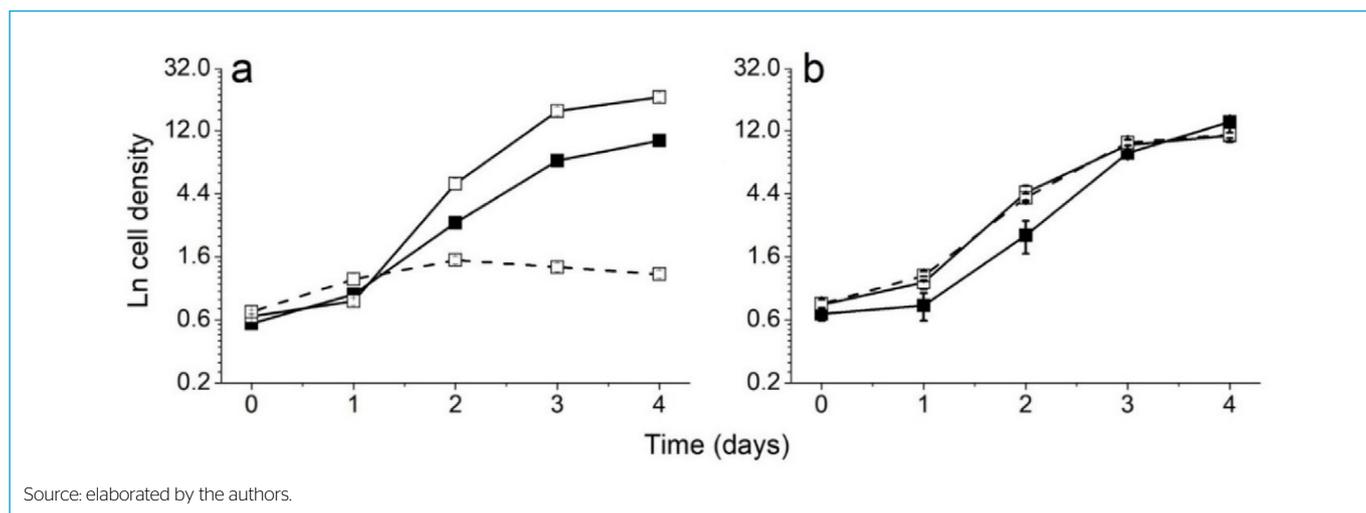


Figure 6 - Ln of the daily cell densities of *Chlorella vulgaris* in (A) vinasse centrifuged at 20% and in (B) vinasse adsorbed by smectite clay and activated charcoal at 60% optimally treated. Symbols: controls in BG11 medium and treated vinasse diluted with distilled water (solid lines) or with BG11 medium (dashed lines).

Table 3 - Mean values (n=3) of growth rates (day⁻¹) of *Chlorella vulgaris* in treatments with 20% centrifuged or 60% adsorbed vinasse diluted with distilled water or with BG11 medium. Standard deviations are represented between parentheses.

Treatments	Centrifuged vinasse at 20%	Adsorbed vinasse at 60%
Dilution with distilled water	1.477 (0.062)	1.089 (0.056)
Dilution with BG11 medium	0.408 (0.020)	1.055 (0.043)

Source: elaborated by the authors.

development of *C. vulgaris*. As for centrifugation, the optimization modified the medium values of growth rates from 1.2 to 1.5 day⁻¹. Given that the vinasse samples used in each study were different, we cannot infer about the significance of differences between the algal growths in centrifuged vinasse at 20%. Therefore, in addition to reducing the amount of materials, energy and equipment used in adsorption or centrifugation, the optimization did not cause any damage to the development of the microorganisms.

In the case of media produced with 20% of centrifuged vinasse, dilution with BG11 instead of distilled water reduced the algal growths, while in the media with adsorbed vinasse the growths remained the same regardless of the diluent. According to Kadioglu and Algur (1992), the excess osmolarity of culture media containing vinasse impairs cell development. Since centrifugation alters the ionic content less than adsorption, diluting the centrifuged vinasse with BG11, the final osmolarity may have exceeded the adaptive capacity of the algal cells. Adsorption reduces the quantity of ions up to 65.6%, as shown in Table 2, so that dilution with BG11 did not adversely affect osmotic cells. Therefore, both the centrifugation and adsorption allow the use of the vinasse without adding nutrients, reducing production costs.

In view of this general picture of the treatments, they were compared in order to note the pros and cons of each one and to discuss what would be most economically and environmentally viable for a sugar-alcohol plant to treat vinasse intending its use in algal cultivation. The considered aspects are reported in Table 4.

Table 4 - Evaluation and comparison of the various aspects involved in the vinasse treatments of centrifugation and adsorption by smectite clay and activated carbon.

Parameter	Centrifugation	Smectite clay and activated charcoal
Treated vinasse ^a	97%	40%
Materials for treatment	NaOH for pH correction	Smectite clay, activated charcoal, HCl for pH correction
Waste generated in treatments ^b	0.02 kg of centrifugation cake L ⁻¹ vinasse	0.10 kg of clay, 0.06 kg of charcoal and - 0.60 kg of adsorbed materials L ⁻¹ of vinasse and a porous membrane
Equipment used in treatment	Centrifuge	Mechanical mixer, decantation tank and filtration system
Stages of treatment	Centrifugation and supernatant collection	Mixing vinasse with smectite clay, decantation, supernatant collection, charcoal filtration
Processing time	0.2 h using 2,510 g	- 8 h
Treated vinasse in cultures	20%	60%
Algal growth	1.477 day ⁻¹	1.089 day ⁻¹
Algal cell yield in 96 h ^c	2.0 10 ¹² cells m ⁻³	1.1 10 ¹² cells m ⁻³

^aPercent (v/v) of the final treated vinasse in relation to vinasse at the beginning of the process; ^bsmectite clay and activated carbon could be possibly reutilized if an elution step is considered, which would result in less material used, but more water consumption and effluent generation. This possibility was not tested in this study; ^caccording to the tests with treated vinasse diluted with distilled water to *C. vulgaris*.

Based on the comparison of the optimized vinasse treatments in Table 4 and the results of algal cultures performed in this study and available in a previous one (CANDIDO; LOMBARDI, 2017), the adsorption resulted in greater modifications of the vinasse, which allowed its use in algal cultures in concentrations up to 60%. However, as perceived in the optimization tests, the need for materials

and equipment is lower if only centrifugation is performed, a process that eliminates mostly the particulate materials. Beyond that, according to Candido and Lombardi (2018), the use of raw vinasse in microalgal cultivation requires much lower concentrations of the residue and generates a highly contaminated biomass with yeasts, other fungi and bacteria, hence the importance of centrifugation.

Besides the general demand of liquids for the production of culture medium be lower in the centrifugation, other economic aspects are advantageous in this treatment, as less materials and equipment required, greater durability of the equipment involved, fewer processing stages and less total time needed. Moreover, since industrial centrifuges are already used in the ethanol production process (SALES *et al.*, 1986; SAUZE, 1973), the adoption of vinasse centrifugation would not face any cultural restriction and, eventually, if the equipment were similar, the necessary investment could be minimized. In the present treatments' comparison, we must consider that both processes, the centrifugation and the treatment with smectite clay and activated charcoal, were done on a laboratory scale basis. On an industrial scale, there may be technologies that improve both processes, but the need for high amounts of materials in the second treatment is undeniable and economically unfeasible.

In environmental terms, centrifugation is also advantageous in comparison to the smectite clay and activated charcoal, since the residue of the centrifugation is composed mainly by yeasts, which can be marketed as protein source for animal feed (POLYORACH; WANAPAT; WANAPAT, 2013). The composition from this centrifugation residue could be confirmed by the observations under the optical microscope and by the way the yeasts are separated from the fermented cane in the plant (SALES *et al.*, 1986; SAUZE, 1973).

From the algal growth data presented in Candido and Lombardi (2017) or obtained with the optimized treatments in this study, the procedural analyses and comparisons described here, it is possible to infer that the centrifugation, although simple, is sufficient for the improvement of the residue characteristics necessary

for the development of algae. Therefore, we can infer that centrifugation is more effective, considering general aspects, than other complex physicochemical treatments that require many materials, such as adsorption, and then the use of raw vinasse that, according to the literature, requires dilutions up to seven times greater. Finally, this research is a contribution to analyze in a procedural way an alternative use of vinasse in relation to soil fertilizer, enabling the economy with the production of culture media and the generation of a biomass with high added value.

CONCLUSION

Higher quantity of materials and equipment, waste generated and processing times for the smectite clay and activated charcoal treatment were required when compared with the centrifugation, considering both optimized treatments. This leads to questioning the viability of the adsorption process when extrapolated to large scale, whereas the centrifugation is more straightforward in these respects. In addition, our results demonstrated that the dilution of treated vinasse with distilled water is sufficient for algal growth, requiring no addition of nutrients in the culture media. Therefore, it can be said that the use of centrifugated vinasse diluted only with water is a more sustainable process for the industrial plant production than the adsorption, since it is a possible destination for the residue and a way to generate profits from algal biomass commerce.

AUTHORS' CONTRIBUTIONS

Candido, C.: Conceptualization, Formal Analysis, Data Curation, Writing — First Draft, Writing — Review & Editing. Bernardo, A.: Conceptualization, Formal Analysis, Supervision, Writing — Review & Editing. Lombardi, A. T.: Conceptualization, Formal Analysis, Funding Acquisition, Supervision, Writing — Review & Editing.

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