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Evaluation of sugarcane vinasse as a medium for enhanced *Chlorella* sp. growth, lipids production, and process integration

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Abstract

The sugarcane industry generates vinasse waste that could have serious environmental effects if not properly disposed of. This study investigated the use of vinasses generated in the sugarcane industry in Arequipa, Peru, as a growth medium for the microalga *Chlorella* sp. and lipid production. The results demonstrate that the best culture conditions for this microalga in a 4-L photobioreactor included a pretreatment of crude vinasse and an inoculum size of 1.0×10^6 cells mL^{-1}. In these conditions, *Chlorella* sp. attained a growth rate of 1.19 day^{-1}, showing high efficiency in nutrient removal from this residue and attaining a lipid content of 11.5 mg L^{-1} when cultured with a vinasse concentration of 10%. However, the highest lipid productivity (2.6 mg L^{-1} day^{-1}) was recorded in cultures of 20% vinasse. Overall, our results suggest that it is possible to integrate microalgae culture as a biological strategy to properly dispose of the vinasse generation of Arequipa's sugarcane industry; thus, modern technologies can be used for the purpose of valorizing this agro-industrial residue.

Keywords Agro-industrial residue · Bioprocess · Microalgae · Photobioreactor

Introduction

The distillation of ethanol from sugarcane molasses is a significant industrial activity in several countries. Particularly in Peru, the nationwide production of ethanol reached 58 million liters by 2020 (PRODUCE 2021). Vinasses are the main liquid by-product of the sugarcane industry and are generated in a proportion of 9–14 L for each liter of distilled alcohol (España-Gamboa et al. 2011). Vinasse is a dark brown liquid with a high content of organic/inorganic compounds, nutrients, and minerals (España-Gamboa et al. 2011) and a high polluting capacity due to its acid pH (3.5 – 5.0), as well as its biochemical oxygen demand (BOD) and chemical oxygen demand (COD). The index of its polluting character ranges between 35–50 and 100–150 g L^{-1}, respectively (Pant and Adholeya 2007). To date, the rich nutrient content of vinasses has been used as fertilizer for sugarcane crop production. However, the direct discharge of vinasses can negatively affect the physicochemical properties of soil and groundwater, generating a severe environmental impact (Altenhofen et al. 2017). Therefore, the U.S. Environmental Protection Agency forbids the inappropriate disposal of vinasses into rivers, lakes, oceans, and soil without prior conditioning treatment (Colin et al. 2016). Nevertheless, in Peru, small and medium-sized distillery companies do not have the appropriate plants for effectively treating their vinasses before discharging them into waterbodies. Hence, it is evident that there is a need to seek creative solutions for the valorization and sustainable disposal of this agro-industrial residue in this country.

Recently, the circular economy based on reduction, reuse, and recycling is an approach that describes the valorization of agro-industrial residues as inputs for the production of value-added active components (Nagarajan et al. 2020). In this regard, the valorization of vinasses through biomass and high-valuable metabolite production by microalgae is an important strategy to dispose of this residue and comply with government regulations

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(Nagarajan et al. 2020; Fernández et al. 2021). Such approaches also simultaneously reduce the costs of microalgae production, since nutrients such as carbon, nitrogen, phosphorous, and minerals represent 50% of the total cost of microalgal production (Zhang et al. 2016). Today, vinasses generated from different feedstocks, such as sugarcane, beet, grape, and corn, have been used as nutrient sources for culturing microalgae in different countries (Carrilho et al. 2016). Different microalgae genera, such as *Chlorella*, *Scenedesmus*, *Chlamydomonas*, *Neochloris*, and *Micractinium*, have been cultured in vinasses. However, each microalga shows different growth patterns and biochemical composition due to the different capacities of each strain, as well as the different culture strategies used (Choix et al. 2021). For instance, the concentration of vinasse and the inoculum size of microalga are critical factors in successfully valorizing this residue (Bohutskyi et al. 2016; Li et al. 2017).

Several years ago, Olguín et al. (2015) demonstrated that the effluents of diluted vinasses can be used as nutrient sources with the potential for lipid production by *Chlorella*. A few years later, Tan et al. (2018) demonstrated that vinasses with a high sugar content can also stimulate lipid production in microalgae growing under a mixotrophic regime. In particular, under a mixotrophic growth regimen, microalgae can assimilate organic and inorganic carbon, attaining higher biomass and valuable-compound production than when cultured under a heterotrophic and autotrophic regime (Pang et al. 2019; Patel et al. 2020). Thus, the use of industrial residues to support microalgal biomass production with high lipid content could constitute a sustainable strategy for valorizing industrial by-products in an environmentally friendly way (Engin et al. 2018). Furthermore, microalgae have attributes, such as resistance, that make them candidates for further valorization systems of agro-industrial residues, combining the environmental benefits of using waste with the production of biomolecules and/or biomass of commercial interest (Candido et al. 2022).

Considering that microalgae culture supported by agro-industrial residues is considered a strategy to valorize nutrient content and produce highly valuable metabolites, this research aimed to evaluate the use of sugarcane vinasses generated in the region of Arequipa, Peru, as a culture media. Specifically, vinasses can be used to support microalgal biomass and lipid production by the native microalga *Chlorella* sp., which is a sustainable strategy for valorizing this agro-industrial residue. Furthermore, the effects of the concentration of vinasses and inoculum size on the growth and lipid production of *Chlorella* sp. were studied. The cultivation of this microalga in vinasses was also scaled up in a 4-L photobioreactor.

Materials and methods

Microalga

The Chlorophyceae strain was *Chlorella* sp. isolated from Chucarapi, Cocachacra, Arequipa-Peru (latitude 17°4′24" S, longitude 71°43′18" W) and identified according to Bellinger and Sigee (2015). This microalga is maintained and protected at the Laboratory of Genetics of the Professional School of Biology at the Universidad Nacional de San Agustín de Arequipa (UNSA) and cultured in BG11 medium (Rippka et al. 1979) at 24 ± 2 °C, 12 h/12 h light/dark regimen, with a light intensity of 48 µmol photons m^{-2} s^{-1}. The substance was stirred by aeration supplied by an air pump operating at a flow rate of 2.4 L min^{-1}, using cotton and activated carbon for air filtration.

Sugarcane vinasses

Crude vinasses were supplied by a local industry located in the Cocachacra district of Arequipa, Peru. A total of 100 L of crude vinasses were collected in the outlet of the separation column and preserved at –15 °C until their use. The physicochemical composition of the crude vinasses is shown in Table 1. Before experimentation, samples of the crude vinasses were centrifuged at 3000 x***g*** for 10 min. Subsequently, the centrifuged vinasses were diluted by the addition of distilled water at the following proportions: 5, 7.5, 10, 15, 20, 25, 30, 35, and 40%. The composition of the centrifuged vinasses is shown in Table 4.

Table 1 Composition of crude sugarcane vinasses

Parameters	Unit	Crude vinasse
pH	–	3.98
Temperature	°C	90
Absorbance at 570 nm	AU	12041
Turbidity	NTU	9473.3
True color	CU	44676
Electric conductivity	µS cm^{-1}	28,735.632
Total suspended solids	mg L^{-1}	4258
BOD	mg L^{-1}	58350
COD	mg L^{-1}	76,261.6
Nitrate	mg L^{-1}	12.4
Nitrite	mg L^{-1}	< 0.0013
Ammoniacal nitrogen	mg L^{-1}	144
Kjeldahl organic nitrogent	mg L^{-1}	988
Potassium	mg L^{-1}	10916
Sulfate	mg L^{-1}	3720
Phosphate	mg L^{-1}	195
Residual distillery flow	m^3 h^{-1}	1.82

Experimental setup

First, the suitable inoculum size of *Chlorella* sp. cultured in sugarcane vinasses was determined. Briefly, from a culture of *Chlorella* sp. with a cell density of 2.0×10^7 cells mL^{-1}, 250 mL of centrifuged vinasse diluted at 5, 7.5, and 10% with distilled water (treatments), as well as 250 mL of BG11 medium (control), were inoculated until they reached an initial cell density of 5.0×10^4, 1.2×10^5, 1.0×10^6, and 1.8×10^6 cells mL^{-1}, respectively. The pH of each treatment was adjusted to 7.0 using a 5 M NaOH solution. Each culture was carried out using flat plate glass bioreactors (Supplementary Fig. 1) and maintained at $25\pm2°$ C, with a light intensity of 60 µmol photons m^{-2} s^{-1} under a regimen of 12/12 h light/dark over the course of 7 days. Bioreactors were stirred by a constant air flow supplied through air pumps.

Subsequently, the appropriate concentrations of centrifuged vinasses of a specific pH (7 or 8) to support the highest growth of *Chlorella* sp. were evaluated. Therefore, 250 mL of centrifuged vinasses diluted at 10, 15, 20, 25, 30, 35, and 40% with distilled water (treatments) and 250 mL of BG11 medium (control) were inoculated with the inoculum size of *Chlorella* sp. previously selected. Each culture was carried out and maintained under the aforementioned conditions. In both experimental setups, the cell density of *Chlorella* sp. was determined each 24 h using a Neubauer hemocytometer.

In the second set of experiments, 4 L of centrifuged vinasse diluted at 5, 10, 15, 20, and 25% were inoculated with the inoculum size of *Chlorella* sp. and the vinasse concentration formerly selected (Fig. 1). The pH was adjusted to 7.0 with the addition of a 10 M NaOH solution. Each culture was conducted at 25 ± 2 °C, with a light intensity of 60 µmol photons m^{-2} s^{-1} under a regimen of 12/12 h light/dark for 6 days. The physicochemical characterization of the vinasses was determined every third day.

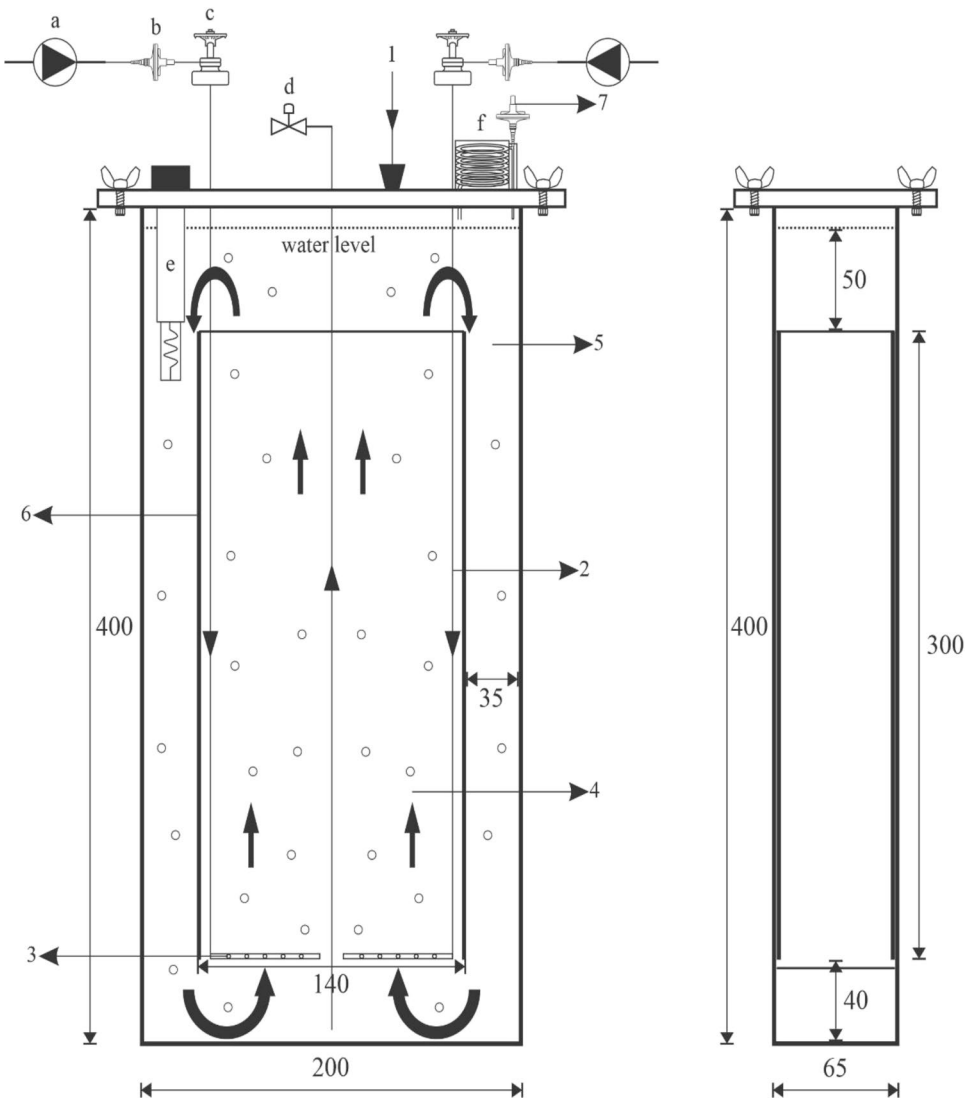

Fig. 1 Schematic diagram of the lab-scale airlift flat plate photobioreactors. [a] air pumps; [b] hydrophobic membrane filter; [c] pressure regulators; [d] sample outlet; [e] regulator thermostat; [f] Water distiller. [1] sample inlet; [2] Gas inlet; [3] Gas diffuser; [4] Liquid upflow; [5] Liquid downflow; [6] Riser; [7] Gas outlet

Analytical methods

Vinasses characterization was determined according to the standard method SMEWW-APHA-AWWA-WEF (2015/2017): Total Suspended Solids (Dried at 103 – 105 °C); BOD (5-Day BOD Test); COD (Closed Reflux, Colorimetric); Nitrate (Cadmium reduction method); Nitrite (Colorimetric method); Ammoniacal nitrogen (Ammonia-selective electrode method); Potassium (Direct air-acetylene flame method); Total Nitrogen (Nitrogen macro-Kjeldahl method); Sulfate (Turbidimetric method); and Phosphate (Ascorbic acid method). The efficiency of nutrients removal was calculated using Eq. 1.

$$\text{Efficiency of nutrients removal } (\%) = (C_i - C_n)/C_i \times 100 \quad (1)$$

where C_i is the initial concentration of nutrients, C_n is the final concentration of nutrients.

The yield and productivity of biomass, as well as the specific growth rate of *Chlorella* sp. in each treatment were determined through the Eqs. 2, 3, and 4 according to Gao et al. (2018).

$$\text{Yield } \left(Y; \text{ cells mL}^{-1}\right) = X_{\max} - X_1 \quad (2)$$

$$\text{Productivity } (P; \text{ cells mL}^{-1}\text{day}^{-1}) = (X_{\max} - X_1)/\ \Delta t \quad (3)$$

$$\text{Specific growth rate } (\mu; \text{ day}^{-1}) = (\text{Ln}X_{\max} - \ \text{Ln}\ X_1)/\ \Delta t \quad (4)$$

where $X_{\max}$ and X_1 are the maximum concentration and initial concentration during the period of cultivation, respectively. Δt is interval of time (in days) between $X_{\max}$ and X_1. The specific growth rate was determined during the period of exponential growth phase.

At the end of experimental time (6 days), from the 4-L glass bioreactors, 1 L of sample was taken of each treatment and was acidified to pH ≤ 2 with 6 M H_2SO_4 solution. Subsequently, the concentration of lipids was determined using the EPA Method 1664 (Cheng et al. 2017). The yield and productivity of lipids in each treatment were calculated according to Eqs. 5 and 6.

$$\text{Yield of lipids } \left(Y_L; \text{ mg L}^{-1}\right) = L_{\max} - L_1 \quad (5)$$

$$\text{Productivity of lipids } (P_L; \text{ mg L}^{-1}\text{day}^{-1}) = (L_{\max} - L_1)/\Delta t \quad (6)$$

where $L_{\max}$ and L_1 are the maximum concentration and initial concentration of lipids in the culture media, respectively. Δt is the interval of time (in days) between $L_{\max}$ y L_1.

Statistical analysis

Each experiment setup was performed in triplicate and repeated thrice (n = 9). The data from each treatment from the three replicates were combined for analyses of variance using Fisher's least significant difference (LSD) post hoc analysis with significance $p < 0.05$, using Statistica 6.0 software (StatSoft).

Results

Inoculum size of *Chlorella* sp. cultured in centrifuged sugarcane vinasses

Chlorella species inoculated at 5.0×10^4 and 1.2×10^5 cells mL^{-1} showed low cell density cultured in each vinasse concentration used (5, 7.5, and 10%) with respect to the BG11 medium (control) during all experimental times (Fig. 2a, b). However, this microalga inoculated at 1.0×10^6 cells mL^{-1} attained a cell density statistically similar to BG11, recording 16.0 (BG11), 16.2 (5% vinasse), 15.8 (7.5% vinasse), and 16.4 (10% vinasse) natural logarithms (ln) of cell density^{-1} at 7 days. (Fig. 2c, lowercase analysis). Meanwhile, when cultured at 1.8×10^6 cells mL^{-1}, *C. vulgaris* only grew up to the fourth day of incubation; subsequently, its cell density decreased in each vinasse concentration evaluated (Fig. 2d).

Similarly, the maximum biomass productivity attained by *Chlorella* sp. was also directly proportional to the growth curves recorded for each concentration of vinasse used (Supplementary Table 1). Thus, the inoculum size of *Chlorella* sp. with a concentration of 1.0×10^6 cells mL^{-1} was selected for further evaluation of the growth and lipid production from the centrifuged sugarcane vinasses.

Growth of *Chlorella* sp. using centrifuged sugarcane vinasses with different pH

Chlorella sp. cultured in vinasses recorded a growth indirectly proportional to vinasse concentration used either with pH 7 (Fig. 3a) or pH 8 (Fig. 3b). In both pHs this microalga had the capacity to grow with a concentration of 10 to 25% of centrifuged vinasses. In contrast, concentrations of 30; 35; and 40% of vinasses decreased the growth of *Chlorella* sp. during all incubation time. At the end of experimental time, cultured in pH 7 *Chlorella* sp. recorded a cell density of 16.51 ± 0.06; 16.29 ± 0.10; 15.95 ± 0.07; 15.43 ± 0.22; 15.04 ± 0.12 ln of cell density^{-1} growing in BG11 medium; 10; 15; 20; and 25% of vinasses, respectively (Fig. 3a). Similarly, growing under pH 8 the cell density attained by this microalga was 16.51 ± 0.06; 16.06 ± 0.03; 15.58 ± 0.09; 15.62 ± 0.29; 15.66 ± 0.12 ln of cell density^{-1}, respectively (Fig. 3b). In both pHs, the cell density of *Chlorella* sp. attained in these vinasses concentration was statistically similar at 7 days (Fig. 3a, b lowercase analysis). Correspondingly, the highest biomass yield, and productivity as well as specific growth rates were obtained when *Chlorella* sp. was growing 10; 15; 20; 25% of centrifuged vinasses (Table 2).

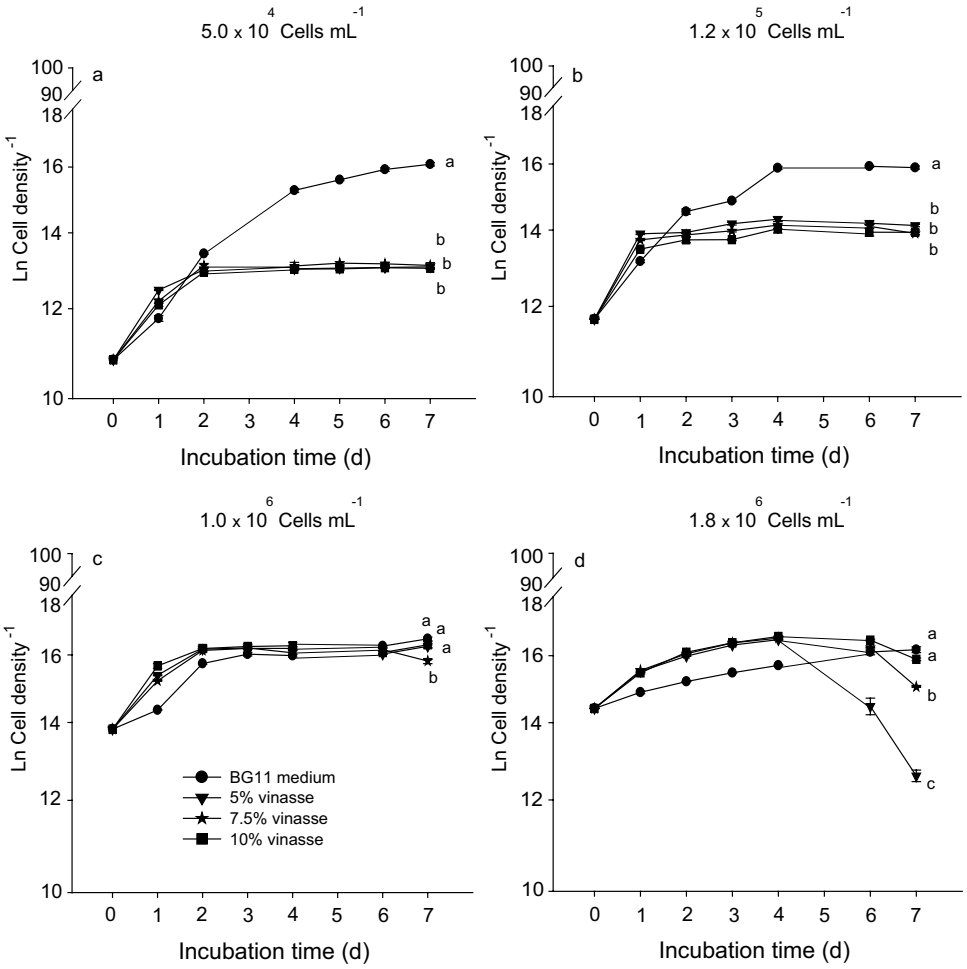

Fig. 2 Cell density (natural logarithm) of *Chlorella* sp. inoculated in centrifuged sugarcane vinasses with different inoculum size. Points denoted by different lowercase letters differ significantly when *Chlorella* sp. was growing in different concentration of centrifuged sugarcane vinasses ($n=9$). Statistical analyses were performed using Analysis of Variance (ANOVA) and Least Significant Difference (LSD) post hoc analysis at $p<0.05$. Bars represent standard error

Lipid production by *Chlorella* sp. from centrifuged sugarcane vinasses

Figure 4 shows the lipids produced by *Chlorella* sp. as a function of the vinasse concentration. When cultured under each vinasse concentration used, *Chlorella* sp. recorded the highest lipid production at the end of the incubation period (7 days). Growing with a load of 10% of vinasses, this microalga attained the highest lipid production (15.4 ± 2.3 mg L^{-1}), followed by a culture of 20% (12.3 ± 1.98 mg L^{-1}) and 15% vinasse (10.9 ± 2.0 mg L^{-1}). However, there were no significant differences between these treatments (Fig. 4, lowercase analysis). Conversely, the concentrations of 25 and 5% of vinasses induced the lowest lipid production by this microalga. Likewise, the highest productivity and yield lipid by *Chlorella* sp. were also recorded in the cultures with loads of 10 and 20% of vinasses, respectively (Table 3).

The physicochemical characteristics of vinasses after supporting the growth of this microalga, as well as the removal efficiency of nutrients such as nitrogen, phosphorous and carbon oxygen demand (COD) from the vinasse concentration used, are shown in Table 4.

Discussion

This study evaluated the use of sugarcane vinasses generated in the region of Arequipa, Peru, as a culture medium to support microalgal biomass and lipid production by the native microalga *Chlorella* sp. as a sustainable strategy to valorize this by-product.

Our results demonstrate that centrifuged sugarcane vinasses can support biomass and lipid production by *Chlorella* sp. This can be attributed to suitable nutrient composition, such as carbon, nitrogen and phosphorous, as well as the capacity of this microalga to endure the specific physicochemical characteristics of this residue. Nonetheless, the inoculum size of *Chlorella* sp. is a vital factor in producing biomass from agro-industrial residues. The low cell density recorded by this microalga inoculated in small inoculum sizes (5.0×10^4 and 1.2×10^5 cells mL^{-1}) in each vinasse concentration evaluated could be because their growth was surpassed by other microorganisms, such as bacteria and yeast, since non-sterile vinasses were used in this study. In a previous study, Bohutskyi et al. (2016) demonstrated that increasing the algal inoculum has a significant effect on

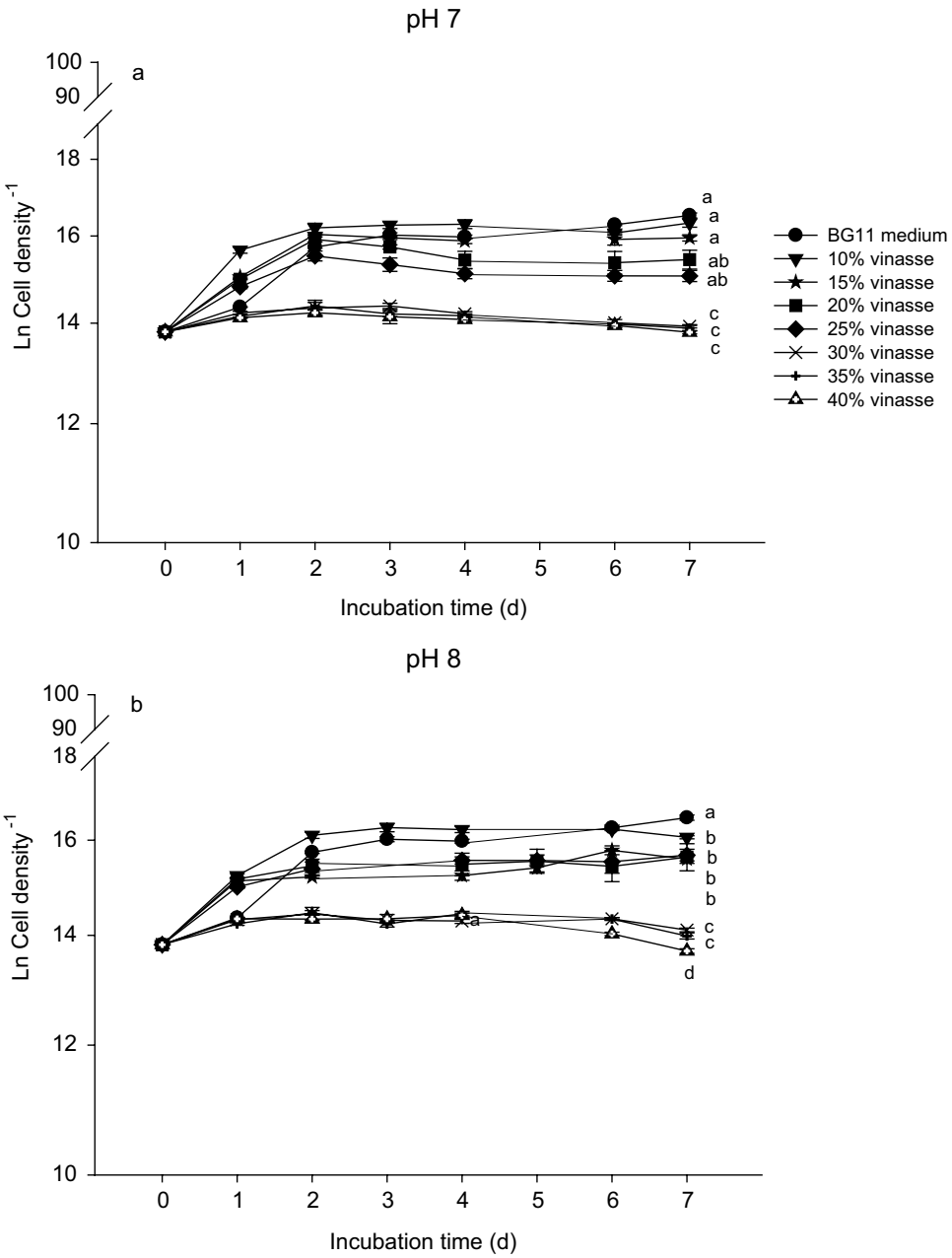

Fig. 3 Cell density (natural logarithm) of *Chlorella* sp. inoculated in centrifuged sugarcane vinasses with different pH. Points denoted by different lowercase letters differ significantly when *Chlorella* sp. was growing in different concentration of centrifuged sugarcane vinasses ($n = 9$). Statistical analyses were performed using Analysis of Variance (ANOVA) and Least Significant Difference (LSD) post hoc analysis at $p < 0.05$. Bars represent standard error

relations between microalgae and wastewater-borne bacteria, especially in competition for organic carbon and nutrients. Meanwhile, when inoculated at 1.0×10^6 cells mL^{-1}, *Chlorella* sp. recorded the highest cell densities and biomass productivities in each vinasse concentration assessed, indicating that in this inoculum size, the growth of *Chlorella* sp. was not surpassed by other microorganisms (Supplementary Fig. 1).

Li et al. (2017) demonstrated that *C. vulgaris* 1067 attained a higher cell density from post hydrothermal liquefaction wastewater when cultured with a high rather than low inoculum size. Surprisingly, the cell density of *Chlorella* sp. inoculated at 1.8×10^6 cells mL^{-1} decreased after 4 days of incubation in each concentration of vinasses. This could have happened due to fact that the ideal nutrient ratio for supporting microalgae growth from agro-industrial residues (127 carbon/16 nitrogen/8 phosphorous; Fernández et al. 2021) was modified in each dilution. Thus, the nutrient ratio used in this study could not support the growth of this inoculum size after 4 days. These results demonstrate the importance of selecting the appropriate inoculum size for each microalga strain to grow and produce biomass from sugarcane vinasses.

Each microalga strain has its own pH, ideal to maximize its growth. In this study, *Chlorella* sp. recorded a performance similar to the production of biomass when cultured in

Table 2 Biomass yield, productivity, and specific growth rate of *Chlorella* sp. cultured in centrifuged sugarcane vinasses

pH	Vinasse concentration (%)	Biomass yield (cells mL^{-1})	Biomass productivity (cells mL^{-1} day^{-1})	Specific growth rate (day^{-1})
7				
	Control – BG11 medium	$5.78\times10^{6}\pm3.0\times10^{5}$a	$2.89\times10^{6}\pm1.5\times10^{5}$a	0.96 ± 0.02b
	10	$1.00\times10^{7}\pm2.0\times10^{6}$a	$5.01\times10^{6}\pm1.0\times10^{5}$a	1.19 ± 0.10a
	15	$8.26\times10^{6}\pm3.4\times10^{5}$ab	$4.13\times10^{6}\pm1.7\times10^{5}$ab	1.11 ± 0.02a
	20	$7.23\times10^{6}\pm8.2\times10^{5}$ab	$3.61\times10^{6}\pm4.1\times10^{5}$ab	1.05 ± 0.05a
	25	$4.57\times10^{6}\pm1.0\times10^{5}$bc	$2.29\times10^{6}\pm5.4\times10^{5}$bc	0.85 ± 0.10b
	30	$7.21\times10^{5}\pm4.9\times10^{4}$c	$3.60\times10^{5}\pm2.4\times10^{4}$c	0.26 ± 0.15c
	35	$7.58\times10^{5}\pm2.3\times10^{4}$c	$3.79\times10^{5}\pm1.1\times10^{4}$c	0.28 ± 0.07c
	40	$5.13\times10^{5}\pm1.4\times10^{4}$d	$2.56\times10^{5}\pm7.2\times10^{4}$d	0.21 ± 0.05c
8				
	Control – BG11 medium	$1.39\times10^{7}\pm1.5\times10^{6}$b	$1.99\times10^{6}\pm2.1\times10^{5}$b	0.39 ± 0.02c
	10	$8.97\times10^{7}\pm1.1\times10^{6}$a	$4.49\times10^{6}\pm5.8\times10^{5}$a	1.15 ± 0.06a
	15	$2.98\times10^{6}\pm1.7\times10^{5}$b	$1.49\times10^{6}\pm8.7\times10^{4}$b	0.69 ± 0.02b
	20	$4.17\times10^{6}\pm1.3\times10^{5}$bc	$2.08\times10^{6}\pm6.6\times10^{5}$bc	0.81 ± 0.12b
	25	$3.83\times10^{6}\pm1.2\times10^{5}$c	$1.91\times10^{6}\pm6.0\times10^{5}$c	0.78 ± 0.13b
	30	$8.75\times10^{6}\pm4.4\times10^{5}$d	$4.38\times10^{5}\pm2.2\times10^{4}$d	0.31 ± 0.12c
	35	$8.92\times10^{6}\pm1.4\times10^{5}$d	$4.46\times10^{5}\pm7.3\times10^{4}$d	0.32 ± 0.04c
	40	$6.71\times10^{5}\pm2.3\times10^{4}$e	$3.35\times10^{5}\pm1.1\times10^{4}$e	0.25 ± 0.07c

Different lowercase letters differ significantly when *Chlorella* sp. was growing in different concentration of centrifuged sugarcane vinasse. Statistical analyses were performed using Analysis of Variance (ANOVA) and Least Significant Difference (LSD) post hoc analysis at $p<0.05$. ± represents standard error ($n=9$)

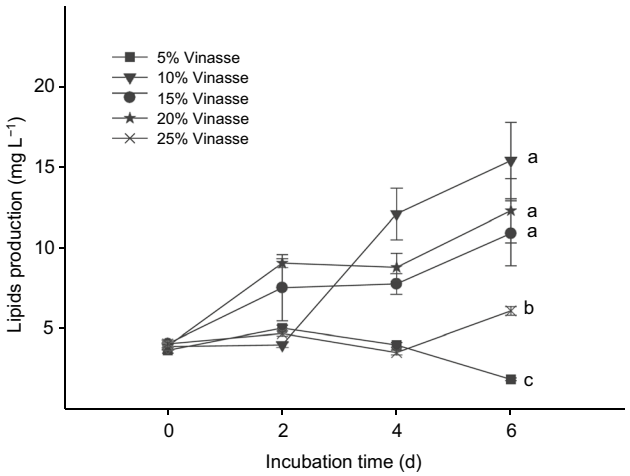

Fig. 4 Lipid production by *Chlorella* sp. from different concentrations of centrifuged sugarcane vinasse. Points denoted by different lowercase letters differ significantly (n = 9). Statistical analyses were performed using Analysis of Variance (ANOVA) and Least Significant Difference (LSD) post hoc analysis at $p<0.05$. Bars represent standard error

centrifuged vinasses of either pH 7 or 8. This result could be explained by *Chlorella* sp. being a robust strain that can grow in a wide pH range (Mayo 1997). Previously, Santana et al. (2017) reported the growth of non-axenic *Micractinium* sp. and *Chlamydomonas biconvexa* at pH 8 for the purpose of reducing contamination by heterotrophic microorganisms. In particular, *Chlorella* has the potential to endure the stressful conditions of agro-industrial residues because of its capacity to mediate anti-oxidative defense through the activities of various reactive oxygen species (ROS) scavenging enzymes, such as ascorbate peroxidase (APX; EC 1.11.1.11; Osundeko et al. 2014). To date, different methods of vinasse purification are well known.

Table 3 Lipid productivity and yield of *Chlorella* sp. cultured in centrifuged sugarcane vinasse

Vinasses concentration (%)	Lipid productivity (mg L^{-1} day^{-1})	Lipid yield (mg L^{-1})
5	0.7 ± 0.1b	1.4 ± 0.1b
10	2.1 ± 0.8a	11.5 ± 4.4a
15	1.1 ± 0.6b	6.8 ± 3.3a
20	2.6 ± 0.2a	8.4 ± 3.1a
25	0.3 ± 0.2c	2.0 ± 0.2b

Different lowercase letters differ significantly when *Chlorella* sp. was growing in different concentration of centrifuged sugarcane vinasse. Statistical analyses were performed using Analysis of Variance (ANOVA) and Least Significant Difference (LSD) post hoc analysis at $p<0.05$. ± represents standard error ($n=9$)

Table 4 Physicochemical characterization and efficiency nutrient removal by *Chlorella* sp. from centrifuged sugarcane vinasse at 10%

Parameters	Unit	Environmental regulations			Centrifuged vinasse			Removal efficiency	
		LMP	VMA	US EPA	V10%	V10%-P	V10%-P-F	First (%)	Second (%)
pH		6–9	6–9	6	7.15	6.25	7.71	–	–
Temperature	°C	35	< 35		20.9	26.9	26.9	–	–
Absorbance at 570 nm	AU				0.462	0.548	0.576	–	–
Turbidity	NTU				16.37	18.53	21.7	–	–
True color	CU				4518	5424	6187	–	–
Electric conductivity	$\mu S\ cm^{-1}$				4590	4570	4650	–	–
Total suspended solids	$mg\ L^{-1}$	350	500	45000	12.6	28.9	22.9	–	–
BOD	$mg\ L^{-1}$	500	500	45000	3140	3500	1614	–	48.6
COD	$mg\ L^{-1}$	1000	1000		8092.3	5155.7	3901.9	36.3	51.8
Nitrate	$mg\ L^{-1}$				9.76	1.72	0.887	82.4	90.9
Nitrite	$mg\ L^{-1}$				< 0.0013	< 0.0013	0.0175	–	–
Ammoniacal nitrogen	$mg\ L^{-1}$				1.27	0.124	0.226	90.2	82.2
Kjeldahl organic nitrogent	$mg\ L^{-1}$				140	335.8	111.2	–	20.6
Potassium	$mg\ L^{-1}$				1014	1074	1011	–	0.3
Sulfate	$mg\ L^{-1}$				358	326	322	8.9	10.1
Phosphate	$mg\ L^{-1}$				18.5	4.46	0.297	75.9	98.4
Residual distillery flow	$m^3\ h^{-1}$				–	–	–	–	–

Values for centrifuged vinasses before *Chlorella* sp. cultivation (V10%) and after *Chlorella* sp. cultivation at 3rd day (V10%-P) and 6th day (V10%-P-F). Values of removal efficiency at 3rd day (*first removal*) and 6th day (*second removal*). Environmental regulation (*LMP*: maximum allowable limit; *VMA*: maximum admissible value; *US EPA*: United States Environmental Protection Agency)

For instance, the filtration process with activated carbon can remove or diminish the load of phenolic compounds, allowing the growth of microalgae from the nutrimental characteristics of these effluents (Candido and Lombardi 2017; Choix et al. 2021). In our work, the pretreatment strategy included centrifugation and dilution to create an appropriate vinasse to allow for light penetration and to increase the photosynthetic activity of microalgae (Candido and Lombardi 2020). Nonetheless, the toxic effects of vinasses were observed in this study, since the increment of vinasse concentration (30, 35, and 40%) induced low biomass yield and productivities of *Chlorella* sp. The above could be attributed to the presence of phenolic compounds and melanoidins, since these compounds have toxic effects on algae growth (España-Gamboa et al. 2011).

In addition, the high level of organic material in vinasses could induce an osmotic effect in some microalgae, causing cell degradation (Kadioğlu and Algur 1992). Furthermore, each microalga strain has a different capacity to endure the harsh conditions of each agro-industrial residue (Choix et al. 2021). For instance, Marques et al. (2013) claimed that sugarcane vinasses are highly toxic in concentrations higher than 4% to *Chlorella vulgaris*. Likewise, Barrocal

Table 5 Comparison of growth rates (μ; day^{-1}) of *Chlorella* sp. obtained in this study and results reported by other researchers

Strain	Culture conditions	Growth rates (day^{-1})	Reference
Chlorella vulgaris	Vinasse (0.2%)	0.76	Marques et al. (2013)
Spirulina platensis	Vinasse (0.8%)	0.15	Budiyono et al. (2014)
Chlorella vulgaris	Vinasse (30%)	0.563 ± 0.014	Candido et al. (2016)
Chlorella vulgaris	Vinasse (2%)	0.34 ± 0.01	de Melo et al. (2018)
Micractinium sp.	Vinasse (10%)	1.01 ± 0.01	Engin et al. (2018)
Chlorella sp.	Vinasse (100%)	0.57 ± 0.02	Choix et al. (2018)
Scenedesmus sp.	Vinasse (100%)	0.57 ± 0.03	Choix et al. (2018)
Chlorella vulgaris U162	Vinasse (100%)	0.58 ± 0.04	Choix et al. (2018)
Scenedesmus obliquus U169	Vinasse (100%)	0.51 ± 0.02	Choix et al. (2018)
Messastrum gracile	Sugarcane molasses	0.22	Tedesque et al. (2021)
Chlorella sp.	Vinasse (10%)	1.19 ± 0.10	This study

et al. (2010) reported a decrease in biomass production of *Spirulina maxima* proportional to an increase in beet vinasse concentration. In another study, Budiyono et al. (2014) indicated that concentrations of vinasses higher than 0.8% inhibited the growth of *S. platensis* because the dark color and turbidity inhibited the penetration of light in the culture medium. The above findings suggest that *Chlorella* sp., as used in this study, has the ability to grow when supplied with a load of centrifuged sugarcane vinasses of up to 25% (Reference Table 5). This latter factor highlights the vital activity of determining the ideal concentration of each residue to maximize microalgal biomass production.

It should also be noted that *Chlorella* sp. showed the ability to produce lipids as a bioproduct of commercial interest for the industry due to the nutrient content of sugarcane vinasse. According to Heidari et al. (2016), the most important parameter for the success of any bioprocess based on microalgae is the production of lipids. Regardless, the different dilutions of vinasses evaluated in this study could have modified the nutrient ratio, thus inducing distinct growth patterns and biochemical compositions (Choix et al. 2018). This could explain the high growth and lipid content of this microalga when cultured in 10% of centrifuged sugarcane vinasses, suggesting that this concentration is suitable for lipid production by this microalga. As mentioned in the methods section, we worked with centrifuged non-sterile vinasses. Thus, the microbiota of this residue might also have contributed to the lipid content, thus hindering the accurate determination of lipid production by *Chlorella* sp. from this residue. This topic needs further investigation, but the result is similar to one reported by Barcia et al. (2020), who found that 10% of tequila vinasses induced the highest biomass productivity of microalgae-yeast flocs.

Previously, Yang et al. (2015) stated that the addition of vinasses as organic and inorganic carbon sources can simultaneously trigger biomass and lipid production. In another study, Tan et al. (2018) reported that wastewater as a carbon source enhances biomass and lipid production by *C. vulgaris*. Similarly, under our experimental conditions, *Chlorella* sp. assimilated the highest content of nitrogen when cultured in 10% sugarcane vinasses, recording the highest efficiency nutrient removal and confirming that in this concentration, the physiological performance of this microalga was appropriate. Recently, Rahimi and Jazini (2021) found that *Chromochloris zofingiensis* cultured in 1.2 g L^{-1} of vinasses almost completes the consumption of COD, TOC, nitrogen, potassium, magnesium, phosphorous, and sulfur of a particular medium. However, in this study, the growth of *Chlorella* sp. cultured in 10% of non-sterile vinasses was not surpassed by yeast and bacteria (Supplementary Fig. 1). The incidence of these microorganisms in nutrient assimilation should also be evaluated further.

In this study, after supporting the culture of *Chlorella* sp., the content BOD_5, COD, and potassium (K^+) in vinasses still remained high, which allows for the supernatant to be reused as a fertilizer in the sugar cane agroindustry following the concept of a circular bio-economy. Finally, our results demonstrate that the nutrient composition of centrifuged sugarcane vinasses can support the growth of *Chlorella* sp., which is native to Arequipa, Peru, thus providing a suitable strategy for the industrial sector of this country.

Conclusions

Overall, the use of vinasses from Arequipa's sugarcane industry as a growth medium for *Chlorella* sp. resulted in the simultaneous reduction of organic and inorganic compounds and high biomass productivity. Moreover, the growth of *Chlorella* sp. along with the other microorganisms of this residue, could enable lipid production in this native strain. Ultimately, the results show the feasibility of integrating microalgae culture with the sugarcane industry for the purpose of valorizing this agro-industrial residue.

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Authors contributions statement MAMC performed and analyzed all experiments and drafted the manuscript. MVV assisted in monitoring and analysis of the experimental data. FAF and FJC served as critical reviewer and wrote the manuscript. All authors read and approved the final manuscript.

Data availability The datasets analyzed during the current study are available from the Institutional Repository of Universidad Nacional de San Agustín de Arequipa (UNSA; http://repositorio.unsa.edu.pe/handle/UNSA/9726), but restrictions apply for the availability of these data which were used under license of UNSA. However, data are available upon reasonable request and permission of Universidad Nacional de San Agustín de Arequipa.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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