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RIVERS OF TUCUMAN CONTAMINATION BY VINASSE SPILLS. ALTERNATIVE TO REDUCE THE LEVELS OF THIS POLLUTANT AND PROMOTE ITS USES

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ABSTRACT

Sugar and alcohol industrial sector, produces ETHANOL, which is used for fuel, food, beverage, pharmaceutical and chemical industries; SUGAR, sold for food, BAGASSE, solid waste grinding to obtain paper and electricity and VINASSE, which is the main liquid waste generated during the production of ethyl alcohol. Discharge of this type of untreated effluents produced water eutrophication of rivers characterized by nutrient enrichment in an aquatic ecosystem. The aim of this work is to deal the problem of pollution of rivers of Tucuman proposing an alternative to decrease the amount of vinasse produced in the process and promote its many uses.

INTRODUCTION

Decreases in oil reserves and gas fields around all over the world justify the deepening of studies to render viable the larger-scale use of new energy sources.Biofuels are fuels of biological origin, derived from renewable organic biomass, where biomass represents a potential source of carbohydrates for microbial fermentation. Interest in its use has increased to the extent that governments seek to reduce and even eliminate dependence on fossil fuels, to ensure future, greater energy security, while benefiting the environment. The momentum of bioethanol in Argentina, from sugar cane was shaped legally with Law 26,093, "Regime Regulation and Promotion for Sustainable Production and Biofuels Uses", in 2010 and established that must supply with at least 5% ethanol to all gasoline, a percentage which was gradually increased to 12% in 2016. This meant an opportunity for the province of Tucuman, in the north of Argentina, to expand its industrial production counting with nearly 300,000 hectares planted with sugar cane, 15 sugar mills and 11 distilleries (Figure 1). Sugar and alcohol industrial sector, produces ETHANOL, which is used for fuel, food, beverage, pharmaceutical and chemical industries; SUGAR, sold for food, BAGASSE, solid waste grinding to obtain paper and electricity and VINASSE, which is the main liquid waste generated during the production of ethyl alcohol. Discharge of this type of untreated effluents produced water eutrophication of rivers characterized by nutrient enrichment in an aquatic ecosystem (Photo 1). Chemical composition of vinasse (Table1). The great power of contamination of the vinasse is favored for several reasons: .

1. Existence of unpleasant odors.
2. High acidity, affecting biochemical conditions of soils and water.
3. High degree of concentration of volatile and fixed solids, which favors sedimentation processes where they are evacuated.
4. High biochemical oxygen demand (BOD), which is defined as the amount of oxygen that the microorganisms responsible for the stabilization (oxidation) of organic matter require, its value represents a measure of the concentration of biodegradable matter in water.

There are several ways of using vinasse.

a. Production of unicellular protein, through aerobic fermentation.
b. Methane gas production, through anaerobic fermentation.
c. Concentration (around 60º Brix), with the following possibilities of use
- Components of animal rations.
- Using yeast as fertilizer.
- Incinerated to produce fertilizer.
d. Agricultural use of the "in nature" residue, totally or partially substituting mineral fertilizations.

Photo 1

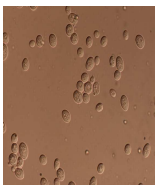
pH	5,2
Conductivity	28,7 mS/cm
QOD	99100 ppm
BOD	40800 ppm
Total Solids	11,10%
Ca	0,26%
Mg	641 ppm
K	1,42%
Na	760 ppm
Cu	4,5 ppm
Zn	2,9 ppm

Table 1

Figure 1

MATERIALES Y MÉTODOS

Two culture media were employed in the present study: YPS proliferation medium (yeast extract 10 g/L, peptone 10 g/L and sucrose 50 g/L) for reactivation and propagation of yeasts; and YPS fermentation medium (yeast extract 10 g/L, peptone 10 g/L and sucrose 250 g/L) for fermentation. Twenty-nine strains isolated from sugarcane molasses and grapes were assayed for their ability to produce ethanol. Yeast samples were aseptically collected from local sugarcane mills (Tucuman, Argentina) and vineyards located in Salta (Argentina), plated individually on YPS agar supplemented with antibiotics (ampicillin 20 g/L, tetracycline 10 g/L, chloramphenicol 20 g/L, and eritromicin 20 g/L) to suppress bacterial contaminants, serially diluted, plated and growth at 30°C for 24 h. The ability to produce ethanol was assayed by triplicate after inoculation of 50 mL YPS fermentation medium with 0.50 g/L biomass in 200 mL bottles. The yeast growth was evaluated by dry weight. The supernatant was separated and stored for determination of sugars using the volumetric method of Fehling Causse-Bonnans and ethanol by Rezex Organic Acid HPLC with precolumn, mobile phase 10 mM H_2SO_4, flow rate 0.6 mL/min, 55°C, Gilson 305 pump, detector LKB Model 2142, differential refractometer, and recorder/ integrator Shimadzu CR3A.



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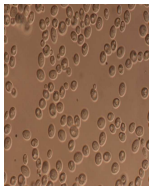

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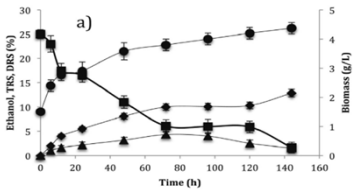
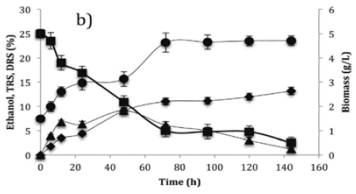
RESULTADOS

The ability to grow and produced ethanol was evaluated in 29 yeast strains isolated from both sugarcane molasses (17 strains) and grapes (12 strains) using YPS fermentation media, starting with an initial inoculum concentration of 0.5 g/L. Table 2 shows the origin of isolates, appearance of colonies, and the final ethanol concentration. Results showed that 13 strains produced reasonable amounts of ethanol oscillating between 5 and 13%. The most outstanding results were achieved with strains A2 (12.87%), A10 (13.20%) and A11 (13.20%); all of them isolated from sugarcane molasses. Figure 2 shows the course with time for total reducing sugars (TRS), direct reducing sugars (DRS), ethanol concentration and biomass. The tendency was similar in the three strains, although the final ethanol concentration was slightly higher in strains A10 and A11.

Isolate	Origin	Appearance of the colony	Ethanol (%)
C1	Grapes-Calayate	Small, circular, brown	3.20 ± 0.21
C2	Grapes-Calayate	Small, circular, brown	5.66 ± 0.32
C3	Grapes-Calayate	Small, circular, brown	4.42 ± 0.22
C4	Grapes-Calayate	Small, circular, brown	3.21 ± 0.14
C5	Grapes-Calayate	Small, circular, brown	3.11 ± 0.12
Q1	Grapes-Calayate	Big, round, buttery, white	5.86 ± 0.28
A1	Molasses	Medium, round, buttery yellow	11.2 ± 0.74
A2	Molasses	Medium, round, buttery yellow	12.87 ± 0.83
A4	Molasses	Medium, round, buttery yellow	8.45 ± 0.41
A5	Molasses	Medium, round, buttery yellow	5.71 ± 0.28
A9	Molasses	Medium, round, buttery yellow	11.87 ± 0.68
A10	Molasses	Medium, round, buttery yellow	13.20 ± 0.81
A11	Molasses	Medium, round, buttery yellow	13.20 ± 0.88
J1	Molasses	Big, round, creamy	5.00 ± 0.29
J3	Molasses	Small, yellowish	2.10 ± 0.14
J6	Molasses	Small, yellowish	4.70 ± 0.18
J7	Molasses	Big, round, creamy	4.26 ± 0.17
J8	Molasses	Small, yellowish	2.10 ± 0.02
J9	Molasses	Big, round, creamy	1.50 ± 0.2
J10	Molasses	Medium, round, buttery yellow	0.70 ± 0.02
J11	Molasses	Medium, round, buttery yellow	3.51 ± 0.14
J13	Molasses	Big, round, creamy	1.50 ± 0.10
J14	Molasses	Big, round, creamy	1.86 ± 0.13
YN1	Grapes-North Yacochuya	Big, round, creamy	5.87 ± 0.21
YN2	Grapes-North Yacochuya	Big, round, creamy	6.40 ± 0.22
YS1	Grapes-South Yacochuya	Big, round, creamy	7.66 ± 0.24
YS2	Grapes-South Yacochuya	Big, round, creamy	4.33 ± 0.15
T	Grapes-Tolombon	Medium, round, white	1.60 ± 0.13
AN	Grapes-Animana	Big, yellow	2.40 ± 0.16

Table 2





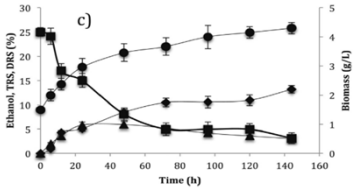


Figure 2: Course with time during the growth of Saccharomyces cerevisiae strains a) A2, b) A10 and c) A11 in YPS fermentation medium at 30 °C. TRS (■); Ethanol (◆); DRS (▲); Biomass (●). Results are the media of three fermentations and bars represent mean ± standard deviation.

Alcohol percentage	10%	11%	12%
Total cane consumption (%)	47,22	46,50	45,77
Steam consumption at distillery (Kg. steam/liter of alcohol)	4,50	4,25	4,00
Liters of vinasse per liter of alcohol ($L_{vinasse}/L_{alcohol}$)	13	11	9

Table 3

Microorganism	Origin	Time fermentation	Concentration of Ethanol	Total cane consumption	Steam consumption at distillery	Liters vinasse per liter of alcohol
	CALSA-ARGENTINA Company	10 h	10%	47,22%	4,5 Kg. steam/liter of alcohol	13 $L_{vinasse}/L_{alcohol}$
Saccharomyces cerevisiae A2	Isolated from molasses	10 h	12%	45,77%	4 Kg. steam/liter of alcohol	9 $L_{vinasse}/L_{alcohol}$

Table 4

CONCLUSIONES

It was possible to isolate 29 different yeasts from samples of molasses, cane juice from different sugar mills of Tucuman, canefield soil and grapes from the region of Salta (north of Tucuman).

Of the total yeasts isolated from samples of molasses, juices and grapes, 16 produced ethanol percentages lower than 5% while the remaining 13 produced ethanol concentrations higher than 5%.

Isolates A2, A10 and A11 were selected as good ethanol producers with ethanol concentration values recorded of 12.87, 13.20 and 13.20% respectively.

It was observed that the A2 strain showed a homogeneous growth in liquid medium, this characteristic is compatible with the technology currently used in the industry. These results showed to strain A2 as a candidate to be used in the industrial production of ethanol without needing to make technological changes.

Strains A10 and A11 showed a flocculent nature in liquid medium.

Isolates A2, A10 and A12 were taxonomically identified and the analysis of the sequences allowed to assign a 100% identity with Saccharomyces cerevisiae.

The scaling of this yeast strain with high production of ethanol, naturally isolated from the environment is a key point in the sustainable circuit to improve technological and industrial level.

From the environmental point of view when using the strain Saccharomyces cerevisiae A2 would be achieved to reduce in 30% the levels of vinasse generated passing from an average of 13 Liters of vinasse / Liter of alcohol to 9 Liters of vinasse / Liter of alcohol which would confer a significant environmental benefit.

Therefore increasing the fermentative power by at least two points means:

-Increase Production Capacity.

-Lower energy consumption for distillation.

-Decrease the liters of vinasse produced per liter of alcohol.

These characteristics make the strain Saccharomyces cerevisiae A2, a producer of bioethanol with potential environmental, energy and economic benefits, to project it on an Industrial scale.

When comparing both strains, it was observed that Saccharomyces cerevisiae A2 produces a higher percentage of ethanol (12%) than the commercial strain of baking (10%) at the same time of fermentation (10h) and would reduce the levels of vinasse by 30% from 13 Lvinasse / Lalcohol to 9 Lvinasse / Lalcohol which would confer a significant environmental benefit.(Table 3 and 4).