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Optimization of Fermentation: Valorization of Vinasse: Case of the Nodiscam Distillery in Mbandjock (Cameroon)

Obono F[1], Bomba FA[1], Metsebo J[2]*, Goka C[3] and Goudoum A[4]

[1]Department of Environmental Sciences, National Advanced School of Engineering, University of Maroua, Cameroon

[2]Department of Hydraulics and Water Management, National Advanced School of Engineering, University Cameroon

[3]Laboratory of Microbiology and Antimicrobial Substances, Faculty of Science, University of Dschang, Cameroon

[4]Department of Agriculture Livestock and Derived Products, National Advanced School of Engineering, University of Maroua, Cameroon

***Corresponding author:** Metsebo J, Department of Hydraulics and Water Management, National Advanced School of Engineering, University Cameroon, Cameroon, Email: jmetsebo@gmail.com

Research Article

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Abstract

The distillery molasses vinasse is an organo-mineral effluent which we have sought to recycle in fermentation to optimizing the fermentation and reducing its environmental impact. The vinasse produced by the NODISCAM distillery is directly discharged into the receiving environment at a daily flow rate of 120m3. The results of physicochemical analyzes show that it is acidic with a pH of 4.2; a temperature of 85.5°C at its outlet from the distillation columns. The conductivity, TDS, TA and degree Brix obtained are respectively 17349µS/cm, 8.37mg/l, 0.2°GL and 9oB; the interpretations of these parameters conclude that the vinasse is recyclable in fermentation. The five culture and fermentation tests carried out with yeast inoculum (0.1g, 0.2g, 0.3g and 0.4g) show that, for test 1 (yeast culture on vinasse) the vinasse is nutritional supplement for saccharomyces cerevisiae. Test 2 shows that the addition of a source of sugar to the previous test makes it possible to obtain an optimal yeast population necessary for fermentation, mainly for samples E6(0.3g) and E7, E8 (0.4g). Test 3 ,4 and 5 on the optimization of inputs: acid, DAP and urea show, for their part, that the addition of 0.9ml sulfuric acid during culture has the effect of prolonging the latency time of yeast the results of optimization of DAP (0.2g) without acid show that there is maximum consumption of sugar 18oB (E8) the yeast population and the rate of alcohol have values greater than or equal to the standard for all samples, a viability rate of 80,33% for sample E8 and alcohol rate after fermentation of 8oGL. Test 5 on optimization of urea show that the amount added does not really influence the results of previous test because urea is provided in part by DAP and there for optimal at 0.2g of DAP. Finally, we will say that, with recycling of the vinasse in fermentation, we optimize the fermentation in acid, DAP, urea and consequently we reduce the environmental impact of the vinasse.

Keywords: Optimization; Fermentation; Valorization; Vinasse; Process

Introduction

Agro-food industries around the world and in Cameroon in particular use and consume large quantities of drinking water in their processes. However, during the various production activities, large volumes of liquid effluents are generated. These effluents are wastewater, sometimes untreated and discharged in an uncontrolled manner into natural ecosystems, causing pollution in the receiving [1].

Environmental pollution, one of the major concerns today, has increased in developing countries where, unlike in industrialised countries, a large proportion of environmental pollutants are agricultural by-products considered as waste [2]. The characteristics of industrial discharge effluents vary greatly from industry to industry Wael, et al. In vinasse effluent, temperature, total dissolved solids are usually high, with levels that can be higher than those of domestic wastewater Benyakhlef M, et al. [3], with pH ranging from 4 to 13 [4]. In Mbandjock, where the NODISCAM distillery is located, we undertook to optimize the fermentation of NODISCAM by recycling the vinasse produced during fermentation in order to also reduce its environmental impact.

Materials and Methods

Physical and Chemical Analysis of Vinasse

The vinasse studied corresponds to the period between 10 May and 5 June of the 2020-2021 campaign. A daily sample (at the exit of the distillation columns) was taken and analyzed in order to monitor its variability. The quantity of vinasse rejected per day was estimated from the fermenters using the following formula:

The physico-chemical parameters (temperature, pH, electrical conductivity, TDS, alcohol content and Brix degree) were measured on site according to the methodological requirements described by Rodier J, et al. [5]; Sablayrolles M, et al. [6] using A multifunction pH meter (pH/EC/TDS) of the brand HANNA series HI98129-HI98130; a probe thermometer of the brand HANNA, a boiler of the brand DUJARDIN-SALLERON and a refractometer of the brand alpha ATAGO.

4.2. Yeast Culture and Fermentation

Equipment: For the yeast cultures, 1.5liter glass beakers were used as fermentation tanks. They were cut at the top to promote oxygen supply during the process. Identical beakers were also used as fermenters. The fermenters are equipped with a small plastic pipe for the evacuation of the CO_2 generated during fermentation.

The plant biomass (molasses) used came from the sugar refinery of the Cameroon Sugar Company (SOSUCAM); the biological material consisted of vinasse and PINNACLE DISTILLERS YEAST (S), the latter in freeze-dried form. The strain is Saccharomyces cerevisiae. The monitoring of the cultures and fermentations includes five parameters: the BRIX degree, the pH, the viability rate of the yeasts, the yeast population and the alcohol rate, which are measured using the following equipment A pH meter for measuring pH, a refractometer for determining Brix level, an electron microscope and the NAUBAUER hematimeter for cell count.

Methodology: Proportions of the different musts and initial concentrations of sugars in the CMs and fermenters. Two types of must were prepared: weak must for yeast culture and strong must for fermentation. The volume of must in each bioreactor being 1l, the proportions are summarized in the (below) Table 1.

	Weak must	Strong must	Tank foot
Volume (ml)	1000	625	375
Molasses quantity (g)	210	370	/
Volume vinasse (ml)	855	380	/
Sugar concentration (°B)	23	37	19 - 20

Table 1: Proportions of different musts for the tests.

Five (05) tests were performed with inoculums of 0.1g, 0.2g, 0.3g and 0.4g of yeast. The first test consisted solely of yeast culture and for tests 2, 3, 4 and 5 we carried out the culture and then the fermentation. For test 1, a single series of four samples (E1 to E4) was performed. Finally, we performed a series of 8 samples (E1 to E8) with a representative of two samples per yeast inoculum for tests 2, 3, 4, and 5.

After the preparation of the different musts, the culture was carried out in batch mode with the inputs. The yeast strain was acclimatized to the sugar substrate in each case. The cultures and fermentations took place at room temperature in the laboratory (26-29°C). The culture time was 16hours, which was necessary to obtain an optimal yeast population for fermentation. Fermentation lasted 24hours.

The different proportions and the different tests carried out for the cultures and fermentations are shown in Tables 2 & 3, respectively.

Tests	Test 1	Test 2	Test 3	Test 4	Test 5
Vinasse	855ml				
Yeast	0.1g ; 0.2 g ; 0.3g ; 0.4g				
Molasses	-	210 g	210 g		
Acid	-	-	0.9 ml	-	-
DAP	-	-	-	0.2 g	0.2 g
Urea	-	-	-	-	0.2 g
pH	4.2	4.5	4.1	4.5	4.5

Table 2: Composition of the culture wort for tests 1, 2, 3, 4 and 5.

Sample N°.	E1	E2	E3	E4	E5	E6	E7	E8
VPC* (ml)	375							
Volume of strong wort (ml)	625							
Molasses quantity (g)	370							
BRIX (°B)	37							
pH	4/5							

Table 3: Composition of the Fermentation Wort from Tests 2, 3, 4 and 5.

Results and Discussions

Results

Physico-Chemical Characteristics of the Vinasse: Volume of vinasse rejected. The average variations in the volume of vinasse produced for the period from 10 May to 5 June are presented in Figure 1-6 below:

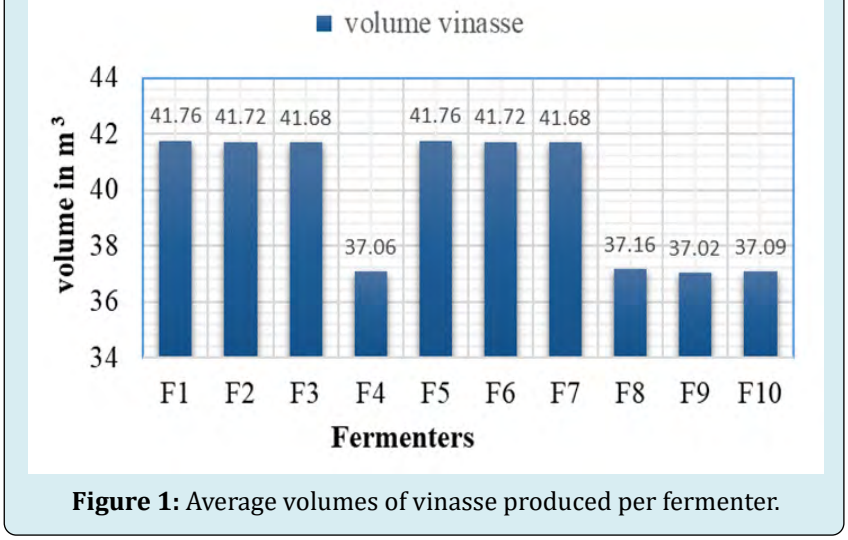

Figure 1: Average volumes of vinasse produced per fermenter.

It appears from this Figure 1 that the volumes oscillate between 37m³ and 42m³. The lower value is 37.02m³ and the maximum value is 41.76m³. The fermenters F4, F8, F9 and F10 have lower volumes compared to the fermenters F1, F2, F3, F5, F6 and F7 this difference is due to the funtoning of the fermenters which is 35m³ for the fermenters F4, F8, F9, F10 against 40m³ the fermenters F1, F2, F3, F5, F6 and F7 Table 4.

Chemical characteristics of vinasse

Parameters	Vinasse used in this study (average)	Analyses of vinasse literature
Temperature (°C)	85.8	31 - 33
pH	4.2	4.3
Conductivity (µS/cm)	10930	4700
TDS (mg/L)	7.86	/
BRIX (°B)	9	/
TA (°GL)	0,2	/

Table 4: Physico-chemical analyses of the vinasses used during the study and data extracted from the following references [7-9].

Experimental Results:

➢ **Test 1:** Effect of not adding sugar

- Variation in Brix degree

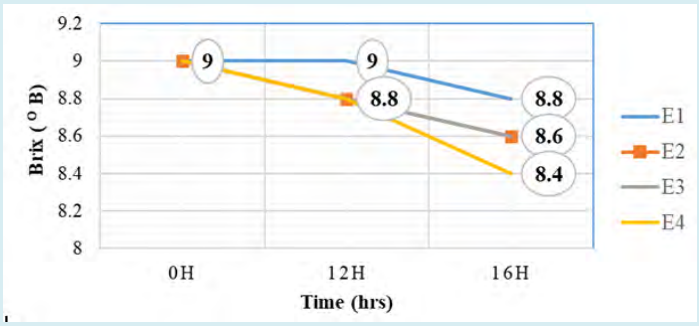

Figure 2: Variation of sugar consumption with time (Test 1).

There is a slight decrease in Brix level overall (Figure 2). The Brix degree varies from 9°B to 8.8°B for sample E1, from 9°B to 8.6°B for samples E2, E3 and from 9 °B to 8.4 °B for sample E4. The yeast consumes sugar by the yeast.

- Variation in the yeast population

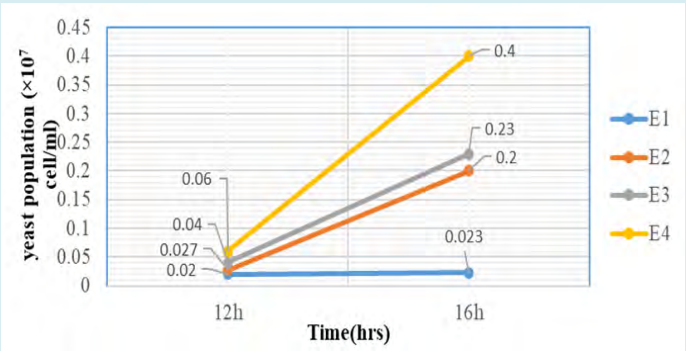

Figure 3: Variation of the yeast population with time (Test 1).

It can be seen from this Figure 3 that the population is almost stable for sample E1 (0.02×107 to 0.023×107) and varies considerably at E2, E3 and E4.

The low variation of the yeast population at E1 is due to the fact that its lag time is high. The variations observed at E2, E3 and E4 are due to the fact that the small fraction of sugar contained in the vinasse was consumed and the cells grew.

➢ **Test 2:** Effect of adding a sugar source: molasses Figure 4

- Variation in Brix degree

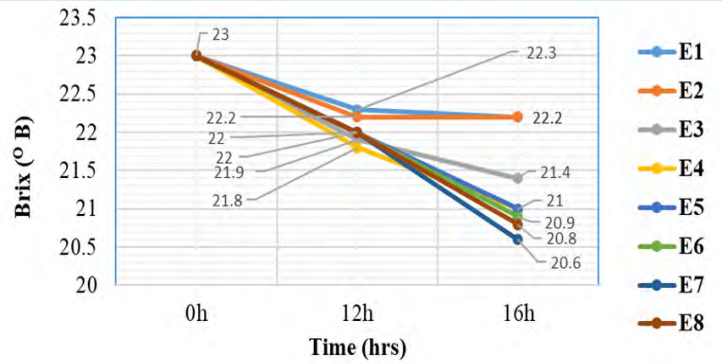

Figure 4: Variations in sugar consumption as a function of time (Test 2).

The maximum variation is from 23°B to 20.6°B for sample E7 and the minimum is observed for samples E1 and E2 (23°B to 22.2°B), followed by samples E8, E6, E4, E5 and E3 with consumptions ranging from 23 to 20.9°B, 20.8°B, 21.4°B, 21°B and 21.4°B respectively.

- Variation of the yeast population and alcohol level after culture Figure 5

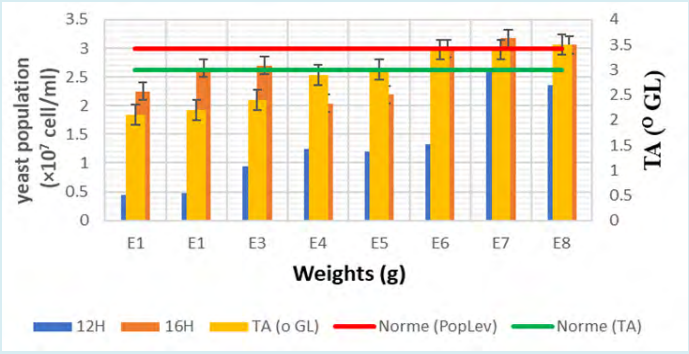

Figure 5: Variation of the yeast population and the alcohol level according to the samples (Test 2).

Samples E1, E2, E3, E4 and E5 have a yeast population below the norm at 16 hours of culture, i.e. 3×107cells/ml, whereas samples E6, E7 and E8 have values above or equal to the norm, respectively 3× 107cell/ml, 3.17× 107 and 3.06× 107. On the other hand, samples E1, E2, E3 and E4 have values below the standard for alcohol content, whereas samples E5 to E8 have values above or equal to the standard.

➢ **Test 3:** addition of sulphuric acid (Figure 6)

- Variation in Brix degree

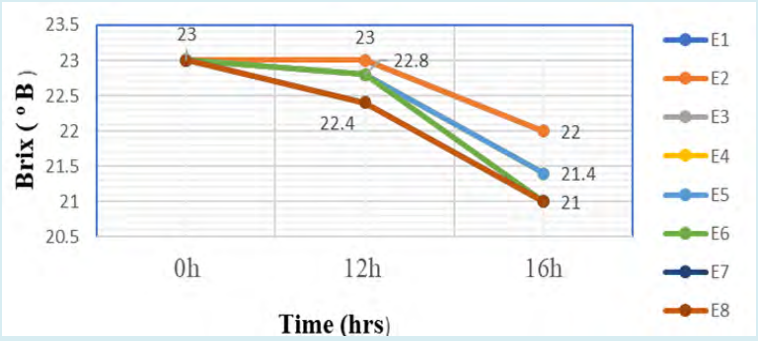

Figure 6: Variation of sugar consumption with time (Test 3).

The Brix values of all samples decreased slightly at 16 hours of cultivation and are lower than in the previous test. The low sugar consumption is due to the high lag time caused by the addition of sulphuric acid to the medium.

- Variation in yeast population and alcohol content after culture (Figure 7).

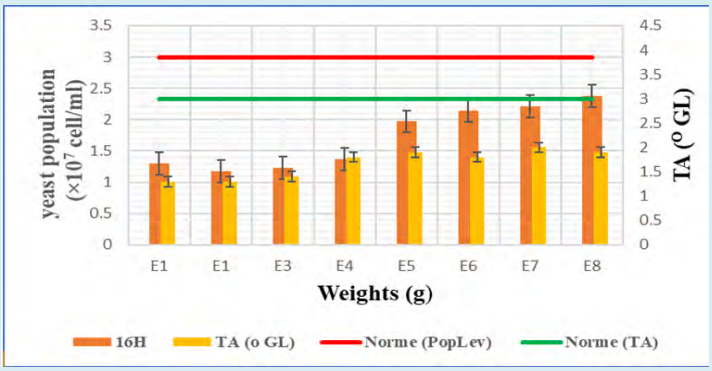

Figure 7: Variation of the yeast population and alcohol content according to different sample masses (Test 3).

According to these results, all samples are below the standard for alcohol content (< 3°GL) and also below the standard for yeast population (< 3×107cell/ml) at 16 hours of culture.

➢ **Test 4:** Addition of DAP 0.2g

- Change in viability rate

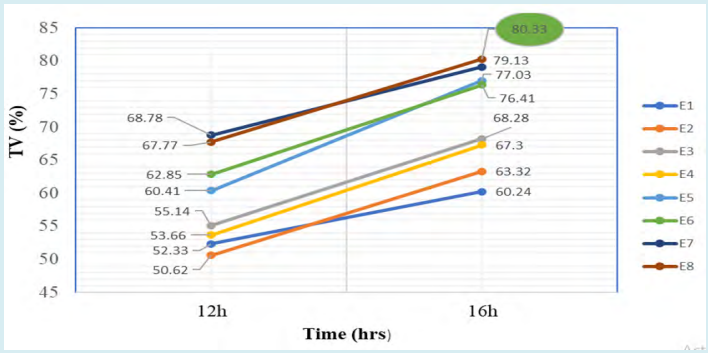

Figure 8: Variation of the viability rate of the samples with time (Test 4; DAP 0.2g).

The results in Figure 8 show that the viability rate of the samples is increasing for all samples. With a maximum of 80.33% values within the standard for sample E8 and a minimum of 60.24% for sample E1 (0.1g).

- Variation in alcohol content at the end of fermentation

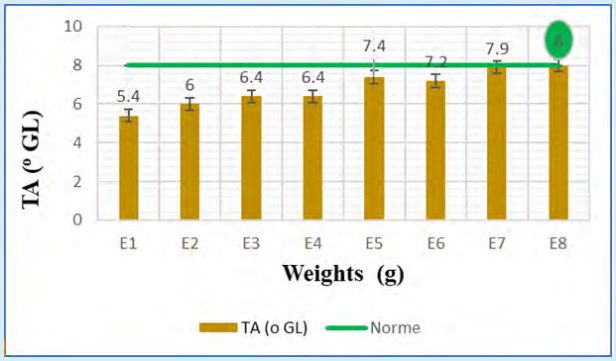

Figure 9: Variation in alcohol content according to the samples after 24 hours of fermentation (Test 4; DAP 0.2g).

It can be seen from the Figure 9 that the maximum alcohol content is produced by sample E8 with a value of 8°GL which is within the norm (8°GL - 11°GL), whereas the rest of the samples produced an alcohol content below the norm.

- **Test 5:** Addition of urea 0.2g (Figure 10).

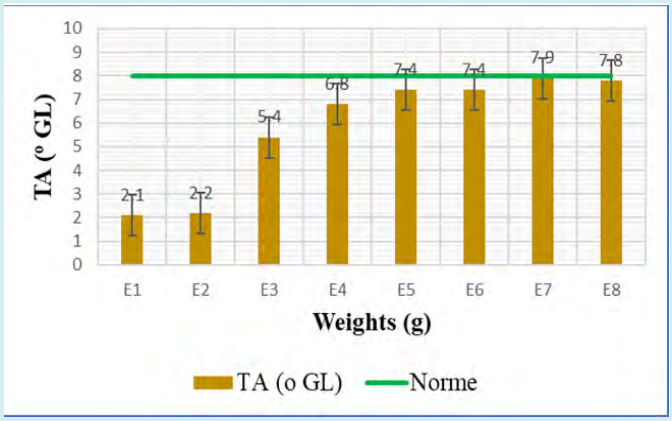

Figure 10: Variation in alcohol content after 24 hours of fermentation (Test 5; DAP 0.2g; urea 0.2g)

From these results, it can be seen that, in contrast to test 4, the yield decreased considerably with a maximum end-fermentation alcohol level of 7.9°GL for sample E7.

Discussions

Physico-Chemical Parameters: The quantity of vinasse is significant and justifies its recycling in fermentation: the total quantity of vinasse produced during the year is 4585.26m^3 with an average of 39.83m^3 per fermenter per day. This gives a daily volume of vinasse of 120m^3 rejected for 3 fermenters produced per day. The high temperature of the vinasse is due to the steam (103°C) used in the wine distillation process. It does not harbour pathogenic micro-organisms and is therefore practically sterile at this temperature [2]. The acidity (Ph=4.2) of the vinasse is mainly due to the use of high amounts of concentrated sulphuric acid used in the preparation of the different musts Noukeu, et al. [9], but this pH is favourable to yeast growth and fermentation [10]. In our case, the alcohol content of the vinasse (average = 0.2°GL) is not a problem for yeast growth. The respective mean values for conductivity and TDS are 10930µS/cm and 7.86mg/L. The electrical conductivity results are different from those found by Nandy, et al. 2002 who analysed several effluents from the food processing industry in India. These results show a high mineralization due mainly to the organic load and the presence of many ions contained in the vinasse [8]. Finally, the variations in Brix degree can be explained by the fact that when the concentration of the strong must varies, this also leads to a variation in the Brix of the vinasse.

Experimental results: In test 1, it was found that the yeast found sugar and nutrients necessary for its growth in the medium (vinasse). Therefore, the vinasse was considered a nutritional supplement and a culture medium for the yeast. On the other hand, the small variations in Brix degree make it possible to assert that the proportion of fermentable sugar in the vinasse would not allow for optimal development of the yeast. [11]. For test 2, a clear difference can be observed compared to the previous test; in terms of Brix degree, the largest difference recorded is for samples E7 and E8, which contain 0.4 g of yeast. As for the yeast population, the maximum concentration allowed for fermentation was reached, as was the end-of-culture alcohol level. The addition of sulphuric acid to test 3 led to an inhibition of the yeast activity. This inhibition was reflected in the high lag time of the samples, in contrast to test 2. These results are in agreement with [10], who states that the lower the pH, the higher the production of sulphites: SO_2=$HSO3^-$=SO_3^{2-} the equilibrium is then shifted towards the production of SO_2 at low pH, SO_2 being the most active against micro-organisms. Finally, the results of test 4 show that the use of DAP as a nutrient for the yeast is necessary for its metabolism and for its maximum activity. Phosphorus allows the yeast to produce cellular components such as nucleic acids but also to improve the productivity of Saccharomyces cerevisiae during ethanol production [12]. They also show that this quantity is optimal because the yeast is more productive with an alcohol content after fermentation of 8°GL and a viability rate of more than 80% for the E8 sample. Regarding the latter, the addition of urea $CO(NH)_2$ as a nitrogen source is necessary for the cell metabolism of yeast, it is used in the synthesis of amino acids, nucleic acids as well as some vitamins, but it can react with ethanol and form ethyl carbamate, which decreases the yield [12-16].

Conclusion

At the end of this study, it appears that industries discharge their untreated effluents into the natural environment. It is in this context that this work, located at the heart of the problem of effluent discharges from agri-food industries, has the objective of optimizing fermentation by recovering the vinasse produced during fermentation in order to reduce the environmental pollution of waste. To achieve this overall objective, we have shown that the vinasse produced at the exit of the distillation columns and directly conveyed to the receiving environment has a daily flow rate of 120m^3. The results of the physico-chemical analyses, mainly pH, conductivity, TDS, TA and alcohol content, led to the conclusion that these values are favorable to the activities of the fermenting yeast; these values are respectively 4.2, 17340µS/cm, 8.37mg/l, 0.2°GL and 9°B. The results of the different tests showed that recycling the vinasse during fermentation would first reduce the consumption of inputs (acid, DAP, urea, water) during the preparation of the different musts and also reduce the impact of the vinasse on the natural environment. The amount of inoculum that gives the best results is 0.4g/l; without the addition of acid, 0.2g/l of DAP during the yeast culture. With these values, the yeast population (values ≥3×107cells /ml), the alcohol level (values ≥3°GL) and the cell viability rate (80.33%) after culture were reached; as well as the alcohol level 8°GL after 24 hours of fermentation. Overall, by recycling its vinasse produced during fermentation, the NODISCAM achieves a double gain: not only does it optimize the consumption of acid (0.0 l), DAP (0.2g/l during yeast culture; 0.26g/l during fermentation) and urea (0.2 during yeast culture). But also dispose of its waste (vinasse) wisely, reducing its environmental impact.

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