REDUCTION OF STILLAGE's VOLUME IN THE PRODUCTION OF ETHANOL BY RECIRCULATION APPLYING LIQUID-LIQUID EXTRACTION.

Castro, G. A.[a], Caicedo, L. A.[a]

[a] *Dpt of Chemical Engineering, Facultad de Ingeniería, Universidad Nacional de Colombia, Sede Bogotá, Ciudad Universitaria, Cll 45 Kra 30 Bogotá, Colombia*

Abstract

The recirculation of the stillage (or vinasse) to the stage of fermentation, in the production of alcohol, is a technique of source reduction very applied in this industry, but it presents the inconvenience of it turns limited because with her they can feed to the process by-products of the fermentation that remain in the stillge after the distillation they are inhibitory for the yeast and they also go accumulating when applying several recirculations in series. In this work was defined the acetic acid, as the main inhibitor of the yeast and the effect of different concentrations of this on the fermentation was evaluated, it was observed that any level of the acid affects the biomass and starting from 2.5 g/L it begins to diminish the production of alcohol. The extraction liquid-liquid was applied, on the stillage, as separation technique of this acid, with that were improved the recirculation possibilities, since, without the separation and after five recirculations, the biomass diminished until in 70% and the productivity of alcohol until in 30%, while when the extraction was applied, the biomass was affected in 42% and the productivity of alcohol didn't diminish; during the five recirculations, a descent in the consumption of water of dilution of 56% was achieved and in the generation of stillage of 44%.

Keywords: Ethanol production, fermentation, distillation, stillage, vinasse, liquid_liquid extraction.

1. Introduction

The great demand of alcohol that actually we can observe causes, as response of the industry, a high production with the subsequent generation of residues as the vinasse or stillage, to which it is necessary to give her the importance that is deserved, as pollutant potential or source of by-products if this way it is wished.

As answer to the world critical environmental situation, to the gradual decrease of the fossil fuels and the possibility in the country of developing alternative energies using renewable resources, the government determined to send the Law 693 of 2001, where is regulated the addition of anhydrous ethanol to the gasolines in 10% starting from 2006, according to this it is necessary to increase the production of this alcohol, until levels initials of 750 million liters / year, (Program of Carburating Alcohols).

As agricultural sources for the obtaining of the ethanol there are the sugary ones (cane of sugar, beet), the starch sources (barley, banana tree, yucca), the lignocellulosics (wood, cotton). The physicochemical characteristic of all sources are different whose causes differences in the adaptation operations for a common later treatment in the three cases, whichever it is the source of sugar for the fermentation, the processes have the high production of liquid effluents of the distillation in common, denominated in this particular case, vinasses or stillage, Durán de Bazúa in 1993 defined the vinasse like "a dilution of substances like mineral and organic salts with relative value and with potential for diverse uses", thanks to his organic high load (6.5.7.6%), CaO (4.57%) and K_2O (1.06-1.53%) (GEPLACEA / PNUD / ICIDCA 1990), among other substances, that make it appropriate of being used as energy source, protein source, mineral salts source for fertilizers and concentrate for animals.

In the conventional process of production of alcohol the vinasse production oscillates between 13 and 15 l/l ethanol, with this and if there are a requirement of 2 million liters of ethanol / day approximately, according to the Carburating Program of Alcohol, the quantity of the effluent per day could ascend to 30 million liters, that it is an important quantity and that could harm a lot in the environment, if it isn’t managed appropriately like some evidences show us for example the experiences of the Cauca`s river Valley, that which can provoke the cancellation of the Environmental License in these distilleries.

As alternative to reduce the vinasse volume has been propossed the recirculation (recycling) from the same ones to the fermentation stage, like dilution water, however the presence of inhibitors like the acetic acid, limits their application. The present work evaluates the effect of the concentration of acetic acid on the alcoholic yield in the fermentation and the use of extractants agents of the acid to improve the recirculation possibilities.

2. Materials and Methods

2.1 Culture

The used culture was a *Saccharomyces cerevisiae* ATCC26603, donated by the Institute of Biotechnology of the National University of Colombia (IBUN), in liofilized state, it was resuspended in peptoned water and it was sowed in a growth medium (yeast extract 1%, peptone 2%, glucose 1%, maltose 1%, agar 2%, water 93%, all percentages w/w) at 30ºC for 48 h, starting from this cultivation broth was prepared the main cultivation (peptone 1%, sucrose 2%, K_2HPO_4 0.2%, $MgSO_4$ 0.001%, $(NH_4)_2SO_4$ 0.2%, water 96.5%, pH 4.5) and was inoculated with 15% of the previous broth and it was renewed monthly.

2.2 Fermentation

The inoculum for the fermentation, constituted 15% of the broth of fermentation, both were compound of cane molasses 16.5%, water 83.5%, $(NH_4)_2SO_4$ 0.01%, the pH was adjusted at 4.5, with a solution 15% w/w of H_2SO_4, the time of preparation of the inoculate was of 14 h and that of the fermentation of 20 h, in a reciprocating agitator with temperature control at 30ºC and 110 rpm.

2.3 Vinasse obtention

The fermented must obtained was distilled in a Büchi Brinkmann distillator with ballon of 1 L of the Physicochemical Analysis Laboratory of ICTA (Food Science and Technology Institute, National University of Colombia), during a time that oscillated between 12 and 16 min., to atmospheric pressure, for each one of the volumes of 250 ml of broth, of this volume 15% was distilled, to obtain 37.5 ml of solution of alcohol and the vinasse that remained in the ballon.

2.4 Recycling Tests

The vinasse obtained in each distillation (212.5 ml approximately) was centrifuged at 900 g during 15 min., later was take a quantity of vinasse equivalent to 70% of the water of dilution of the following fermentation (128 ml); in the cases in that extraction was not applied, with this was prepared the following fermentation medium, in the other case it was taken to the extraction stage.

2.5 Extractant agent selection
Recipients with cover of 25 ml were used they were loaded with 10 ml of diluted solution of acetic acid in water and different quantities of solvent or organic phase, in proportions of watery phase to organic phase of 4:3 and 1:1, the recipients were shaked during 1 h to 170 rpm, later were put to rest to reach the equilibrium, during 12 h at 25ºC, after this time were taken 2 ml of the watery phase for analysis, this way were evaluated five extractant agents and among them one was chosen keeping in mind the cost, the distribution coefficient and the toxicity on the yeast.

2.6 Extraction tests on the vinasse
The extraction on the vinasse was carry out in erlenmeyer of 500 ml, with the obtained vinasse of the distillation, as watery phase (128 ml) and the defined solvent previously, as organic phase, in proportion 1:1, the increase of the contact area made sure putting the erlenmeyers in the reciprocating agitator to 180 rpm, during 1 h, after which they were stopped to reach the equilibrium in funnels of decantation of 500 ml in an incubator at 25°C, during 12 h.

2.7 Analysis
The kinetics of the fermentation was verified by analysis of sugars and etanol at different fermentation times by high performance liquid chromatography (HPLC) in a Waters chromatography system, with a detector of refraction index, each sample was centrifuged 900 g during 15 minutes, later it was diluted ten times, starting from the second fermentation a solid phase extraction was applied using Sep pack C18, to retire the extractant agent's residuals that could remain in the recycled vinasse, after the sample was filtered using a 0.22 μm membrane and finally it was injected manually in the cromatograph. The used column was a Sugar-Pack type Waters, as mobile phase it was used water deionized and degasifed with a flow of 0.5 ml / min, the column temperatures and detector were respectively of 84 and 40ºC, the volume of sample was 20 μl.

The quantification of the acetic acid in the vinasse was also made in the same equipment of HPLC, each sample was centrifuged at 900 g during 15 minutes, later it was diluted twice, next it was filtered using a 0.22 μm membrane and finally it was injected manually to the cromatograph. The column used was Shodex KC-811 that requires of a precolumna KG-C, as mobile phase was used water deionized and degasified to a flow of 1 ml / min, the column and detector temperatures were respectively of 30 and 40ºC, the volumen of the sample was 20 μl.

The toxicity of the two extractants agents more promissory was evaluated in the following way: it was prepared a medium of 60 ml and it was inoculated with 10 ml of cultivation broth in exponential phase of growth, this mixture was divided in six falcon tubes, two of them were added with one from the solvents to prove (1 ml), to another couple of them was added the other solvent (1 ml) and the two remaining tubes were left as white. The six tubes were shaked during two hours and then they were left in rest by 8 hours more, after which biomass was determined, using the technique of the camera of Neubawer.

3. Results and Analysis

Initially the fermentative capacity of the yeast was evaluated, being observed a descent of the soluble solids from 14 to 7ºBx after 23 h, results that agree with those of Obregón (2006) that was able to arrive between 5.8 and 7.5ºBx after 22 -24 h, when observing a decrease of 0.5ºBx in the last three hours, it was defined the time of fermentation in 20 h.

Later the inhibitory effect of the acetic acid on the fermentation was studied, for that which were ahead four fermentations in parallel with different concentrations of acetic acid from (0, 2.5, 5 and 7.5 g/L), added to the fermentation medium.

In the figure 1 can observe that for concentrations of acetic acid least than 2.5 g/L, the production of ethanol is increased, seemingly for the associate of this, with the generation of ATP, its synthesis is increased by the energy necessity of the cell of carrying out the phosphates active transport toward the interior of the cell, affected by the solubilization of the ión acetate, of acetic acid, in the lipids of the cellular membrane (Maiorella, 1983); for superior values in the acetic acid concentration the ethanol production diminishes toward 20 g/L (a decrease of 70% regarding the initial value), value a little inferior to the one achieved by Maiorella (30 g/L), under the same inhibition level, maybe because he used glucose like sustrate and a complex mixture of nutritious; the generation of biomass diminishes in 50%, from $5*10^7$ cells / ml to $2.5*10^7$ cells / ml is observed that the biomass is affected by the most minimum concentration in the inhibitor.

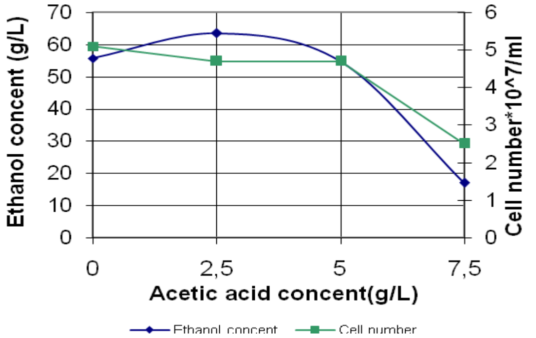

Figure 1 Inhibitory effect of acetic acid on the ethanol and biomass production.

To improve the recirculation possibilities, it is necessary to retire the acetic acid, due to its inhibitory power on the yeast, some techniques have been proven to retire organic matter of the vinasse, as the micro and ultrafiltración (Melzoch, 2001; Kim, 1999, In-Soung Chang, 1994), the electrodialysis (Cheryan, 1995; Decloux, 2002), in the 2001, Melzoch mentioned the use of the liquid-liquid extraction to retire organic matter in the vinasse and that to imply less costs in its application at laboratory level and probably to industrial scale, it was selected in this work; for its implementation it is required initially to select the solvent to use, according to three approaches, low cost, high distribution coefficient and low toxicity, a revision of different extractant agents was made assisting to the first two approaches, look at table 1. Keeping in mind the cost, some of these was discarded and was determined the distribution coefficient of those that don't report it and have low cost, were used two proportions of watery phase to organic phase, 1:1 and 4:3, the results appear in the table 2.

Table 1 Price and distribution coefficients of solvents with extractive capacity of acetic acid.

Solvent	Theoric Distribution coefficient	Source	Price ($/Kg)	Source
Penthanol	4.033	Fahim et. al. 1996	180.000	ICIS Pricing
MIPK	3.444	Correa et. al. 1989	270.000	ICIS Pricing
Isophorone	3.164	Colombo et. al. 1999	6.720	Proquimort
Oleic acid*	-	Briones et. al. 1994	3.250	Proquimort
Castor oil*	-	Offeman et. al. 2006	4.800	Proquimort
Raw palm oil methylic esther*	-	Bujang et. al. 1997	5150	II Seminario Internacional Biocombust.
Kerosene**	-	Yang et. al. 1991	2.800	www.minminas.gov.co
Aliquat 336	2.71	Yang et. al. 1991	1'028.248	ICIS Pricing

*: Are solvents reported with extractive capacity of low molecular weight organic matter
**: Is reported like diluent

Table 2 Distribution coefficients at two proportions watery phase (0.9 g/L of acetic acid) to organic phase.

Solvent	Distribution Coefficient	
	(4:3)	(1:1)
Isophorone	2.3	2.69
Oleic acid	0.3	0.37
Castro oil	0.12	0.19
Raw palm oil methylic esther	2.6	3.1
Kerosene	0.12	0.17

Assisting to the highest distribution coefficients for the isophorone and the raw palm oil methylic esther, it was decided to evaluate with them the toxicity on the yeast, giving as a result that for the first one the number of cells / ml was of $1.1*10^5$ and for the second of $2*10^5$, this bigger value for the methylic esther defined it as the selected solvent, the proportion was used 1:1 to present better distribution coefficients.

To study the effect of the extraction of the inhibitor on the vinasse recirculation were carry out five fermentations in series (F12, F22, F32, F42 and F52) each one of those used 70% of the vinasse of the previous fermentation, to dilute the molasses, is to say, part of the water used originally was substituted in this process, parallelly to these five fermentations, were carried out other ones (F11, F21, F31, F41 and F51) this time applying a liquid. liquid extraction to the vinasse, before recycling it.

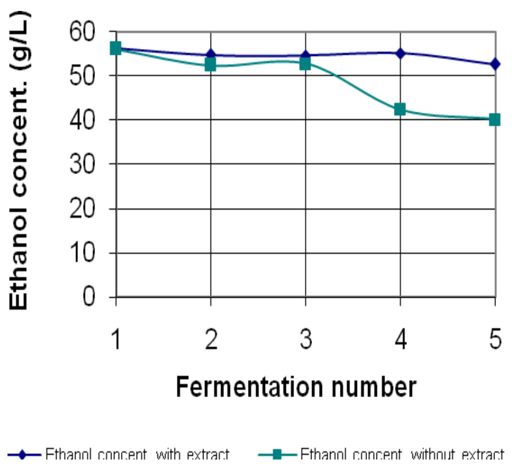

Figure 2 Ethanol final concentration of the fermented musts prepared with vinasse with and without extraction

The figure 2 shows how varies the concentration of ethanol in each one of the five fermentations, with recirculation of 70% of the vinasse, one could see that the recirculation with extraction applied to the vinasse, doesn't have a notorious effect on the achieved concentration of ethanol, is to say, there isn't a decrement in the ethanol concentration in the fermented final must, with the advance of the successive fermentations; for the case of the fermentations without extraction, is to say, it was recycled the vinasse directly only applying the centrifugation (inferior curve figure 2), is posible to see a similar behavior in the first three fermentations, also comparable with that observed for the case with extraction, since the values of concentration of ethanol are reached 55 g/L. The last two fermentations show a descent in the concentration of ethanol until values of 40 g/L.

The figure 3 shows something similar to the figure 2 to the ethanol, this time with the biomass, in this case it presents a descent from the second fermentation, making notorious that the answer of the yeast is more sensitive than that of production of ethanol and when the extraction is not applied in the vinasse, with the one that will be using to prepare the corresponding medium, the quantity of produced biomass is even smaller.

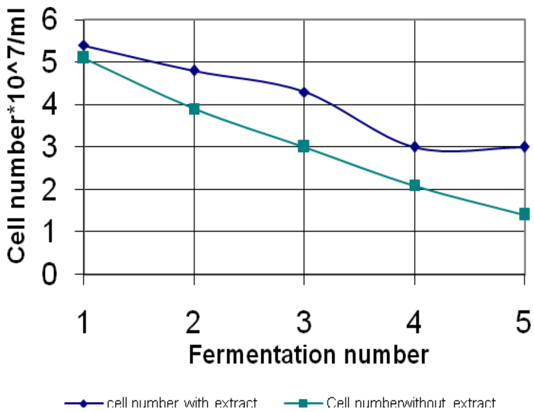

Figure 3 Final biomass in the fermented musts prepared with vinasse, with and without extraction

Besides the inhibitory effect for the acetic acid, one cannot ignore the effect of the increase of the solids in the vinasse, they can includ the not fermentable sugars that remain in the vinasse after the fermentation and the distillation, mineral salts not assimilated by the yeast, are also included, these substances are increased with the advance of the serial fermentations, due to the molasses and the nutrients with those the mediums of fermentation are prepared are rich in these substances (Maiorella, 1984), others are by-products of the metabolism of the yeast like glicerol, propanol, furfural and lactic acid that also inhibit the fermentation and the speed of growth (Navarro, 1989 and Maiorella 1983).

In the figure 4 the increase of solids is observed in the vinaza and in the mediums that were prepared with this, for example for the fermentation 3, there is two mediums, one (M31) (when one speaks of Mxy or Vxy, it refers to the mediums of the fermentation Fxy or to the resulting vinasse of Fxy, respectively) prepared with the vinasse to the one that was applied extraction (V21) and the other one (M32) with vinasse without previous extraction (V22), the first one with 21.45ºBx and the second with 20.99ºBx, each one of these mediums, after being fermented and distilled produce their corresponding vinasses, V31 and V32, with 15.7ºBx and 14.7ºBx, respectively.

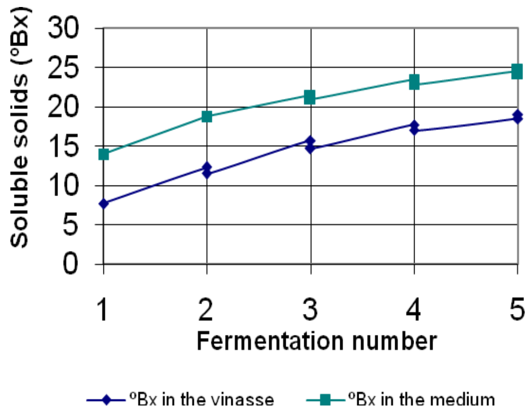

Figure 4 Increase in the soluble solids in the vinasse and the médiums prepared with them.

The soluble solids (fermentable coming from the molasses and not fermentable coming from the vinasses) in the mediums of the fifth fermentation (M51 and M52), reached values of 24.59 and 25.19ºBx, respectively, very close at the toxicity level, of 26% of dry matter presented for Navarro (2000), knowing that of the total of dry matter in the vinasse the 85 to 90%

constitute the soluble solids, the dry matter in the mediums of the fifth fermentation would be of the order of 27 and 27.7%, values that are above the mentioned toxicity value, being this another of the possible reasons for the decrease in the productivity of ethanol and generation of biomass in the fifth fermentation and still for the fourth, where the values of dry matter are from the order of 25.7 and 25% that are very near to the toxicity limit value, provoking the same effect on the two mentioned answer variables. Thatipamala and collaborators in 1992, reached a similar conclusion, since they verified a decrease of 33% (w/w) in the production of ethanol, when the content of solids in the fermentation medium increases from 14% to 27% (w/w).

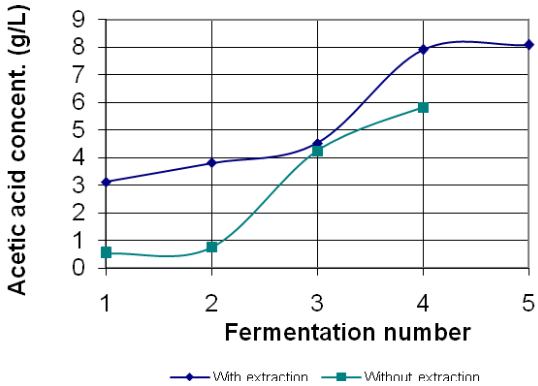

Figure 5 Increase in the acetic acid concentration in the serial fermentation médiums.

Another possible cause of the descent in the productivity of ethanol and of biomass is observed in the figures 2 and 3 respectively and is the gradual increase of the concentration of the acetic acid as the fermentations advance without applying previous extraction to the vinasse and consequently in the fermentation medium, prepared with this; the figure 5 shows how the concentration of acetic acid increases in the mediums prepared with the recycled vinasses in the successive fermentations; for the fourth and fifth of these (without extraction) the values are near to 8 g/L of acetic acid, it is in the area of high inhibition for acetic acid, seemingly this it is the biggest cause for the decrease in the production of ethanol and growth of biomass, in these last fermentations.

When applying the liquid-liquid extraction is retired part of the acetic acid of the vinasse in such a way that the prepared mediums(see inferior curve figure 5) with this, will inhibit in smaller measure to the yeast. In the fermentation 3 is observed a very similar acid concentration in both cases (with and without extraction), that which is not normal, can be due to an error in the determination of one of the two concentrations. The effect of the extraction can be verified in the figure 6, where besides the increase of the acetic acid concentration in the vinasse for the sequence of fermentations can also be observed how in each one of them, after the application of the extraction, the content of the inhibitor decreases, for inferior concentrations to 6 g/L, until in 80%; as the concentration of inhibitor increases, the extractant agent capacity of the methylic ester diminishes, in such a way that at concentrations of 12 g/L of acetic acid the decrease it is of 40% and for a concentration of 14 g/L of 30% only.

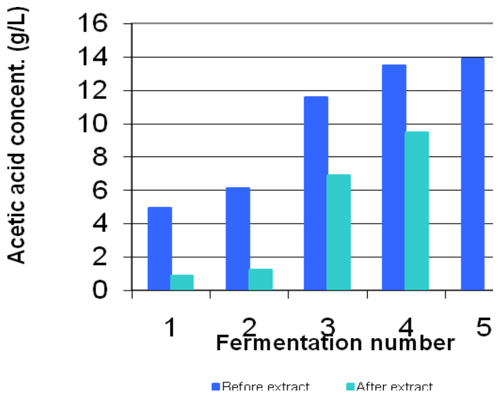

Figure 6 Increase in the acetic acid concentration in the vinasse with the serial fermentations and the extraction effect on the inhibitor concentration.

4. Conclusions

The system of recirculation of the vinasse led to an increase in the concentration of by-products of fermentation, mainly the acetic acid and other compounds that inhibit the action of the yeast. This problem can attenuate applying a liquid extraction to retire this acid, since it affects the productivity of ethanol, initially it increases for concentrations less than 2.5 g/L, starting from this value the productivity diminishes until in 66% for a concentration of 7.5 g/L, for this concentration the biomass is diminished in 50%.

The raw palm oil methylic esther was selected, as the extractant agent of the acetic acid in the vinasse, thanks to present a good distribution coefficient, a low cost and an almost null toxicity on the yeast; in the extraction was preferred to use a proportion of organic phase to watery phase of 1: 1, because this way was achieved a better distribution coefficient that in the case of the proportion 4:3. When applying the extraction on the vinasse to be recycled the productivity of ethanol was increased and also the biomass regarding the early tests without extraction of these.

It is possible to apply more than five serial recirculations of vinasse to the one that previously has been extracted the acetic acid, without detriment of the concentration of ethanol in the fermented must; when not applying the extraction only can be carried out three recirculations, since for the fourth and fifth the concentration of ethanol diminishes in 30%.

The biomass is affected by the most minimum presence in the acetic acid, diminishing its quantity until in 70% when not applying extraction, after the fifth fermentation in series and in 42%, when the extraction is applied, after the same number of fermentations.

The concentration of solids affects the biomass starting from 15% and to the productivity of ethanol in near values to 26%.

During the five fermentations it was achieved a saving of water of 56% and a decrease of the volume of vinasses of 44%.

5. References

1) Bilford H., Scalf R., Stark W., Kolachov P. ; Alcoholic Fermentation of Molasses; Industrial and Engineering Chemistry Research, Vol. 334, Issue 11, 1942
2) Briones, J.A., Mullins, J.C. and Thies, M.C., Liquid-Liquid Equilibria for the Oleic Acid-b - Sitosterol-Water System at Elevated Temperatures and Pressures. Ind. Eng. Chem. Res., 33, 151 (1994).
3) Bujang, K.B.; Ishizaki, A.; Michiwaki, S. and Crabbe, E. Comparison Between Crude Palm Oil Ester and Oleyl-alcohol as the Extraction Media on Product Recovery in ABE Fermentation of Sago Effluent. Annual Report of ICBiotech. Osaka Univ., Japan. 20: 337-344. (1997).
4) Cheryan, M; Parekh, S; Separation of glycerol and organic acids in model ethanol stillage by electrodialysis and precipitation; Process Biochemistry,Vol. 30, Issue 1, 1995, pg. 17-23.
5) Colombo, A., Battilana, P., Ragaini, V. and Bianchi, C.L., Liquid-Liquid Equilibria of the Ternary Systems Water+Acetic Acid+Ethyl Acetate and Water+Acetic Acid+Isophorone (3,3,5-Trimethyl-cyclohexen-1one), J. Chem. Eng. Data, 44, No.1, 35, 1999.
6) Conway, E.J. and Downey, M. An outer metabolic region of the yeast cell. Biochemical Journal 47,347-355,1950.
7) Correa, J.M., Blanco, A. and Arce, A., Liquid-Liquid Equilibria of the System Water+Acetic Acid+Methyl Isopropyl Ketone Between 25 and 55 °C, J. Chem. Eng. Data, 34, No. 4, 415 (1989).
8) Decloux M., Bories A., Lewandowski R., Fargues C., Mersad A., Lameloise M.L., Bonnet F., Dherbecourt B., Nieto osuna L. (2002). Interest of electrodialysis to reduce potassium level in vinasses. Preliminary experiments. Desalination, 146, 393-398.
9) Díaz, M.; Factibilidad técnica de la recirculación de vinazas en la fermentación alcohólica, tesis de pregrado Universidad Nacional de Colombia, 1987.
10) Durán de Bazúa, C. 1993. "Tratamiento biológico de aguas residuales de la Industria de proceso". Facultad de Química. Ciudad Universitaria, 127 pags, México, D.F.
11) Fahim, M.A., Al-Muhtaseb, S.A. and Alnashef, I.M., Liquid-Liquid Equilibria of the Ternary System Water+Acetic Acid+1-Pentanol, J. Chem. Eng. Data, 41, No. 3, 562 (1996).
12) Federación Nacional de Biocombustibles, abc de los alcoholes carburantes en la página web: http://www.minminas.gov.co/minminas/sectores.nsf/2a84e89f4d73f130052567be0052c75a/ffdff

1b298ac766a052571720071cfad?OpenDocument
13) GEPLACEA/PNUD/ICIDCA. 1990. "Manual de los Derivados de la Caña de Azúcar". 2da Edición. Colección GEPLACEA. México, D.F.
14) GEPLACEA/PNUD. 1991. "La diversificación de la agroindustria de la caña de azúcar". 2da edición. Colección GEPLACEA. Serie Diversificación. México, D.F.
15) In-Soung, C; Kwang-Ho, C; Chung-Hak, L; Un-Hwa, P.; Ul-Chan, K.; Sang-Won, K; Jong-Ho, K; Application of Ceramic Membrane as a Pretreatment in Anaerobic Digestion of Alcoholic Distillery Wastes; Journal of Membrane Science, Vol. 90, Issues 1-2, 27; April/94, pg. 131-139.
16) Kim, J; Kim, B; Lee, Ch; Destillery Waste Reciycle through Membrane Filtration in Batch Alcohol Fermentation; Biotechnology Letters 21 (5): 401 – 405 May 1999.
17) Kim, J; Kim, B; Lee, Ch; Kim, S; Jee, H; Koh, J; Fane, A; Development of clean technology in alcohol fermentation industry; Journal of Cleaner Production, Vol. 5, Nº 4, 1997, pg. 263-267.
18) Maiorella, B; Blanch, H; Wilke, C; By-product inhibition effects on ethanolic fermentation by *Saccharomyces cerevisiae.*; Biotechnology and Bioengineering Vol 25, pg. 103-121, 1983.
19) Maiorella, B; Blanch, H; Wilke, C; Feed and component inhibition in ethanol fermentation; Biotechnology and Bioengineering Vol26 Issue 10 Nov. 84 pg. 1155 – 1166.
20) Melzoch, K; Hábová, V; Rychtera, M; Lapisova, K.; Vlcek, R.; Membrane Separation Techniques for Stillage Treatment, www.vscht.cz/kch/kestazeni/12.pdf.
21) Mena, J.; Tratamiento anaerobio de vinazas en un reactor SCFBR, tesis de grado Ingeniería Química, Universidad Nacional de Colombia, 2000.
22) Navarro, A.; Sepúlveda, M; Rubio, M.; Bio-concentration of vinasse from alcoholic fermentation of sugar cane molasses; Waste Management, Vol. 20 pg. 581 – 585, 2000.
23) Obregon, J; Romero, O; Sebrango, C; Curbelo,I; Producción industrial de bioetanol y biomasa de levadura empleando el substrato mezcla de jugos caña energetica mas miel final; Centro Universitario de Sancti Spíritus “ José Martí Pérez”, Cuba 2006.
24) Offeman, R. D. , Stephenson, S. K. , Robertson, G. H. , Orts, W. J.; Solvent extraction of ethanol from aqueous solutions using biobased oils, alcohols, and esters; J. Amer. Chemists Soc., Vol. 83, N° 2, pg. 153-157, 2006.
25) Programa de Alcoholes Carburantes, presentación en el I Seminario Internacional de Alcoholes Carburantes, Jorge Cárdenas Gutiérrez, Medellín, Junio 10, 2004.
26) SAMSON, F. E., A. M. KATZ, AND D. L. HARRIS. Effect of acetate and other short-chain fatty acids on yeast metabolism. Arch. Biochem. Biophys. Vol. 54, pg. 406-423, 1955.
27) Thatipamala,R., Rohani S., Hill G.; Effects of High Product and Substrate Inhibitions on the Kinetics and Biomass and Product Yields during Ethanol Batch Fermentation; Biotechnology and Bioengineering Vol 40, pg. 289-297, 1992.