PGTF
THE PEREZ-GUERRERO TRUST FUND FOR ECONOMIC AND TECHNICAL COOPERATION AMONG DEVELOPING COUNTRIES

FINAL REPORT

Code of the project: 00083233

Title of the project: "Protein Enrichment of Lignocellulosic Residues for Animal Feed from Sugar Industry Effluents"

Coordinator: Cuban Research Institute for Sugar Cane By-products (ICIDCA)

September 2016

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I. Basic Project Information

Number and title of the project:

00083233/ 00091816 Protein Enrichment of Lignocellulosic Residues for Animal Feed from Sugar Industry Effluents

Coordinator: Cuban Research Institute for Sugar Cane By-products (ICIDCA)

Other responsible parties (if any):

Three institutions recognized from Argentina, Mexico and Cuba worked in this project:

- Cuban Research Institute on Sugar Cane By-products (ICIDCA), Cuba.
- Universidad Autónoma Metropolitana (UAM-I), México.
- Facultad Regional Tucumán (FRT), Argentina.

Originally Preview: January 2011 Real: January 2012

Date of completion:

Originally Preview: January 2014 Estimated: September 2014

Reporting period: January 2014 – September 2016

II. Organization of the Project

a) Project objectives

The objective of the project was contribute to the implementation of a methodology technically and economically viable for protein enrichment of lignocelluloses residues (sugar cane bagasse, citrus, etc.) from the growth of yeast *Candida utilis* food in immobilized cell bioreactor, using as carbon source and energy the organic compounds vinasse emitted by the alcohol distilleries, for making an animal feed.

As it can be observed in the project two objectives are supplemented, the first environmental, since, it is related with the mitigation of the polluting load of the residual emitted by the stills (vinasse) and the second is alimentary, since, a product of nutritional high value is obtained, rich in proteins and vitamins for the animal feeding. It also revalues lignocelluloses residuals like the sugar cane bagasse or the solid residuals of the prosecution of citric that can contribute fiber and energy if they are treated appropriately.

In this project three institutions Mexico, Argentina and Cuba were in charge of developing the studies proposed for the definition of the technical. This allowed toquantify particular and general characteristics in the proposed outlines and facilitated to give a multiplier focus to the

problem and to create a solution outline that can adapt to any factory of sugar cane and by-products from any country, for that is also sought with the project that processes an amplifier effect and obtain a transferable technology toward any sugar country.

These objectives were reached completing the following stages:

1. To characterize chemical and physically residual liquids and solids emitted by sugar cane mill, distillery and citric industry.
2. To study and to establish the best conditions in operation of a bioreactor with pure cultivation of yeasts *Candida utilis*, immobilized on lignocellulosic support (sugar cane bagasse or citric residuals) and grown starting from vinasses of stills in laboratory conditions.
3. To development of the scale up of the process in pilot plant bioreactor.
4. To determine the potential of the product obtained as animal feed.

b) Project Outputs

The outputs of the project were:

1. The chemical and physical characterization of the residual liquids and solids emitted by sugar cane mills, distilleries and citrus industry.
2. The optimization of operation conditions of a bioreactor with pure cultivation of yeasts *Candida utilis*, using as support (sugar cane bagasse or citric residuals) and vinasses as carbon source for the grown in laboratory conditions
3. The scale up of process in pilot plant bioreactor.
4. The chemical and physical characterization of the product obtained for animal feed.

c) Project Activities

The activities of the project were distributed in 4 work packages, one of them being the coordination task.

Work packages

Work Pack	Work package title
0	Project Management
1	Chemical and physical characterization of the residual liquids and solids
2	Laboratory study of yeasts production using vinasses as carbon source
3	Pilot plant study of yeasts production using vinasses as source carbon and energy
4	Chemical and physical characterization of the product obtained for animal feed

III. Evaluation of the objectives of the Project. Main technical activities.

WP0 Project Management

In the following pages the main technical activities of the Project are described.

The Initial Project meeting Perez Guerrero "Protein Enrichment of Lignocellulose Residues for Animal Feedfrom Sugar Industry Effluents" with representatives of Cuba, Mexico and Argentina was developed in October 2014 at the Metropolitan Autonomous University (UAM) in Mexico - Iztapalapa attended by the following members of the project:

- Dr. Fidel DomenechLópez, General Coordinator of the Project PGTF
  Cuban Research Institute byproducts Sugarcane (ICIDCA, Cuba)
- Dr. Sergio RevahMoise, Coordinator for Mexico of PGTF Project
  MetropolitanAutonomousUniversityof Iztapalapa(UAM-I, Mexico)
- Dr. Patricia M. Albarracin, Coordinator for Argentina's PGTF Project
  Regional School of Tucumán, National Technological University (UTN, Argentina)
- MSc. Sergio Hernández, Specialist of PGTF Mexico Project

Initial Project meeting Perez Guerrero held in Iztapalapa Metropolitan Autonomous University (UAM-I, Mexico)

AGENDA OF THE MEETING

1. Presentation of the project background.
2. Presentation, discussion and approval of the project objectives. Definition of thetasks of eachparticipant.
3. Definition of actions to be developed in 2015.
4. Presentation and analysis of experimental results developed in the ICIDCA

PROCEEDINGS

Presentation of the project background:

A presentation was made about the project background. The obtained results in collaboration with the Autonomous University (UAM) in Mexico, the IRD of France and the ICIDCA (Cuba) which has more than 10 years, achieving significant results in biofiltration technology to valuing liquid and gaseous effluents of the sugar industry and its derivatives, as important result, there are four publications, participation in 6 conferences and international patent Patent: WO 2006/131643 A3, Enriching method for residues with yeast protein lignocellulose. February 1, 2007.

Agreements taken:

1. To consolidate results of a technology capable of producing a protein-rich animal feed. Studies focus on optimizing the growth of yeast and consequently mitigate the pollution load of vinasse with possible applications as a local solution.
2. To undertake a research protocol where all stages of the process (Methods conservation and propagation of *C. utilis* strain, test methods for characterizing physical, chemical and biological of lignocellulosic residues and waste liquids (slops) detailing methodology for the preparation of the culture medium and fermentation medium (Mass balances for the formulation of the fermentation medium), process control methodologies, parameters monitored during fermentation, devices and experimental conditions and production methodology slops laboratory.
3. To do in Cuba protein enrichment studies using lignocellulosic materials as bagasse. Experiments performed in Argentina using citric bagasse and analyze the possibility of using in Mexico bagasse and stillage obtained from the production of tequila.
4. To conduct experiments in the pilot biofiltration UAM Iztapalapa in Mexico with analysis and control of the plant through respirometry produced CO2 and O2 consumed. The sensors were developed and proposed by the ICIDCA mathematical model is implemented.
5. To design and build in a pilot study and establish the best operating conditions of the bioreactor pilot trickle bed reactor. Part of project financing for the purchase of the necessary accessories will be used for the same work automatically.
6. Argentina side held the same job but with the product obtained from bagasse citric and stillage.

WP1 Chemical and physical characterization of the residual liquids and solids

The characterization of waste water of the sugarmill:

Table 1. Wastewater from derivative plant

		Promedio	Desv. Estan	Xmáx	Xmín	n
DQOt	**g/L**	**71,20**	29,27	168,4	26,4	49
DQOt	**g/L**	49,94	7,68	63,3	38,4	14
pH		**4,47**	0,43	6,4	4	46
ST	**g/L**	52,67	4,15	60,46	45,47	14
STF	**g/L**	12,61	0,90	13,7	11,12	6
STV	**g/L**	38,67	4,29	44,25	33,05	6
SST	**g/L**	**10,70**	5,12	18,03	1,42	14
SSF	**g/L**	3,39	2,91	8,89	0,91	6
SSV	**g/L**	7,31	6,14	17,26	1,47	6
SDT	**g/L**	41,97	8,11	52,54	30,7	6
SDF	**g/L**	9,23	2,67	12,19	4,18	6
SDV	**g/L**	31,08	8,24	40,38	19,1	6
CE	**mS/cm**	**8,36**	2,86	13,41	6,6	5
Sulfatos	**g/L**	15,81	29,53	76	2,893	6
Nitrógeno	**g/L**	**0,21**	0,10	0,322	0,02	6
Fósforo	**g/L**	**0,11**	87,53	181,16	0,189	6
Calcio	**g/L**	0,55	0,34	1,2	0,26	6
STV/ST		0,75	0,02	0,77	0,73	6
SSV/SST		0,68	0,64	2,07	0,28	6

The content of DQO is adequated for developing the animal food in the bioreactor from lignocellulosic residues.

WP2 Laboratory study of yeasts production using vinasses as carbon source

a. Optimizing the growth of food yeast *Candida utilis* using distillery vinasses as a source of carbon and energy

A system on solid media fermentation was evaluated, consisting of a bio-trickling bioreactor packed bed by bagasse, inoculated with yeast *Candida utilis*food, using as substrate stillage obtained at pilot scale.

The experiments were carried out at laboratory level in an experimental system in continuous, consisting of two cylindrical glass columns 2500 mL and 1000 mL settler. The first column acts as bioreactor Solid State Fermentation (SSF), the second working as a filter of yeast cells, that is, retains the cells are carried by the liquid phase and the settler serves clarification of vinasse, depositing in the background cells that reach it and recycling to the first column. It was an experimental design to study the effect of feed flow and the initial concentration of Chemical Oxygen Demand (COD).

Experimental results showed that it is possible to use distilleries supplemented vinasse nitrogen and phosphorus sources for growth of fodder yeast *Candida utilis* using bagasse as solid support, indicating that on the one hand, the vinasse can be to removing 40% of the organic contaminants to the environment and on the other, it is possible to obtain a product with more than 9% protein, which could be used as a dietary supplement for cattle for its balanced fiber content and proteins.

The stillage can be the raw material for other fermentation processes as protein production, given its content B vitamins, trace elements such as Co, Ni, Mn, Mg, Cu, Fe, free amino acids, carbonic acid, mono and disaccharides, D - glucose, D - fructose, sucrose, nitrogen, organic acids, K, Na and other***Saura G. et al, (2002); E. Valdés (2003)***. The current practice of dumping of vinasse is also contrary to the protection of the environment, uneconomic; and an appropriate approach to this problem is finding new ways to use this rich product.

The replacement of molasses from sugarcane as a carbon source in the production of food yeast by vinasse, has an economic and environmental impact. Production costs are reduced yeast and residual organic pollutant load is reduced, residual distilleries highly polluting used, avoiding therefore the immediate treatment of them and maximizing the elements contained in this production base serving also the primary objective to contribute with food program of the sugar industry (AZCUBA) ***Saura G. et al, (2002).***

The aim of this work was to maximize the dose of nutrients and vinasse, for optimal growth of food yeast *Candida utilis* (Torula) and thus the protein enrichment of bagasse. Continuous fermentation product was studied.

II. MATERIALS AND METHODS

2.1- Microorganisms.

Food yeast strain *Candida utilis* L / 3-75-1, from the collection of the Cuban Research Institute Sugarcane By-products (ICIDCA) was used. Strain was transferred periodically inclined Potato Dextrose Agar (PDA) tubes, incubated for 48 hours at 30 ° C and observed after growth on agar stored at 4 oC.

2.2- Inoculum Preparation

500 mL medium was prepared consisting of: sugar molasses 40.6 g / l; (NH4) 2SO4 4.88 g / l, (NH4) 2HPO4 1.42 g / l nutrient yeast 0.067 g / l, 150 ml of medium added three 1000-ml Erlenmeyer, sterilized at 15 psi (121 ° C) for 20 min ., after cooling, each flask was inoculated with a culture of *Candida utilis* wedges, the propagation was carried out on a rotary shaker for 16 to 18 hours at 30 ° C and 200 rpm. At the end of the total cell count propagation is performed.

2.3 Preparation of solid support (integral sugarcane bagasse)

Integral sugar cane bagasse with particle size between 0.54 mm and 3 mm, were packaged in poly bags to be sterilized at 15 psi for 1 hour, in order to reduce the microorganism.

2.4 Preparation of liquid Medium nutritious

The support is complemented by the nutritive liquid medium which consists primarily of vinasse standardized to 50 g COD / L distillery.The formulation of the medium was performed using a program developed in Excel to facilitate the formulation (Table I). Was taken as the basis for calculating a reactor of 2500 ml.

2.5 Components and experimental conditions

2.5.1. FIRST EXPERIMENTAL PHASE: three bioreactors working at different power flows in the liquid phase.

Experiments were developed in an experimental equipment consisting of three cylindrical glass columns 2500 mL, as shown below (see Figure 1):

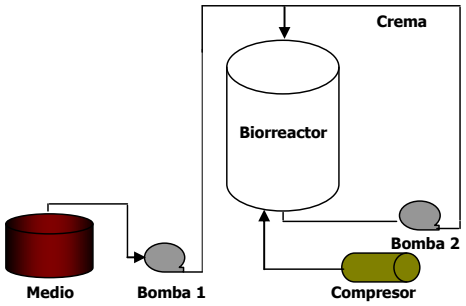

Figure 1. Experimental equipment

Three streams feeding were studied: 100, 200 and 300 mL/h, in columns packed with 250 g of bagasse as solid support. Initial moisture of 72% saturation by the addition of the inoculum and the growth medium (vinasse, (NH4) 2SO4 4.34 g / l and (NH4) 2HPO4 .85 g / L) was achieved. The study was carried out keeping the system air v/kg.m and a temperature of 30 oC.

The columns were washed and left in 70% alcohol v / v overnight. Support (integral cane bagasse sieving mesh between 0.5 and 5 cm) was sterilized in a poly-bag to 15 psi for 30 minutes to reduce the microbial load. Bagasse was allowed to cool and when reached room temperature was added columns. Then each column was inoculated with 500 ml of inoculum and begins to feed air from the bottom of the column. This is followed by feeding the liquid medium until the total volume of the column after the feed is stopped and left for 4 hours before feeding the culture medium. The columns were washed and left in 70% alcohol v / v overnight. Support (integral cane bagasse sieving mesh between 0.5 and 5 cm) was sterilized in a poly-bag to 15 psi for 30 minutes to reduce the microbial load. Bagasse was allowed to

cool and when reached room temperature, the columns was added. Then each column was inoculated with 500 ml of inoculum and begins to feed air from the bottom of the column. This is followed by feeding the liquid medium until the total volume of the column after the feed is stopped and left for 4 hours.

2.5.2 SECOND EXPERIMENTAL PHASE: Behavior of three bioreactors working connected in series

One feed flow 300 mL / h was set for the three columns packed with 250 g of bagasse as each solid support. Initial moisture of 72% saturation by the addition of the inoculum and the growth medium (vinasse, (NH4)2SO4 4.34 g / l and (NH4)2HPO4 .85 g / L) was achieved. The study was carried out by maintaining a system airv/kg.m and temperature of 30 oC.

The columns were washed and left in 70% alcohol v / v overnight. Support (integral cane bagasse sieving mesh between 0.5 and 5 cm) was sterilized in a poly-bag to 15 psi for 30 minutes to reduce the microbial load. Bagasse was allowed to cool and when reached room temperature was added columns. Then each column was inoculated with 500 ml of inoculum and begins to feed air from the bottom of the column. This is followed by feeding the liquid medium until the total volume of the column after the feed is stopped and left for 4 hours before feeding the culture medium. The columns were washed and left in 70% alcohol v / v overnight. Support (integral cane bagasse sieving mesh between 0.5 and 5 cm) was sterilized in a poly-bag to 15 psi for 30 minutes to reduce the microbial load. Bagasse was allowed to cool and when reached room temperature, the columns was added. Then each column was inoculated with 500 ml of inoculum and begins to feed air from the bottom of the column. This is followed by feeding the liquid medium until the total volume of the column after the feed is stopped and left for 4 hours.

After this time, began to pass the liquid phase of the first column to the second column at a flow of 400 mL/h, connecting again through fresh feed to the first column at a flow of 300 mL/h. When the second column reaches 2.5 liters volume, the discharge to the third column is continuous. When it reaches its total volume gravity discharge begins.

PROCESS CONTROL:

Samples of the liquid phase (each reactor outlet) were taken every 4 hours. A 50 ml sample was determined COD, BOD, N, P, etc. Which will be delivered to the laboratory waste and the other was taken into test tubes for microbiological determinations.

Solid samples at baseline and final phase of fermentation to determine you were taken: % Moisture, pH and total cell count.

2.6. Analytical methods

2.6.1. Cell count

The cell count was carried out in Neubauer chambers using a Nikon optical microscope (10x ocular / 20, objective Plan 40 / 0.65). The weighed one gram samples of solid material are added 50 mL of a solution of NaCl at a concentration of 9 g / L. The amount necessary for cell countingis homogenized and is taken from the liquid phase.

2.6.2. Protein determination

The determination of protein content was carried out by the method of Barsnstein (Winton and Winton) [4], the method is based on pure proteins that adhere to the surface of Cu (OH) 2 in nascent state. After washing of the precipitate it is determined by Kjeldahl nitrogen with 6.25 facto takes true protein.

2.6.3- pH measurement

In the solid phase was determined by taking a gram sample, to which was added 25 mL of distilled water, homogenized and reading is made with a potentiometer.

2.6.4. Determination of moisture in the solid support

One gram of wet material is dried in an oven at 100 o C for 24 hours, determining the percent moisture by weight difference before and after drying.

2.6.5. Chemical Oxygen Demand (COD)

This method involves refluxing for 12 min 1 mL of sample, 1 mL of a solution of potassium permanganate (0.25 mol / L), about 1 gram of mercury sulfate and 3 mL of a mixture of acid concentrated sulfuric silver sulfate, was subsequently added 5 mL of distilled water, one drop of indicator Ferroin and excess dichromate is titrated with a solution of ammonium iron sulfate (II) (0,05mol / L).***(Obaya, 1985)***.

The amount of oxidized organic material is proportional to the consumed potassium dichromate. Total COD determinations made during reactor operation, samples were taken daily at the same power and treated effluent.

2.6.6. Biochemical Oxygen Demand (BOD). Oxygen concentration required for biological degradation of organic matter present in the sample tested.

From the above concept has developed a technique that aims to carry out the measurement of oxygen required to oxidize the organic matter in the waste. This technique involves incubating a sample or dilution for a time of 5 days at a temperature of 20 oC. This sample was previously inoculated with a microbial culture obtained from ground, although this step can be bypassed if there is evidence that brings the residual microorganisms.***(Obaya, 1985).***

2.6.7. Determination of Total Nitrogen

After subjecting the sample to digestion in acid medium, are taken 5 mL of the same and fed into a reboiler containing a 6 mL of 50% sodium hydroxide sufficient to ensure a strongly basic medium. The sample is distilled and collected about 50 mL by distillation in an Erlenmeyer flask containing 5 mL of a solution of 4% boric acid. Subsequently by titration with hydrochloric acid 0.02 mol / L total nitrogen in the sample is determined.***(OPS, 1983)***.

2.6.8. Phosphorus determination

Determination of phosphorus is performed by a colorimetric method, after digestion in acidic medium. A portion of the neutralized sample taken previously, is added 1 mL of ammonium vanadate solution 0.25% ammonium molybdate and 5%, is brought to a volume of 25 mL, allowed to stand for 10 minutes and to develop the color thereof was measured in a spectrophotometer at 415 nm. The color intensity of the complex formed is proportional to the concentration. ***(OPS, 1983; Pancreac, 1990)***

2.6.9. Determination of COD removal efficiency (RE), the COD removal capacity (EC) and the load (K).

$$RE = \frac{Ci - Co}{Ci} * 100 \quad (1)$$

$$K = \frac{F * Ci}{V} \quad (2)$$

$$CE = \frac{(C_i - C_o) * F}{V} \quad (3)$$

Where:

RE = COD removal efficiency (%)

K = Feed Charge COD (g/m3.h)

EC = Elimination Capacity (g/m3.h)

Ci = Concentration of COD input (g/m3)

Co = Concentration of COD output (g/m3)

F = Airflow (m3 / h)

V = Volume of the reactor (m3)

III. RESULTS AND DISCUSSION.

3.1 FIRST EXPERIMENTAL PHASE: performance of three bioreactors working at different power flows in the liquid phase.

Fermentation systems in the solid state are characterized by the growth of microorganisms on substrates insoluble in water but in the presence of a liquid phase formed by nutrients dissolved in free water, therefore, depending on the water content free, fermentation of solid substrates transitions from solid-state fermentation, fermentations up fermentations slurries of solid particles in suspension ***(Doelle et al., 1992).***

Therefore, the solid state fermentation has disadvantages regarding submerged systems, which are listed below:

- Its application is limited to microorganisms that grow at low moisture contents.
- The removal of metabolic heat can be a problem, especially when working on a large scale and not the process is controlled.
- The solid nature of the substrate causes problems in measuring fermentation parameters such as pH, temperature, moisture content and the concentration of substrate and products.
- The mass transfer processes are limited by diffusion.
- Many engineering aspects such as reactor design and scaling are poorly characterized.

Great efforts are made in the search for partial solutions to the difficulties mentioned above, some scientists as***Gervais and Bazelin (1986)***experimented with a reactor that allowed the regulation of relative humidity and temperature in circulation since according***Ballio et al., (1964) and Richard-Molards et al., (1985)***, has shown that during the development of the fungus, the variation of water activity (Aw), can influence growth mycelium or spores germination and this can be useful for optimizing production in fermenters with conidia solid substrate where oxygen supplementation and harvesting of conidia, is easier than in liquid fermentation.

In contrast to solid phase fermenters, the bio-trickling bioreactors, solve the problems of drying of the bioreactor (low moisture and water activity), and increased diffusion temperature. Absorption processes gas and regeneration of the liquid phase occur simultaneously. Packed columns generally consist of material which allows the development of a microbial film promotes increased volumetric cell density. Usually the packing specific area (contact area per unit volume of column) is relatively low (100 to 300 m2 / m3) and high void volumes (90 to 95%) are preferred to minimize the pressure drop in column and the risk that the empty space unobstructed by microbial growth.

The liquid contains the nutrients needed to maintain a stable microbial film. The liquid is continuously fed at the top of the column, where it is evenly distributed throughout the bioreactor. The liquid drops in thin layers of packing material while the gas flows upstream. The elimination occurs primarily in the biofilm, according to the process conditions, limitations or transfer reaction.

Control both aspects are very important when the compounds are not volatile oxidation and accumulate in the liquid phase. This is the case of sulfates, nitrates and chlorides, in addition to decreasing the pH during degradation, are inhibitory to microorganisms at high concentrations. ***Devinny JS, et al (1999)***

The biological treatment system that is most similar to that proposed in this paper is to bio-trickling filter, but not passing a gaseous stream for decontamination, but air supplied oxygen for aerobic growth of yeast. Furthermore vinasse (liquid phase) as a source of nutrients that descends through the packing, in this case, sugarcane bagasse is used

Figure 2 shows the results of the removal of chemical oxygen demand (COD) using three feed flows of the liquid phase.

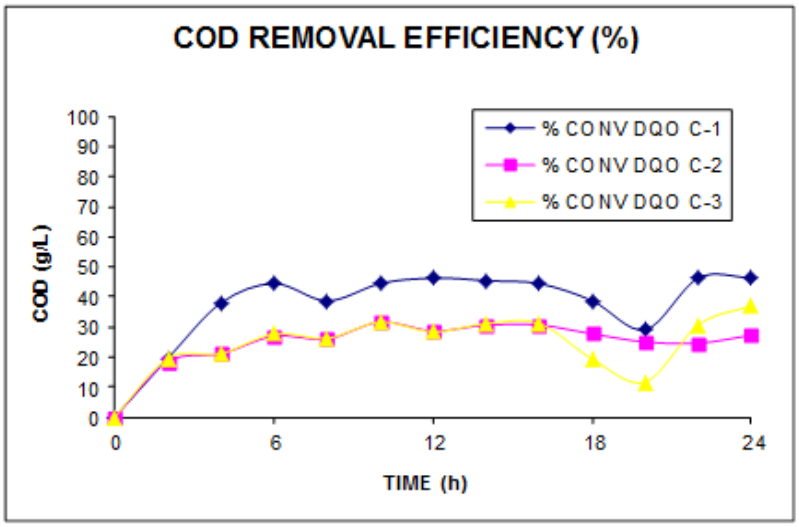

Figure 2. *Behavior of the removal of COD three flows (100, 200 and 300 ml / h)*

It is evident that the highest conversion was achieved in column 1 where the feed flow was stable at 100 mL / h. In this case, was achieved on average 45% removal. Columns 2 and 3 had a similar behavior, achieving a clearance above 30%. It should be observed that the fermentation time is prolonged to only 24 hours, but a stable behavior is observed as for removal. In Figure 3, the behavior of the removal of the Biochemical Oxygen Demand (BOD) is presented.

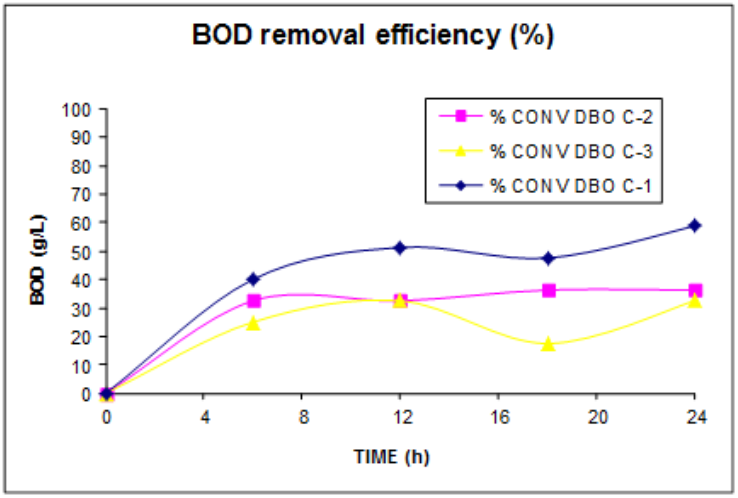

Figure 3. *Removal of Biochemical Oxygen Demand (BOD) during the fermentation.*

BOD removal showed similar behavior to the COD. This effect is due to the yeast Candida utilis is capable of using different organic compounds (sugars, ethanol, organic acids, acetates, etc.) present in the composition of vinasse as a source of carbon and energy***Watteeuw C.M.(1979)***. From a previous screening, a *C. utilis* strain from the ICIDCA collection was selected in liquid medium for its best ethanol elimination rate***Christen P, (1999) and Domenech F, (1999)***. Besides, the growth of *C. utilis* on sugarcane bagasse complemented with glucose, in small packed bed reactors, has been characterized previously***Christen, P. et. al. (1993)***

The yeast *Candida utilis* is classified as food yeast, being an excellent source of protein, vitamins, amino acids and multiple nutrients for animal diet is composed of 50% protein. It is added as a source of protein in animal feed formulations. It is mixed with energy sources and other nutrients.

In spite of the removal of COD and BOD appropriate achieved, it was observed that in the liquid phase, the yeast concentration is increased with time. In Figure 4, the results of determinations of cell counts are shown in time:

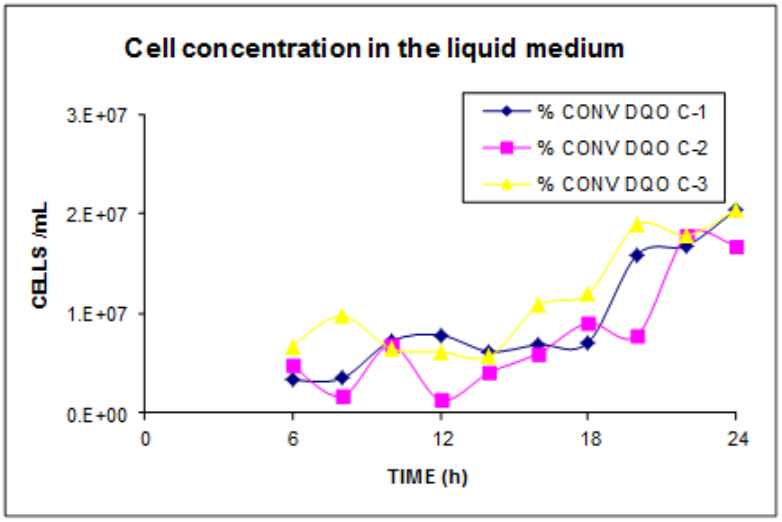

Figure 4. *Results of determinations of cell counts in the course of the fermentation*

The behavior of the three columns was similar, reaching 24 when reaching concentrations above 20 million cells / mL. Clearly, there is considerable loss of cells using a fermentation system with a single column. This behavior indicates that working on a system with multiple columns connected in series, which could prevent the loss of the rising yeast.

In Table 2, the results of cell counts in liquid and solid medium, and accomplished the amount of biomass in the solid medium and had to be achieved if all the cells in the liquid phase is recovered is presented.

Table 2. Cell counts in liquid and solid medium and the amount of biomass in the solid.

COLUMN	Cells in solid medium (Cel / g dm)	Cells in liquid medium (Cel / ml)	Total Cells liquid medium (Cel * flow * 24h)	Cells equivalents /g dm	Total cells/g ms	% Total biomass (g X / 100 g wm)	% Protein (g / 100 g wm)
1	1.45E+09	1.00E+07	2.40E+10	9.60E+07	1.55E+09	15.2	6.8
2	2.30E+09	1.00E+07	4.80E+10	1.92E+08	2.49E+09	24.4	11.0
3	3.05E+09	1.00E+07	7.20E+10	2.88E+08	3.34E+09	32.7	14.7

The percent relative to the dry yeast content is increased from 6.8 in column 1 14.7% in column 3, corresponding to a protein enrichment of bagasse in the order from 6.8 to 14.7 percent respectively.

The behavior of the experiment working as a trickle bed system suggests the possibility of using distillery vinasse supplemented with sources of nitrogen and phosphorus for growing fodder yeast Candida utilis using bagasse as solid support. It was possible to remove 50% of the organic load present in the vinasse.

3.2 SECOND EXPERIMENTAL PHASE: Behavior of three bioreactors working connected in series.

The second experiment was conducted in a shaped by 2500 mL three columns connected in series system. The system remained totally flooded with liquid medium, behaving the fermentation system as a system of immobilized cells. This system has the advantages of reducing fermentation volumes, due to the increase of the liquid phase within the reactor.

Figure 5 shows the results of the removal of chemical oxygen demand (COD) using three fermenters connected in series are shown.

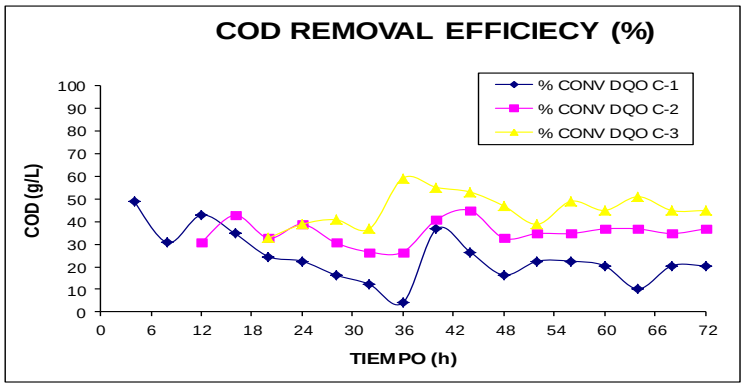

Figure 5. *Behaviour COD removal with three fermenters in series.*

In Figure 5, it is observed that as the fluid passes through the columns COD removal increases. This behavior corresponds to the uptake of nutrient by the growth of yeast in each column. It is achieved 50% removal of COD which provides the liquid medium and the stability of the system is evident within 72 hours. A similar behavior was obtained in the removal of Biochemical Oxygen Demand (BOD).

In Figure 6, the results of determinations of cell counts are shown in time:

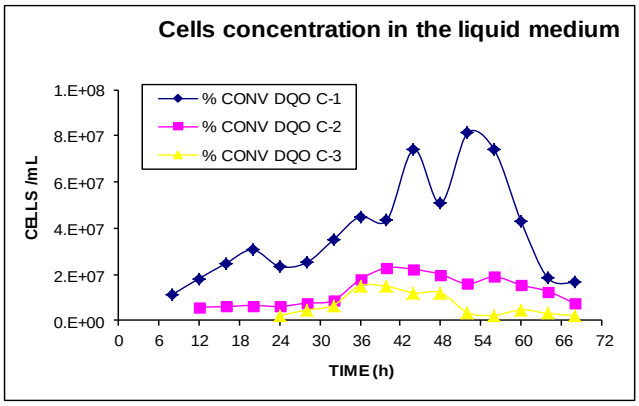

Figure 6. *Results of determinations of cell counts in the course of the fermentation.*

The behavior of the three columns was different, reaching higher concentrations of cells reach in column 1, above 80 million cells / mL. Clearly, the second and third filter served fermenter liquid phase. In the figure shows, as the concentration of cells in the liquid phase decreases considerably, reaching values below 20 million cells / mL.

In Table 3, the results of cell counts in liquid and solid medium, and accomplished the amount of biomass in the solid medium and had to be achieved if all the cells in the liquid phase is recovered presented.

Table 3. Cell counts in liquid and solid medium and the amount of biomass in the solid.

COLUMN	Cells in solid medium (Cel / g wm)	Cells in liquid medium (Cel / ml)	Total liquid medium Cells (Cel * flow * 24h)	Cells equivalents /g dm	Total cells/g ms	Biomass (g X / 100 g dm) (%)	Protein (g / 100 g dm) (%)
1	2.09E+09	4.00E+07	9.60E+10	1.92E+09	4.01E+09	39.3	17.7
2	2.33E+09	2.00E+07	4.80E+10	9.60E+08	3.29E+09	32.2	14.5
3	2.11E+09	2.00E+07	4.80E+10	7.20E+08	2.83E+09	27.7	12.5

As shown, yeast counts on the solid phase remained at similar levels in the three columns (200 million cells / g dry material). However, it was decreasing the liquid phase going from one reactor to another (Column 1 4.01E+09 total cells / mL to 2.83E+09cells / mL in column 3).

At the end of fermentation, yeast similar concentrations were achieved, which considerably increase if the same recirculation system was achieved.

In both fermentation systems were no problems of heat buildup and increased metabolic temperature due to heat generated by microorganisms which leads to inhibition of metabolic activity and drying the medium.

IV. CONCLUSIONS

- It is possible to use distilleries vinasse supplemented with sources of nitrogen and phosphorus for growing fodder yeast *Candida utilis* using bagasse as solid support.
- It is able to remove up to 50% of this organic load in the stillage.
- In the trickle bed reactor is able to retain as many cells on the support in immobilized cell reactor.
- In both fermentation systems were no problems of heat buildup and increased metabolic temperature due to heat generated by microorganisms which leads to inhibition of metabolic activity and drying the medium.

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WP3 Pilot plant study of yeasts production using vinasses as source carbon and energy

a. Experiments on pilot scale bioreactor 20L

An extensive literature search on the subject, which served as the basis for the fulfillment of the objectives was made.

It was activated and put into operation a reactor of 10 liters of biofiltration pilot plant UAM Iztapalapa. Two experiments which will serve as design criteria and operation of a pilot reactor in Cuba, the distillery Jesus Rabi to develop a technology for treating stillage of distilleries and in turn enriching bagasse from sugar cane in systems developed trickle bed fermentation.

The important parameters that define the development of fermentation were studied as it is, the non-addition of sources of nitrogen and phosphorus to support vinasse treatment before fermentation.

Different fermentation systems according to optimize the growth of the yeast *Candida utilis*as stillage substrate using laboratory-produced from cane molasses sugar mill Jesus Rabi were evaluated. To achieve a standardized vinasse, a fermentation medium was prepared to 120 g / L of reducing sugars and inoculated with Sa*ccharomyces cerevisiae* active dry yeast

The experimental results showed that it is possible the use of vinasse from distilleries supplemented with sources of nitrogen and phosphorus for growth of forage yeast *Candida utilis* using bagasse as solid support. It was possible to remove up to 50% of the organic load present in the stillage and moreover, it is possible to obtain a product with more than 9% protein and could be used as dietary supplement for livestock for their balanced content fibers and protein.

A technological procedure efficiently primary treatment of liquid and gaseous effluents released by the Cuban distilleries, as well as the protein enrichment of bagasse to be used as

animal feed was achieved. Material balances and energy corresponding to the selection of appropriate equipment, that would have criteria for a preliminary economic assessment was conducted.
It is possible to obtain 8.0 t / d of a dry product with a protein content of 13.8%, which could be used as animal feed in typical Cuban distilleries. The use of by-products of the sugar mill of waste and ancillary services of the distillery in the proposed technology, enables flexible approach to sustainable production and Sugar Industry.

b. Pilot-scale experiments to find design criteria and operating a larger scale reactor trickle bed fermentation.

The following advances made in the Faculty Regional Tucuman (FRT) can be mentioned:

a) Characterization of bagasse citric, definition of particle size for use as a support and stillage obtained in distilleries Tucuman.

b) Balance of materials, means of crops.

c) Definition of the devices used and experimental designs.

During the stay fermentation system was installed, evaluating the flow medium feeding use in the commune filled with citric bagasse.

Installing an experimental equipment consisting of a cylindrical glass column 2500 mL connected via peristaltic for feeding vinasse and for the backflow of the same pump, the air inlet is installed at the bottom of the column and the output CO2 gases at the top, installing a sensor for quantification. Sterilization tests and column loading were performed the same, the columns were washed and left in 70% alcohol for 24 hours. Citric bagasse, support columns was added. Subsequently, it was started to feed air from the bottom and liquid medium at the top, up to the total volume of the column.

c. Technical training fermentation system

The technical training was developed in the laboratory and pilot gas treatment plant UAM Iztapalapa, with the participation of the Dra. Patricia M. Albarracin of Argentina and MSc. Sergio Hernandez of Mexico.The training was based on the study and demonstration of all experimental steps defined in the experimental protocol. The training served for the three countries have experimental tools, calculation and analysis for the development of the tasks defined in the project.

d. **Theoretical and practical training in the fermentation system.**

The training was developed in the laboratories of the Chair of Organic Chemistry at the Department of Process Engineering and industrial management. 15 graduated students and post-graduate careers of Chemical Engineering, Biomedical Engineering and Mechanical Engineering.

The theoretical and practical training focused on the Materials and Methods used in the system of pilot-scale fermentation. Microorganism used in this study were*Candida utilis* L / 3-75-1 from the collection of ICIDCA. The strain was transferred to Potato Dextrose Agar slants (APD) tubes, incubated for 48 hours at 30 ° C and observed after growth on agar stored at 4 ° C. It was prepared 500 mL of medium composed of: sugarcane molasses, 40.6 g / L; (NH4) 2SO4 4.88 g / L, ammonium phosphate ((NH4) 2HPO4) 1.42 g / L and yeast nutrient 0.067 g / L, 150 mL of medium is added to three 1000-ml Erlenmeyer,

sterilized at 15 psi (121 oC) for 20 min., after cooling, inoculated with wedges each erlenmeyer*Candida utilis* cultivation, propagation was carried out on a rotary shaker for 16 to 18 hours at 30 oC and 200 final propagation rpm. Three inoculawere mixed and determined will count total, viable and budding cells.

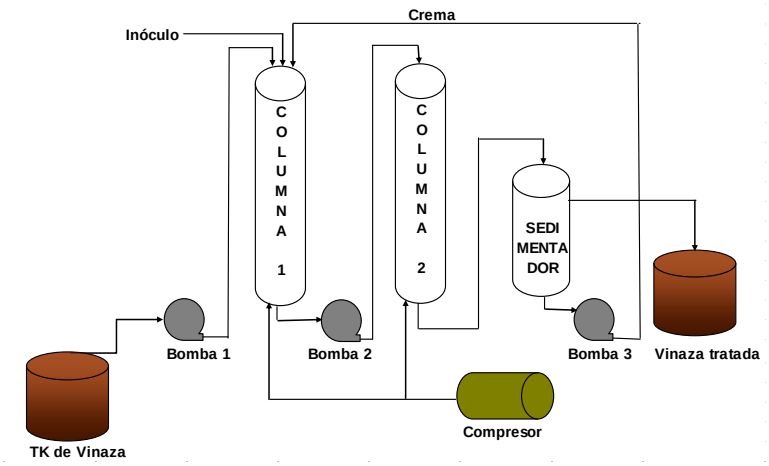
In preparing the liquid medium and support, it was used as an integral support bagasse sugarcane bagasse and citric. The bagasse was sieved through a mesh of 0.5 mm and 5 mm, sterilized at 121 ° C for 30 minutes 700g bagasse this is done with the aim of reducing the microbial load. Then, 225 g were placed in the columns. The support is complemented by the nutrient liquid medium which is formed mainly by distillery vinasse, standardized with water between 25 000 and 50 000 mg / L COD. mass balances necessary for the formulation of the culture media were explained. An spreadsheet in EXCEL prepared to facilitate of the formulation.

The analytical methods used in experiments were the following: the determination of Dry Matter Gravimetric (MSG) of the liquid and solid phase, the determination of total reducing sugars (ART), the pH, chemical oxygen demand (COD), Biochemical Oxygen Demand (BOD), total nitrogen and phosphorus determination were also explained. Practices microbiological control methods were developed, including the total account cells and viable, in solid liquid phase

Meeting of the Cuban specialist Ana Nelis San Juan with specialists from Argentina.
Presentation of the project background and analysis of experimental results developed in the ICIDCA. Date: October 20 to November 5, 2015

A presentation on the project, where the results of the evaluation of a fermentation system drained bed packed with bagasse, inoculated with yeast *Candida utilis*, using as substrate stillage obtained at laboratory scale stand was made.

1. Install fermentation system in the laboratory Bioprocess Regional Tucuman faculty to develop pilot-scale experiments, which will serve as design criteria and operating a larger scale reactor trickle bed fermentation.
2. The ICIDCA bioreactor provided a prototype to be used in experiments with vinasse and bagasse citric. This bioreactor was the basis for the design and construction of new equipment.
   It is mounting an experimental system composed of two cylindrical glass columns connected in series 2500 mL as shown below is proposed.



Details for leaks or spills of gaseous and liquid phases respectively, form filling columns, maintaining technical parameters, etc. are explained

National Technological University Faculty Regional Tucuman, the following actions were developed.

The Industrial Biotechnology Applied to Sugar Sectorcourse was coordinated by the Mg. Ing.PatriciaAlbarracín, and had as objective to define the most suitable for developing the biotech sugar derivatives and sugar technologies.

The course was given to 43 participants, including university professors, doctoral students, masters and engineering and technical personnel linked to the sugar industry.

The following conferences were held:

Sugar cane as raw material biotechnology:
Integrated Production sugar and biotechnological derivatives

Bioproducts for agricultural and veterinary use.
Protein biosynthesis.central metabolic pathways.
Growth of microorganisms. Physicochemical that influence growth factors.
Design and scale bioreactors.
Mass and energy balance of the fermentative processes. Modeling and simulation of bioprocesses.
Economic evaluation in fermentative processes.

Visit to Central Florida. SA, Cruz Alta Department Tucuman

The La Florida sugarmill is a town located in Argentina Cruz Alta Department of the Province of Tucuman, administratively to the commune of La Florida and Louisiana, whose town center is 1 km.

It was found the washing system gas boilers with industrial filters for separating suspended particles and the recovery and reuse of water in washing gas, a practice that could be applied in Cuban mills towers were visited.

Florida brewery tour S.A

A visit to the distillery where knowledge is exchanged on issues related to the alcoholic fermentation was carried out. Cuban and Brazilian experiences were explained on the issue of improving fermentation parameters and the use of mixtures of juices and molasses as substrate in the production of ethanol.

The distillery has serious problems of environmental pollution, as they have implemented systems suitable treatments stillage generated in the distillation.

Visit vinasse treatment plant.

An exchange of experiences on environmental issues was conducted by analyzing different industrial vinasse treatment systems. Cuban experiences of primary treatment of these two biological systems exposed. One where food yeast aerobically grown in liquid medium from

nutrients and organic matter provided by the stillage, as a result, yeast is obtained with high content of protein and vitamins for animal feed and reduced environmental impact. The second system is the anaerobic digestion of vinasse in UASB reactors with the production of biogas, which can have different uses such as renewable energy.

The treatment system stillage is now used in the distillery is the production of compost, this system is not enough, since the volumes of vinasse are extremely large and there is not enough area to allocate for this technique. It was interesting stillage concentration system through the same flasch.

Visit to the Laboratory of Anaerobic Digestion: meeting with Dr. Oscar Morroy and Dr. Florina Martinez, where results of different experiments were analyzed. It managed to copy the design of a pilot UASB reactor to build in the ICIDCA, which will facilitate investigations.

Visit the Laboratory of bioprocesses: meeting with Dr. Felipe Lopez and Hugo Velazco was held. Modeling and simulation systems were analyzed fermentation

Visit the Fermentation Pilot Plant in solid state. An exchange of experiences with Dr. Gerardo Sauceso and Mariano Gutierrez in the development of bioproducts for biocontrol *Metarhizium*and*Tricchoderma*in solid fermentation. It was shown the operation of a solid-phase fermentor pilot scale (20 liters), which provided for the design criteria of a pilot reactor bed immobilized cell drained.

WP 4 Chemical and physical characterization of the product obtained for animal feed. Adaptation of cattle to a balanced feed based on citrus peel.

Citrus growing companies get as waste in industrial process lemon peel, characterized by the difficulty of storage and high moisture content. Large volumes of this waste are a real ecological problem, usually solved by throwing in the river or in a field near the slaughter industry.
For its high fiber content and low cost, this residue can be used to develop an animal food. As a solution to this problem, it was developed an animal feed from lemon peel as a major component at laboratory level. The diets were evaluated microbiological nutritional and energy compared with other foods balanced. Consequently, in this work, the stage adaptation shown the consumption of cattle diets designed

We worked with samples of lemon peel of a citrus industry Tucuman and supplements necessary for balanced animal feed formulation comprising: wet shell (52%), residues of dry pasta (4.6%), cream yeast (35 , 2%), molasses (7.4%), urea (0.4%) and mineral premix commercial (0.4%). Diet was assessed in consumption by cattle donated by a cattle SMEs.

The tests were carried out in the bovine area near Trancas, Tucuman Province between April and June, coinciding with the period of citrus production and scarcity of natural pastures. 4 calves Holando race, clinically healthy, aged and average of 60 days and 85 kg weights (data base is used. Respectively, with two homogeneous groups (two calves per group) were formed to try the following treatments dry):

1) Experimental Treatment: Diet designed (60%) and natural fodder (40%).

2) Treatment Witness: balanced commercial food from cereals (60%) and natural (40%) forage.

It was proceeded according to the steps outlined below:
Step 1: Preparation of diet, nutritional and microbiological evaluation of the same
Stage 2: Selection of animals, pens and feeding adaptation for testing
Stage 3: Application of diet in cattle.
Animals that consume diet designed are housed in a separate pen, while the witnesses are in a collective corral; in both cases the cows are labeled in their ears and have a plastic feeder and water at will. The food is distributed into two fractions one in the morning and afternoon of 2kg each.

The trial consisted of adaptation and acceptance of the diets for six weeks. The replacement is performed gradually from 25% of the usual diet to 100% in the sixth week. acceptability of a diet designed for only in the first week was observed there were rejections of 200 g average per day decreased gradually and disappeared towards the end of the second week.

The daily weight gain was measured by weighing (after fasting period of 18 hours) individual every fortnight. The data showed that the daily weight gain was 430 g average in the first half

to 680 g per day per animal in calves subjected to diet designed at the end of the sixth week of adaptation. While in the witness was 750g average throughout the period.
It is considered that an effluent from the Tucuman citrus industry can be used in formulating a diet for cattle feed being obtained profits similar to those achieved with the usual diet of these animals weight. It would thus be a revaluation of the citrus effluent and a contribution to livestock development in the region in drought period, where scarce natural pastures and coincides with the citrus season.

INTRODUCTION

Argentina in the 2007-2008 harvest produced 1,362,000 t of lemons (20.56% of world production). 1,208 million tons, corresponding to the region of Northwest Argentina (NOA), consisting of the provinces of Tucuman, Salta, Jujuy and Catamarca, the 63.84% [1] They industrialize. The main activity of the industry is the production of concentrated juices, and essential oils, frozen and dehydrated peel pulp produced.

The amount of industrialized lemon in the province of Tucuman had an annual growth in the last decade of 8%, consequently increased their products and discards the same percentage. The products obtained from the citrus industry are for both the domestic market and external. As a byproduct of the process of obtaining the bark citrus juices, membranes, part of the pulp and eventually are fruit seeds. In the case of waste, some companies have in the land of sacrifice and the other flushed with liquid effluents Sali river basin. In both cases the organic matter decomposition generates odors, proliferation of flies and insects, increased pollution load and infiltration into the groundwater levels in the case of land available in sacrifice. Dehydrated lemon peel, is a byproduct of the citrus industry whose main market was getting pectins. Today the fall of this market makes it necessary to study other alternatives. In this case the residue available would be the wet shell due to its high fiber content and its relatively low cost compared to its nutritional value, it could become a nutraceutical. These foods beneficial to health action would make it suitable to develop a functional food veterinarian. These products still lower than those suggested for termination amounts, improve the body condition of ruminants that receive them.

It should be noted that the forage base for the production of beef in the semi-arid plain of Tucuman depressed is given by the use of tropical perennial grasses. Animal nutrition in the dry period is seriously compromised, since both planting winter cereals, such as using agricultural crop residues (corn and soybean), are highly conditioned by rainfall. The economic strategy would be to improve their winter feeding supplements in order to obtain a fat cow or consumption kind to the arrival of spring, where the price of fat cattle increases. Among the energy industry by-products from Tucuman available for livestock feed is citrus peel, with seasonal production.

As an alternative solution to the problem was studied in previous projects, the formulation of a diet feed as the major product using wet citrus peel of the citrus industry in Tucumán. In this work the experience of acceptance and adaptation of diet designed in consumption in cattle shows.

The use of waste from the citrus industry, and lemon peel in support of livestock achieved improvements in the sector, increasing the marketability of the provincial production, promoting social economic development of the region and solving a pollution problem environmental.

MATERIALS AND METHODS

We proceeded according to the steps outlined below:

Step 1: Preparation of diet and nutrition and microbiological evaluation

Stage 2: Experimental design application in animal diet: selection of animals by weight and health status, adaptation of pens and feeding troughs.

Stage 3: Application of diet in cattle.

Stage 1

Diet formulation: Using wet lemon peel and dietary supplements, diet formulation was performed. This was designed based on previous experiences, conducted by our laboratories in 1989, in a confinement of cattle in Ingenio La Florida, Tucuman, thus weight gain of 0.8 kg / animal / day was achieved.

The diet was prepared with: wet peel (52%), corn (4.6%), cream yeast (35.2%), molasses (7.4%), urea (0.4%) and mineral premix commercial (0.4%).

The collection of samples of wet shell was conducted during the months of April, May and June, in a citrus company located in the area Cevil Redondo, Province of Tucuman, Argentina.

It was determined formulated diets: dry matter, protein (protein and amino nitrogen), lipids, crude fiber, ash, carbohydrate and energy value. The parameters were studied using techniques of Official Analytical Chemists Association (A.O.A.C.) [2]. Parallel composition of diets in calcium, phosphorus, iron, lead and microbiological analysis was analyzed according to technical A.O.A.C. [2].

Dietary Supplements: To formulate balanced food sources of protein, vitamins, minerals and carbohydrates, using cream yeast, molasses, corn grain and commercial dietary supplements of vitamins and minerals was used.

Analysis consisted supplements:

Cream Yeast: a portion of about 5% cream yeast output centrifuges deflects a local distillery. The yeast was subjected to cell lysis using a 0.6M NaCl solution [3].

pH was determined and protein and dry matter were analyzed by techniques A.O.A.C. The acidity was determined by titration of neutralization.

Molasses: Molasses is obtained from a sugar mill in the region. In samples analyzed: ash, protein, calcium, iron and lead by methods A.O.A.C .. The total reducing sugars were determined by the method of Lane Eynon- [4].

Stage 2:

Diet was assessed in consumption by cattle donated by a cattle SMEs Trancas area.

The tests were carried out in the bovine area near Trancas, Tucuman Province between April and October, coinciding with the period of citrus production and scarcity of natural pastures. To perform the test 4 calves Holando race, clinically healthy, aged and average of 60 days and 85 kg weights, respectively, with two homogeneous groups (two calves per group) were formed was selected. The first group is made up of animals identified: A052 and A054, while the second group are identified as: A053 and A051 respectively.

Pens so that animals subjected to diet designed they were separated from the rest of the group was redesigned, while the witnesses are in a collective corral. In both cases It will identify them with plastic caravans ears. It was available plastic feeders to facilitate hygiene and to avoid deterioration by acid effect of lemon peels and water at will.

Step 3:

Animals to the following treatments (data on a dry basis) were subjected:
1) Experimental Treatment: Diet designed (60%) and natural fodder (40%).

2) Treatment Witness: balanced commercial food from cereals (60%) and natural (40%) forage.
The food was distributed into two fractions one in the morning and afternoon of 2kg each. The trial consisted of adaptation and acceptance of the diets for six weeks. The replacement is performed gradually from 25% of the usual diet to 100% in the sixth week. Weight Daily Gain (ADG) was measured by weighing (after fasting period of 18 hours) individual every fortnight.

The experimental diet ingredients are slurried with crushed shell and the animals accompanied were provided to facilitate the formation of the rumen and compared with controls. The control diet (commercial feed and hay) were provided separately.

Voluntary intake of dry matter (CVMS) was estimated by pen, through the daily measurement of food rejected.

The clinical status of the animals was monitored weekly by a veterinarian specialist.

RESULTS AND DISCUSSION
Table 4 shows the nutritional values of the formulated diet are observed.

Table 4. Diet Nutritional analysis

Parameters % Dry material	Diet design
Proteins	15.05
Lipids	2.24
Fiber	18.59
Carbs	19.95
Calcium	0.85
Iron	0.05
Sodium	0.63

Yeast cream before being incorporated into the diets, was subjected to a process of cell lysis, suitable for incorporation into animal especially ruminant rations. This procedure was performed because it must take into account the intensive use of yeast in animal feed causes inconveniences by the resistance of the cell wall of the microorganism to enzymatic and chemical attack of gastric juices [5].

In regard to molasses there are numerous history of use in animal rations, but their incorporation should be in small amounts to avoid causing mechanical diarrhea in cattle [6], which was controlled in our case by the addition of hay to the ration.

The mineral traces are added contains high levels of calcium and sodium providing all the necessary requirements of animals for fattening.

The values diet designed in protein, fiber and carbohydrates so reported are suitable for use as food for cattle. This contribution of key nutrients, would be given mainly by the industrial waste wet lemon peel, constituting 52% of the diet.

Table 5 shows the values of microbiological analysis of diet designed observed.

Table 5. Microbiological analysis of diet designed

Parameters	***Design***
NMP total coliform	***<1.8 NMP/g***
E. coli	***<10 UFC/g***
NMP of fecal coliforms	***<1.8 NMP/g***
Salmonella spp	***0/25g***

Table 6. Weight gain (g / day) of calves tested with design diet compared to control animals

	Calves	*1º* Fifteen	*2º*Fifteen	*3º*Fifteen	**Average per animal**	**Average**
Treatment experimental	***A052***	***300***	***530***	***850***	***560***	***683***
	A054	***566***	***850***	***1000***	***805***	
Treatmentwitness	***A053***	***600***	***550***	***680***	***610***	***752***
	A051	***850***	***900***	***930***	***893***	

The data showed that the daily weight gain was 430 g average in the fifteen to 680 g per day per animal in calves subjected to diet designed at the end of the sixth week of adaptation. While in the witness was 750g average throughout the period.

Also of the observations made by the veterinarian specialist it could infer that the animals were in good general clinical status during the period of adaptation to the diet designed.

CONCLUSIONS

The data obtained showed that the diet formulated has an average protein content is 15%, fiber 18.5% and carbohydrate 20%, which would be acceptable values for animal feed, taking into account that the industrial waste, wet lemon peel. It constitutes 52% of the diet and bring the highest percentage of the primary nutrients.

The formulated diet is apt to be subjected to a full test with calves, given its good nutritional and microbiological quality gains achieved similar to those achieved with the usual diet of these animals in the stage of acceptability of the same weight.

It is considered therefore can an effluent from the citrus industry in Tucuman used in formulating a diet for feeding cattle thus achieving a revaluation of citrus effluent and a contribution to livestock development in the region in drought period, where scarce natural pastures and coincides with the citrus season.

Metabolic evaluation of calves diet made with waste citric industry

At previous works It was formulated a balanced food for cattle containing wet orange peel. Once the formulas were analized nutricionally and microbiologically, it was necessary to realice an actual test on animals. We show the metabolic evaluation of calves which tried such diet. The trials were realized in Trancas (Tucuman), between April and June 2015, which coincides with the period of citric production and a shortage of natural grass. Four calves of dutch-argentinian breed, clinically healthy, with average age and weight of sixty days and 85 kg, respectively, were used. Two homogeneous groups were formed (two calves per group) to test the following treatments:

1) Experimental: wet orange peel, corn, yeast, molasses, and urea, and natural forage.

2) Witness: commercial balanced food of soy pellets and corn, and natural forage.

Three blood samples were taken monthly out of each calve during the experiment. It was analized: hematocrit, hemoglobin, glycemia, urea, total proteins, and calcium. We used for glycemia, urea and calcium, an enzimatic method, and for total proteins the Biuret system. In all the parameters studied, the numeric results obtained were within a normal range, in both witness animals and those in the experimental treatment. In the case of the urea, high plasmatic concentrations were observed in the experimental animals, which indicated an excess of proteins in the experimental diet, and a deficit of energy or asynchronism in the degradation of the protein and energy in each portion. On the basis of these results, it was considered important to determine the cause of the elevated values of urea in blood, and thereby reformulate another diet to carry out another live experiment.

A food experiment with citrus peel, treacle and yeast in Dutch-Argentinian calves in Tucumán

Citrus peel are waste of the citric industry. The large amount of such waste presents a real ecological issue.

As a solution to this problem, it has been formulated an aliment for animals which has as its primary component orange peel. The diets were evaluated nutricionally, microbiologically and energetically. This paper shows the figures which belong to an experiment with cattle where the diet designed were used. The diet consisted of: wet orange peel, corn, cream of yeast, treacle, and urea.

The trials were carried out in the cattle area of Zarate, Departamento de Trancas, Provincia de Tucumán, Argentina between the months of April and July, for twelve weeks. Four calves of dutch-argentinian breed, clinically healthy, with sixty days of age and weighting 85kg in average. Two homogeneous groups were formed to test the following treatments :

1) Experimental treatment: designed diet and natural forage

2) Witness treatment: commercial balanced food consisting of cereals and natural forage.

It was measured the daily increase in weight through fifteen days weighing. The figures showed that the increase in weight of the animals in treatment, and of the animals in the witness treatment was similar.

It is considered that a waste of the citric industry of Tucumán can be used together with treacle and cream of yeast, in the formulation of diets for feeding cattle, reaching the increase in weight similar to the one gained by the animals fed with their ordinary diet.

Chemical and physical characterization of the product obtained for animal food.

Mission work Amarilys MSc Carmen Guevara Rodriguez, Esp Technical SilvanoLegrá Mora to Mexico Date: 7. - December 20, 2015

Visit to Mexican pig livestock facilities for evaluating the effect of bioproduct LEBAME in the treatment of excreta and urine to reduce the pollutant load produced by them and their possible use as a food additive in food concentrates and their effect on waste lignocellulosic used as litter in poultry breeding (bagasse, bagasse, rice chips, others), known as chicken manure and chicken manure, and currently used primarily in the production of food concentrates for ruminants.

A conference was imparted and exchanged with industry specialists on the benefits and usability as validations made of bioproduct LEBAME in the treatment of excreta and urine of monogastric and ruminant animals and litters used in upbringings, to reduce the pollutant load produced for them, as well as the productive results obtained with its application in agriculture.

Knowing the results obtained in the wastewater after treatment, fecal coliforms and biochemical oxygen demand (BOD) levels are higher than admitted by the Mexican NOM - 001 SEMARNA -1996T that establishes the maximum permissible limits contaminants in wastewater discharges into waters or domestic use.

Two research protocols were performed for the 2 experiments performed in two pig units located one in the state of San Luis Potosi and another in the state of Oaxaca in order to evaluate the effect of LEBAME on the population of "coliforms faecalis" present in wastewater, the emission of ammonia and BOD in these waters.

TREATMENTS

T1 Control (10 liters of wastewater as is).

T2 LEBAME 5ml diluted in 5 liters of wastewater.

T3 LEBAME 10ml diluted in 5 liters of wastewater.

Each treatment will be determined within 30 days from the first day that the samples were prepared:

I. The presence of coliform

I. The biochemical oxygen demand (BBO)

The results obtained are shown below, and as shown, the concentration of coliforms remained ditto for the three treatments, not BOD which was higher in the treatment where we apply LEBAME 1ml / liter of wastewater. In the treatment where LEBAME applied twice per liter of water BOD result it was lower, and very similar to that water was not added at all.

The results obtained in the laboratory analysis, the concentration of coliforms remained ditto in the three treatments, but not the DBO was far superior to the control treatment where we apply LEBAME 1 and 2 ml / liter of wastewater.The increase in BOD can be given by the

addition of microorganisms, during the establishment and multiplication. In new studies will determine whether the beneficial prevail and / or regenerators provided by the bioproduct.

The knowledge gained will help the technological optimization of bioproduct based on efficient microorganisms for agricultural and environmental use, developed in the ICIDCA, the results can be applied in livestock productions AZCUBA and other organisms as well as food production, contributing to mitigate the environmental impact and efficiently increase production of meat, milk and egg.

It will provide the possibility to use organic waste with better quality as raw materials for feed especially ruminants, the production of organic fertilizers (liquid and solid), import substitution, saving chemical fertilizers, replacing lime production poultry to combat flies and odors produced by manure and urine of animals and in the treatment of wastewater from pig production contributing to the development of agriculture and unsustainable sugarcane sugarcane.

During its development the effective microorganisms synthesize amino acids, nucleic acids, vitamins, hormones and other bioactive substances beneficial to any ecosystem. When increase its population in the middle, the activity with beneficial natural microorganisms is also increased and the microflora in general is enriched, balancing ecosystems, inhibiting the growth of pathogens, harmful microorganisms that cause putrefaction, which have been widely used in the agricultural sector both in soils and crops, organic waste treatment, waste sewage, drastically reducing pests (flies), eliminating odors caused by the decomposition of excreta and urine, and disease prevention.

It arises that reduce odors as nitrification-denitrification favor, which removes nitrogen including ammonia, which is often responsible for bad odors. They also use hydrogen sulfide and methyl mercaptan odors associated (putrefaction) as final recipients loads of the respiratory chain.

Its use has been approved in several important countries, including the United States, the Department of Agriculture included all microorganisms present in EM® within the category of G.R.A.S. (Generally Recognized As Safe). (ENVIRONMENTALLY SAFE). Knowing that the production of efficient Microorganisms craft are local solutions in which most of the time not containing microorganisms and the production process is not reproducible. Researchers ICIDCA have been given the task of producing inocula are known for efficient production of microorganisms with known strains in an optimal and reproducible production process for producing the byproduct consisting LEBAMED microorganisms culture collection of *Bacillus subtilis* strain collection of ICIDCA B / 23-45-10 Nato, *Lactobacillus bulgaricum B* / *Saccharomyces cerevisiae* 103-4-1 and L-7/25/12, which is produced from an inoculum of the aforementioned microorganisms, and water cane end Miel through a fermentation process, which is in the process evaluation.

Results obtained

Porcine facilities dedicated to reproduction, growth and fattening pigs visited and a food plant where lignocellulosic residues poultry prey. Treatment plants of residual installed in pig farms were also visited.

Knowing by results shown above in wastewater obtained after treatment, fecal coliforms and biochemical oxygen demand (BOD) levels are higher than admitted by the Mexican NOM - 001 SEMARNA -1996T which establishes the maximum limits allowable contaminants in wastewater discharges into waters or domestic goods.

Two research protocols were performed for the 2 experiments performed in two pig units located one in the state of San Luis Potosi and another in the state of Oaxaca in order to evaluate the effect of LEBAME on the population of "coliforms faecalis" present in wastewater, the emission of ammonia and BOD in these waters.

First experiment: Farm Play the CP Roberto Zermeño, located in Villa de Reyes in San LuisPotosi in Mexico.

View broods área Farm

The calculus of production waste (excreta) were first performed as animal mass and live in each unit weight, to estimate the need for bio-product to be applied throughout the excreta produced in the unit.

Table 7. Total production excreted in the reproduction unit CP Roberto Zermeño

Etapas	Categorías	Población porcina	% Etapa	Peso Promedio (kg)	Peso Total (kg)	Tasa diaria de excresión (%PV)	Producción de excretas (kg/animal)	Producción de Excretas Total (kg/dia)
Reproducción	Hembras lactantes	176	1,63	220	38,720	8,08	17,78	3,129
	Hembras gestantes	774	7,15	220	170,280	3,35	7,37	5,704
	Hembras vacias	50	0,46	200	10,000	5,04	10,08	0,504
	Numero de vientres	**1000**	**9,24**	**213,33**				
	Sementales	18	0,17	300	5,400	2,93	8,79	0,158
	Lechones	1600	14,79	2,7	4,320	9,00	0,24	0,389
	Subtotal	**2618**	**24,20**	**172,01**				
Crías	Destete	3200	29,58	14,6	46,720	8,60	1,26	4,018
	Subtotal	**3200**	**29,58**	**14,60**				
Finalización	Crecimiento	2600	24,03	40,0	104,000	7,11	2,84	7,394
	Finalización	2400	22,19	77,5	186,000	6,95	5,39	12,927
	Subtotal	**5000**	**46,22**	**44,03**				
Total de la población		**10818**	**100**		**565,440**			**34,223**

	ton/día	ton/mes	ton/año	ton/m³	m³ /día
Excreta production unit	34,22	1060,82	12,4903		
Density of excreta				1,00	34,22

Samples wastewater coming from the lagoon sedimentation phase the waste treatment plant were taken, as shown in following photos.

Settlers

Table 8. Result Report reproduction unit CP Roberto Zermeño

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INFORME DE RESULTADOS

No. DE INFORME:	22-0097 A 22-0099
FECHA DE INFORME:	2016, enero 25
CLAVE DE LA MUESTRA:	22-0097 A 22-0099

CLIENTE:	GRUPO TECNOLOGICO DE ENERGIA RENOVABLE S.A. DE C.V.
DIRECCIÓN:	1ER RETORNO UNIVERSITARIO ACCESO 1 INT. 3-A, LA PRADERA QUERETARO QRO. CP 76769
TELÉFONO/FAX:	442 135 69 72
ATENCIÓN A:	ING. KARLA ORDAZ

LUGAR DE MUESTREO:	VER RESULTADOS
TIPO DE MUESTREO:	CLIENTE
FECHA DE MUESTREO/HORA:	CLIENTE
RESPONSABLE DE MUESTREO:	CLIENTE
FECHA RECEPCIÓN/HORA:	2016, ENERO 13 / 16:00 h

PARÁMETRO	RESULTADO	UNIDADES	MÁXIMO PERMISIBLE	FECHA DE ANÁLISIS	MÉTODO DE ANÁLISIS
22-0097 (AR SLPT1 11 ENERO)					
Coliformes Fecales	<300	NMP/100 mL	N.E.	2016, enero 14	NMX-AA-042-1987
Demanda Bioquímica de Oxígeno	71.60	mg/L	N.E.	2016, enero 14	NMX-AA-028-SCFI-2001
22-0098 (AR SLPT2 11 ENERO)					
Coliformes Fecales	<300	NMP/100 mL	N.E.	2016, enero 14	NMX-AA-042-1987
Demanda Bioquímica de Oxígeno	107.60	mg/L	N.E.	2016, enero 14	NMX-AA-028-SCFI-2001
22-0099 (AR SLPT3 11 ENERO)					
Coliformes Fecales	<300	NMP/100 mL	N.E.	2016, enero 14	NMX-AA-042-1987
Demanda Bioquímica de Oxígeno	86.60	mg/L	N.E.	2016, enero 14	NMX-AA-028-SCFI-2001

OBSERVACIONES

EL SÍMBOLO "<" INDICA EL LÍMITE DE CUANTIFICACIÓN DEL MÉTODO.

COLIFORMES FECALES: "ANÁLISIS REALIZADO EN : CALDO EC, TEMPERATURA : 44 ± 0.5 °C, TIEMPO: 24 h"

N.E. NO ESPECIFICADO

PARÁMETROS ACREDITADOS ANTE LA ENTIDAD MEXICANA DE ACREDITACIÓN

ecosistemas industriales
ACEPTADO
FECHA 160126
CONTROL DE CALIDAD

ATENTAMENTE

Lourdes M.L.

TEC. LOURDES MAQUEDA LÓPEZ
SIGNATARIO AUTORIZADO

FIN DEL INFORME

EL PRESENTE INFORME SOLO ES VALIDO EN ORIGINAL CON LAS FIRMAS AUTORIZADAS Y AMPARA ÚNICAMENTE LAS MUESTRAS QUE SE INDICAN
EL PRESENTE INFORME NO DEBE REPRODUCIRSE EN SU TOTALIDAD SIN PREVIA AUTORIZACIÓN DE ECOSISTEMAS INDUSTRIALES, S.A. DE C.V.
Y NO PODRÁ SER REPRODUCIDO PARCIALMENTE
ACREDITACIÓN: AG-204-035/12, VIGENTE A PARTIR 2012-08-17

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Second experiment: Farm Tepetapla Santiago, located in Oaxaca, dedicated to the breeding and development of pig fattening 21día until 12 weeks of age (84 days)

Main entrance of the piggery

The calculation of production waste (excreta) was performed according to the animal mass and body weight in each unit, to estimate the need for bioproduct to be applied throughout the excreta produced.

Table 9. Total production excreted in Santiago Farm development unit Tepetapla in Oaxaca.

Etapas	Categorías	Población porcina	% Etapa	Peso Promedio (kg)	Peso Total (kg)	Tasa diaria de excresión (%PV)	Producción de excretas (kg/animal)	Producción de Excretas Total (kg/dia)
Crías	Destete	7500	53,57	14,6	109,500	8,60	1,26	9,417
	Subtotal	**7500**	**53,57**	**14,60**				
Crecimiento	Crecimiento	6500	46,43	40,0	260,000	7,11	2,84	18,486
	Subtotal	**6500**	**46,43**	40,0				
Total de la población		**14000**	**100**		**369,500**			**27,903**

	ton/día	ton/mes	ton/año	ton/m³	m³ /día
Producción de excretas en la unidad	27,90	864,993	10,184595		
Densidad de las excretas				1,00	27,90

Wastewater samples from the final oxidation pond treatment plant waste, where the water falls from the settlers were taken.

Final oxidation pond

Settlers

It was used for making samples carafes (gallons of 5 liters) with a hole at the top, which was left open for exchange with air.
TREATMENTS
T1 Control (3 liters of wastewater as is).
T2 LEBAME 3ml diluted in 3 liters of wastewater.
T3 6ml of LEBAME diluted in 3 liters of wastewater.
Each treatment was determined at 30 days from the first day that the samples were prepared:
• The presence of coliform
• The biochemical oxygen demand (BBO)

The results obtained in the laboratory analysis, the concentration of coliform iden remained in the three treatments, but not the BOD was far superior to the control treatment where we apply LEBAME 1 and 2 ml / liter of wastewater.

It is worthwhile noting that the increase in BOD can be given by the addition of microorganisms, as they settle and multiply can increase the BOD.

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INFORME DE RESULTADOS

No. DE INFORME:	22-0100 A 22-0102
FECHA DE INFORME:	2016, enero 28
CLAVE DE LA MUESTRA:	22-0100 A 22-0102

CLIENTE:	GRUPO TECNOLOGICO DE ENERGIA RENOVABLE S.A. DE C.V.
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LUGAR DE MUESTREO:	VER RESULTADOS
TIPO DE MUESTREO:	CLIENTE
FECHA DE MUESTREO/HORA:	CLIENTE
RESPONSABLE DE MUESTREO:	CLIENTE
FECHA RECEPCIÓN/HORA:	2016, ENERO 13 / 10:00 h

PARÁMETRO	RESULTADO	UNIDADES	MÁXIMO PERMISIBLE	FECHA DE ANÁLISIS	MÉTODO DE ANÁLISIS
22-0100 (AR OAX1 15 ENERO)					
Coliformes Fecales	<300	NMP/100 mL	N.E.	2016, enero 14	NMX-AA-042-1987
Demanda Bioquímica de Oxígeno	182.00	mg/L	N.E.	2016, enero 14	NMX-AA-028-SCFI-2001
22-0101 (AR OAX2 15 ENERO)					
Coliformes Fecales	<300	NMP/100 mL	N.E.	2016, enero 14	NMX-AA-042-1987
Demanda Bioquímica de Oxígeno	332.00	mg/L	N.E.	2016, enero 14	NMX-AA-028-SCFI-2001
22-0102 (AR OAX3 15 ENERO)					
Coliformes Fecales	<300	NMP/100 mL	N.E.	2016, enero 14	NMX-AA-042-1987
Demanda Bioquímica de Oxígeno	209.00	mg/L	N.E.	2016, enero 14	NMX-AA-028-SCFI-2001

OBSERVACIONES
EL SÍMBOLO "<" INDICA EL LÍMITE DE CUANTIFICACIÓN DEL MÉTODO
COLIFORMES FECALES: *ANALISIS REALIZADO EN: CALDO EC, TEMPERATURA: 44 ± 0.5 °C, TIEMPO: 24 h*
N.E. NO ESPECIFICADO
PARÁMETROS ACREDITADOS ANTE LA ENTIDAD MEXICANA DE ACREDITACIÓN

ecosistemas industriales
ACEPTADO
FECHA 160126
CONTROL DE CALIDAD

ATENTAMENTE
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TEC. LOURDES MAQUEDA LÓPEZ
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Y NO PODRÁ SER REPRODUCIDO PARCIALMENTE
ACREDITACIÓN: AG-304-03/15, VIGENTE A PARTIR 2013-08-17

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The genetic piggery breeding San Diego Union, in the state of San Miguel del Valle belonging to Kasto group where we arranged future experiments was visited.

Main entrance of the Farm

A plant producing feed for beef cattle and milk, where one of the ingredients in the food constitutes the chicken manure (composed of excreta, urine) and lignocellulosic residues used in raising litter of broilers was visited.

Dehydrator store manure chicken manure

Store manure chicken manure

Dehydrator

In exchange with producers and specialists argue that this residue provides high protein content and other elements in the formulation despite the process of thermal dehydration that lasts between 15 and 20 min, which enters at a temperature of 120 degrees out with a temperature of 70 -80 degrees and a humidity of 10-12%.

It was evaluated the possibility of applying the LEBAME chicken manure, because not receive any microbiological before reaching the treatment plant feed. This action could not be executed by the time factor.

Final product

Main results obtained:

1. Obtained vinasse laboratory scale, which were characterized later his, the proposed experiments were performed. It was confirmed that this waste is highly aggressive to our environment, and that a pretreatment prior to dumping necessary.

2. The spreadsheet developed in EXCEL, with material balances and the bioprocess conditions allowed the formulation of liquid media, solid media and inoculum.

3. It proved to be possible to use stillage of distilleries, supplemented with sources of nitrogen and phosphorus for growth of the yeast Candida utilis forage using bagasse as solid support, indicating that on the one hand, it is possible decontamination vinasse , reaching remove 40% of the polluting organic matter to the environment and on the other, it is possible to obtain a product with more than 9% protein, which could be used as a dietary supplement for cattle for its balanced fiber content and proteins.

4. Experimental results and statistical analysis shows that the best operating conditions on a pilot scale are:

• Working to flow 0.6 L / h.
• Working with COD concentrations of 50 g / L.
• Working with load values of 12 g / Lh.

These results are the basis for the proposed design of an industrial plant.

5. The process was developed together To Autonomy Metropolitan University of Iztapalapa, Mexico transferred to the Regional School of Tucuman in Argentina working citrus peel with satisfactory results, corroborating the initiative of South - South Cooperation.

IV. Dissemination activities

Project site within the ICIDCA WEB page, showing project activities www.icidca.cu/ Bioproducts***"Technologies for obtaining food supplements and veterinarians to improve efficiency in animal production"***

Thesis of chemical engineering degree.ISPJAE, "Primary Treatment of distillery stillage Using Candida utilis on a Bed Drained System".Geidy Suárez Valdés.

V. Financial information

Funds Source	**TFPG**
Total budget	35 000,00
Executed Budget	29.561,75
Available Budget	5.438,25
Execution (%)	84,5

VI. Briefly description of the lessons learned during the period

1. Combining several objectives to decrease the costs of the achievement of results. This recommendation is valid for achieving the objectives in a foreign place like at the national industry where we have to accomplish visits up country.
2. The establishment of multidisciplinary groups at the companies contributes to guarantee the sostenibility of the activity
3. Joining efforts with the participating institutions and AZCUBA for fulfillment of objectives
4. To implicate factors that intervene in the executive decisions of introduction of results and benefit of services at the national industry with commitments of projects of AZCUBA's Program to join forces and to contribute to the fulfillment of the objectives.

Dra. Georgina Michelena
Project Coordinator

Havana, September 6th, 2016