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# Use of Sugar Cane Vinasse as Substrate for Biosurfactant Production Using *Bacillus subtilis* PC

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The sugar cane was first grown produce exported from Brazil during the colonial period. Nowadays the cultivation of cane sugar became relevant for its final inclusion of ethanol in the Brazilian energy matrix. Among the effluents generated in the sugar industry vinasse has high content of organic matter, coupled with nutrients such as nitrogen, phosphorus and potassium as well as minerals such as zinc, iron, manganese and others. These characteristics make it possible to use as a substrate in fermentation processes. The biosurfactants spite of its wide field of use in the environmental area when retrieving soil in the oil industry which is widely used in oil recovery wells and in the food industry and health, industrial production is restricted by the high cost of operations that involve your getting in that respect is very important to find alternatives to reduce these costs, using substrates that would be discarded in the environment or underutilized, leveraging content available carbon in these waste (solid or liquid) and minimizing the impact to the environment. The aim of this study was to use vinasse, biosurfactant production by *Bacillus subtilis* PC, in order to reduce production costs and minimize the environmental impacts generated by these effluents. In tests, the experimental design was the central planning central composite - DCCR, $2^n$ with the number of independent variables equal to 3 (pH, concentration of vinasse and amount of inoculum), with replicas at the midpoint and axial points, using agitation 120 rpm and temperature 30 °C. Measurements were made of the Surface Tension (ST) using KSV Sigma 700 tensiometer by the Wilhelmy method, measures Emulsifying index ($E_{24}$), using castor oil. The use of vinasse in the fermentation process for the production of biosurfactant without supplementation of salts and nutrients were obtained values of surface tension reduction on the order of 32 $mN.m^{-1}$ and the emulsifying index $E_{24}$ to 51.10 %. The operating conditions where the response variable (surface tension) reached the lowest value was 55 % (v/v) concentration of vinasse, 20.40 % (v/v) inoculum concentration, pH value 6.5. The experiment showed that the inoculum concentration had the greatest effect with respect to the data of ST. The vinasse presented potential as substrate for the production of biosurfactants.

## 1. Introduction

Concern for the environment has mobilized some segments of society. Governmental and industrial sectors have been preparing to implement environmental policies aimed at reducing the negative impacts on nature. The agro-industrial residues constitute a potential source of environmental impact in addition to requiring large investments in pollution control treatments represent significant losses of raw materials and energy (Acorsini, 2010).
An interesting alternative would generate from these waste products of commercial value rather than investing only in treatment for final disposal. According Morita et al. (2007) for industrial processing of renewable resources or waste it in useful compounds, has received much attention from the environmental point of view. The resources currently available are: oil, starch, sugar, cellulose and lignocellulose from plants, and a variety of organic waste from agriculture and related industries (Acorsini, 2010).
Several researchers tested, in their experiments, alternative carbon sources in obtaining biosurfactants Fox and Bala (2000) used potatoes as substrate fermentation with *Bacillus subtilis* ATCC 21332, Ishi, Bharucha and Desai (2008) tested serum manufacture cheese and molasses, Raza, Khalid and Khan (2007), used as carbon sources frying oil, refinery effluents from vegetable oils and molasses Nawawi; Alam and Jamal (2010) and Sarubbo et al. (2012) used the residue pressing palm seed in the production of biosurfactant.

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According to Leal (2007) Brazilian agribusiness is one of the most productive and competitive in the world, by presenting a unique combination of climatic conditions, land area and abundant natural resources. Currently, alternative waste recovery through its use has been greatly encouraged, as they can contribute to the reduction of environmental pollution, and to enable economic recovery of the waste making it a byproduct and thus adding value to the process of industrialization (Camili and Cabello, 2008). Christofoletti et al (2013) point that there are still many technological alternatives for sugarcane vinasse destination, like combustion, yeast production, livestock feed production, incineration, which attests to the fact that many efforts have been directed to allocate, properly, the large volume of this waste. Thus, new studies and green methods need to be developed aiming at recycling and disposing sugarcane vinasse.

The sugar cane was first cultivated product from Brazil during the colonial period. From the inclusion of ethanol in the Brazilian energy matrix, the cultivation of sugarcane became relevant for the production of this fuel. Sugarcane-to-ethanol plants currently lack suitable options for value-added processing of co-products, especially vinasse, which is a leftover following ethanol recovery (Nitayavardhana and Khanal, 2010), the amount discharged by distilling can vary from 10 to 18 L stillage per liter of ethanol produced, depending on the technological conditions for the distillation. (Rosseto, 1987; Silva, Griebeler and Borges, 2007), which has higher pollution load, with varying Biochemical Oxygen Demand (BOD) 20,000-35,000 $mg.L^{-1}$.

The composition of vinasse is quite variable and depends on the raw material used, the type of distillate being obtained and the fermentation process employed, features lots of organic matter with variable mineral composition and predominance of potassium (K) between cations (Magalhães, 2010). According Silva *et al.* (2011) the carbon compounds and nitrogen assimilable by the microorganisms and is widely available, therefore it could be used for production of microbial biomass.

Surfactants are surface active agents which reduce the free energy of the system to replace most of the high energy molecules at the interface, thus lowering the surface and interfacial tension of liquids, they consist of a hydrophobic portion and a hydrophilic group, is also known as amphiphilic substances (Muligan, 2005; Batista, 2010).

Surfactants of microbial origin have recently attracted great interest because they have advantages compared to their similar chemical as biodegradability, environmental compatibility, effectiveness in adverse environmental conditions, lower toxicity and ability to be produced from renewable sources. This interest increases when considering the wide field of industrial application of these compounds is very diverse, especially, chemical and oil industries for pharmaceutical, food, agriculture, etc. (Accorsini, 2010).

In recent decades, many microorganisms have been reported as producing various types of surfactants (Barros *et al*., 2007). Surfactants produced by different strains of *Bacillus subtilis*, and are obtained by processes under less aggressive environmental point of view, affect significantly the degradation of aliphatic and aromatic hydrocarbons (Barros et al. 2007), among other applications.

The present study utilized the stillage from sugar cane to produce biosurfactant from *Bacillus subtilis* PC, aiming to take advantage of carbon concentration and salts present in this waste as alternative substrate in the production of this byproduct.

## 2. Materials and Methods

### 2.1 Culture medium

The micro-organism used was *Bacillus subtilis* PC, isolated on local soil and part of the collection of strains of the Laboratory of Environmental Biotechnology - LABAM in the Federal University of Sergipe - UFS. The pre-inoculum was prepared Himedia bacteriological peptone, 2 % (w/v) sterilized at 120 °C and 1 atm for 15 min. After inoculation, the medium was placed in a shaker (shaker Certomat ® BS-T) at 120 rpm and 30 °C for 24 h.

The stillage used as carbon source for inoculum preparation and fermentation medium was collected in a industry of sugar and ethanol in the municipality of Capela in the state of Sergipe in northeastern Brazil. The inoculum was prepared in 100 mL of vinasse into a 250 mL erlenmeyer flask, autoclaved at 120 °C and 1 atm for 15 min. After cooling, was added 10 mL of pre-inoculum, and then subjected to agitation in a shaker at 120 rpm and 30 °C for 12 h.

### 2.2 Emulsifying index

The E24 was determined in test tubes with screw cap by adding 2.0 mL of a castor oil 3.5 mL of the supernatant, mixing with stirrer tube for 2 min. and allowed to stand for 24 h. The index is calculated from the ratio between the height of the emulsified layer (cm) and total height of the column of liquid (cm), as shown in Eq(1) (BICCA; FLECK; AYUB 1999 apud PASTORE, 2010).

$$E_{24} = \frac{height\ emulsion\ layer}{total\ height} x100 \quad (1)$$

**2.3 Surface tension**

To measure the surface tension one tensiometer KSV Sigma 700 was used. The analyzes were performed by the method with a platinum plate iriada perimeter of 40.0 mm, length of 19.9 mm and a thickness of 0.10 mm, called Wilhelmy plate. The analysis conditions were: average sample volume 20 mL, at room temperature and standard deviation measurements concerning 5 ≤ 0.20 $mN.m^{-1}$. The equipment was calibrated always at the beginning and end of analysis by measuring the surface tension of distilled water, which is around 72 $mN.m^{-1}$.

**2.4 Experimental design in test termentation**

The experimental design used in the tests was the central planning rotational central composite - CCRD, $2^n$ with the number of independent variables equal to 3 (pH, concentration of vinasse and inoculum concentration), with replicas at the midpoint and axial points, and the value of $\alpha$ calculated according to the number of independent variables by the relation $(2^n)^{1/4}$, resulting in 1.673 for this study. The levels and the values of the independent variables used (Table 1).

*Table 1:* Levels and variables used in the tests fermentation stillage.

| Independent variables | Levels | | | | |
|---|---|---|---|---|---|
| | -α | -1 | 0 | +1 | +α |
| Concentration of vinasse (%) | 12.95 | 30,00 | 55.00 | 80.00 | 97.04 |
| Concentration of inoculum (%) | 3.6 | 7.0 | 12.0 | 17.0 | 20.40 |
| pH | 5.66 | 6.00 | 6.50 | 7.00 | 7.34 |

## 3. Result and Discussions

The use of a natural substrate without mineral supplementation as fermentation medium is thoroughly tested by researchers Joshi et al (2008) tested using the biosurfactant production of sugar cane molasses in four strains of *Bacillus subtilis* -. K51, R1, and 20B HS3, which had surface tension values of 29.67, 29.33, 30.33 and 30.67 $mN.m^{-1}$, respectively, but in this experiment the authors used as inoculum Luria Bertani culture medium, a synthetic medium with concentration of salts and nutrients subsidiaries.

The Pareto diagram (Figure 1) obtained from the experimental design (Table 1) indicates that the inoculum concentration was the variable that had the greatest effect on the results of surface tension on the conditions under which the tests were conducted.

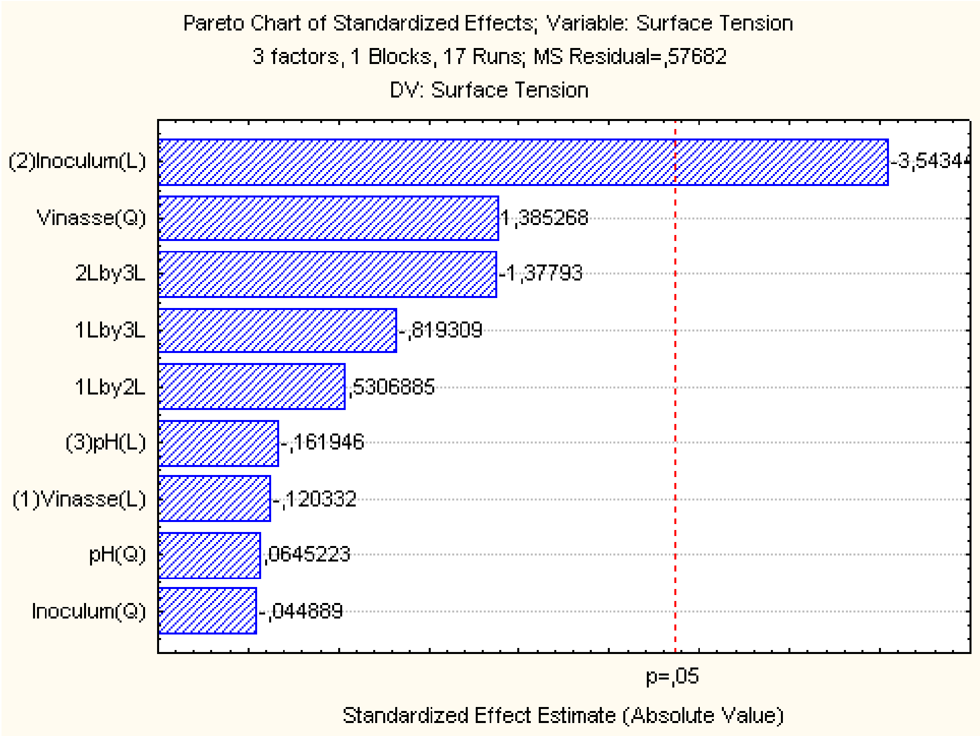


*Figure 1: Pareto chart for the proposed experiment*

The assessment of response surface considering the influence of variables inoculum concentration and concentration of vinasse to a pH value of 6.5 (Figure 2), indicate that the best growing conditions are in the direction of an increasing concentration of inoculum above 20 %, the concentration of vinasse stabilizing at around 50 %, the operating conditions of fermentation of 120 rpm and 30 °C.

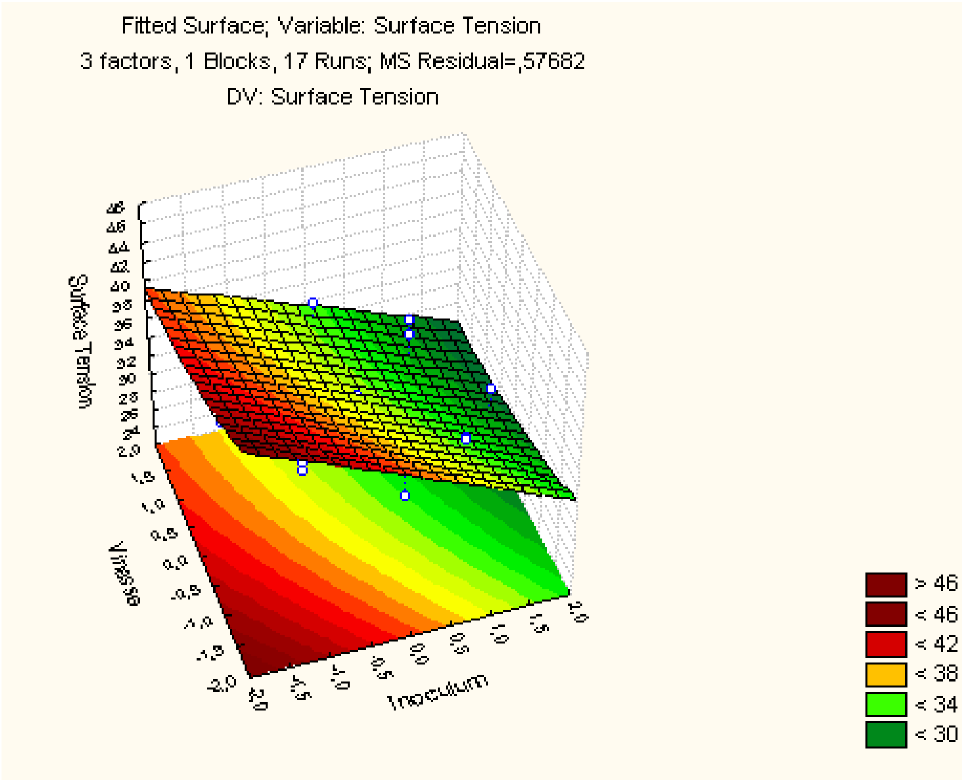


*Figure 2: Response surface for tests at pH 6.5 for the variables stillage and inoculum.*

The values of surface tension, the lowest value was 32.78 mN.m$^{-1}$, which corresponds to a decrease of 54.47 % over the surface tension of distilled water whose reading was 71.997 mN.m$^{-1}$ and 13.90 % when compared to the fermentation medium free of cells. This result directly reflects the conditions in which this trial was conducted in relation to the concentration of vinasse (55 %) and inoculums (20.4 %) used and the pH (6.5) of the fermentation medium. Other studies using *Bacillus subtilis* and alternative sources of carbon in the production of surfactant had surface tension values similar to those found in this study. Oliveira et. al. (2013) used cashew clarified juice with mineral supplementation using *Bacillus subtilis* LAMI005 with results ranging from 30 to 34.7 mN.m$^{-1}$ strain superficial. Al-Bahry et. al. (2013) tested molasses as the carbon source with different compositions of salts of *Bacillus subtilis* strain B20, the authors obtained 27 mN.m-1 for surface tension. Faria et. al (2011) tested glycerol residue of the biodiesel process and the strain *Bacillus subtilis* LSFM-05 obtaining surface tension 29.5 mN.m$^{-1}$ after 36 h of fermentation, reaching 32.1 mN.m-1 after 72 h. However, in all these studies both the inoculum and the fermentation medium used was supplemented with minerals.

The monitoring of emulsification index $E_{24}$ was conducted in the fermented broth as an indication of the presence of components capable of emulsifying oil or hydrocarbons, in this experiment castor oil was used and the results obtained are shown in Figure 3. The larger $E_{24}$ were obtained in the fermentation times of 9, 24 and 72 h, reaching values on the order of 50 %. Pereira et. al. (2013) in a study with *Bacillus subtilis* strains isolated from oily sludge, obtained $E_{24}$ 48.4 %, utlizando 10 g.L$^{-1}$ glucose as carbon source for one of the strains studied. Oliveira et. al (2013) obtained values of $E_{24}$ 58 %, with the fermentation of cashew apple juice supplemented with ammonium sulphate using *Bacillus subtilis* strain LAMI005.

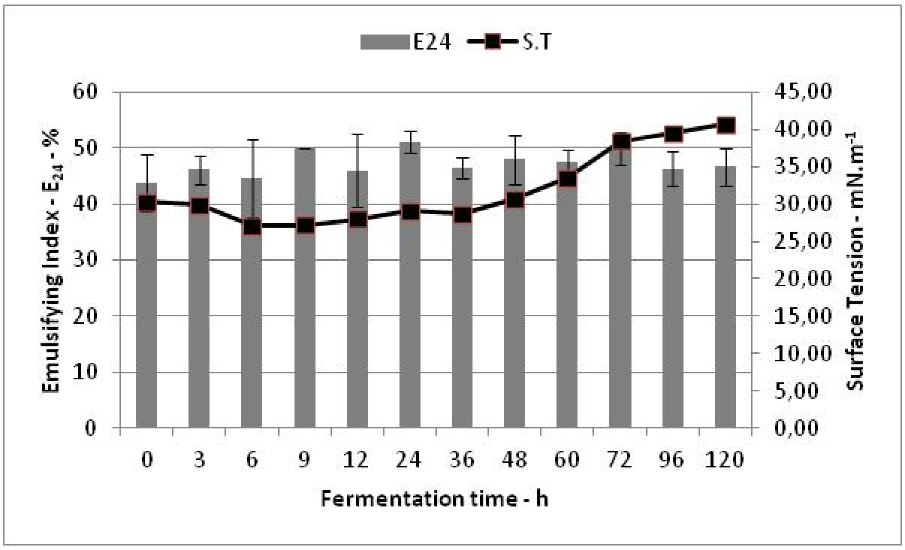


*Figure 3: Monitoring the emulsification index during fermentation*

## 4. Conclusions

The use of vinasse as substrate in the fermentation process for the production of the biosurfactant, as well as inoculum fermentation media without supplementation of nutrients and salts, biosurfactant production was favorable, providing surface tension values of the 32 $mN.m^{-1}$, indicating that supplementation of salts and the carbon source contained in the stillage can be investigated in further studies.

In experimental design, the inoculum concentration had the highest effect the values of surface tension. The operating conditions where the response (surface tension) variable has reached the lowest value were 55 % (v/v) amount of vinasse, 20.40 % (v/v) inoculum concentration, pH value 6.5. The stirring rate and temperature used in the experiments were kept constant at 120 rpm and 30 °C.

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### References

Accorsini F.R., 2010, Isolation of yeasts from a specialized consortium and evaluation of its potential in the production of biosurfactants in alternative carbon sources (Isolamento de leveduras de um consórcio especializado e avaliação de seu potencial na produção de biossurfactantes, em fontes alternativas de carbono), MSc Dissertation, Universidade Estadual Paulista, Jaboticabal, Brazil.

Al-Bahry S.N., Al-Wahaibi Y.M., Elshafie A.E., Al-Bemani A.S., Joshi S.J., Al-Makhmari H-S, Al-Sulaiman H.S., 2013, Biosurfactant production by bacillus subtilis B20 using date molasses and its possible application in enhaced oil recovery, International Bioterioration & Biodegradation, 81, 141-146.

Barros F.F.C., Quadros C.P., Maróstica Júnior M.R., Pastore G. M., 2007, Surfactin: technological and functional chemical properties for food applications (Surfactina: Propriedades químicas tecnológicas e funcionais para aplicações em alimentos), Quim. Nova, 30, 2, 409-414.

Batista R.M., Rufino R.D., Luna J.M., Souza J.E., Sarubbo L.A.,2010, Effect of mediom components on the production of a biosurfactant from Candida tropicalis applied to the removal of hydrophobic contaminants in soil, Water Environ. Res., 82, 5, 418-425.

Bueno S.M., Silva N. S., Garcia-Cruz C. H., 2010, Study of biosurfactant production in the fermentation broth (Estudo da produção de biosurfactante em caldo de fermentação), Quim. Nova, 33, 7, 1572-1577.

Camili, E. A., Cabello C., 2008, Valuation of the processo of flotation in the treatment of originated manipueira of the production of flour cassava, Revista Energia na Agricultura, Botucatu,23, 1, 32-45.

Christofoletti C.A.,Esher J.P., Correia J. E.,Marinho J.F.U.,Fontanetti C.S., 2013, Sugarcane vinasse: environmental implications of its use, Waste Manang., 33, 2753-2761.

Faria A. F., Teodoro-Martinez, D. S., Barbosa G. N. O., Vaz B. G., Silva I. S., Garcia J.S., Tótola M.R., Eberlin M.N., Grossman M., Alves O.L., Durrant L.R., 2011, Production and structurual characterization of surfactin ($C_{14}$/$Leu_7$) produced by *Bacillus subtilis* isolate LSFM-05 grown on raw glycerol from biodiesel industry, Process Biochemistry, 46, 1951-1957.

Fox S.L.; Bala G.A., 2000, Production of surfactant from *Bacillus subtilis* ATCC 21332 using potato substrates. Bioresource Technology, 75, 235-240.

Joshi S., Bharucha C., Jhas S., Yadav S., Nerurkar A., Desai A.J., 2008, Biosurfactant production using molasses and whey under thermophilic conditions. *Bioresource Technology*, 99, 195-199.

Leal R. M. P.,2007, Effect of irrigation with treated wastewater effluent on chemical properties of an Latosol cultivated with sugarcane (Efeito da irrigação com efluentes de esgoto tratado em propriedades químicas de um Latossolo cultivado com cana de açúcar), MSc. Dissertation, Universidade de São Paulo, Piracicaba, Brazil, 109 p.

Muligan C.N., 2005, Environmental applications for biosurfactants. Environ. Pollut., 133, 183-198.

Nawawi, W. M. F. W.; Jamal, P.; Alam, Z., 2010, Utilization of sludge palm oils as a novel substrate for biosurfactant production. Bioresource Technology, 101, 9241-9247.

Nitayavardhana S., Khanal S.K., 2010, Innovative biorefinary concept for sugar-based ethanol industries: Poduction of protein-rich fungal biomass on vinasse as an aquaculture feed ingredient, Bio. Tech., 101, 9078-9085.

Oliveira D.W.F., França I.W.L., Felix A.K.N., Martins J.J.L., Giro M.E.A., Melo V.M.M., Gonçalves L.R.B, 2013, Kinetic study of biosurfactant production by *Bacillus subtilis* LAMI005 grown in clarified cashew apple juice, Colloids and Surfaces B: Biointerfaces, 101, 34-43.

Pastore N.S., 2010, Evaluation of different nitrogen sources and sucrose concentration of citric acid production by Aspergillus niger liquid using the pressing of the cassava as substrate (Avaliação de diferentes fontes de nitrogênio e concentração de sacarose na produção de ácido cítrico por A*spergillus niger* usando manipueira como substrato). Dissertação MSc Dissertation, Universidade Estadual do Oeste do Paraná,Brazil,67 p.

Pereira J.F.B., Gudiña E.J.,Costa R., Vitorino R.,Teixeira J.A.,Coutinho J.A.P.,Rodrigues L.R.,2013, Optimization and characterization of biosurfactant production by *Bacillus subtilis* isolates toward microbial enhanced oil revovery applications, Fuel, 111, 259-268.

Raza Z.A., Khan M.S., Khalid Z.M., 2007, Evaluation of distant carbon sources in biosurfactant production by a gamma ray-induced *Pseudomonas putida* mutant. Process Biochemistry. 42, 686-692.

Rosseto A.J.,1987, Agricultural use of by-products and wastes of the sugar and alcohol industry (Utilização agronômica dos subprodutos e residuos da indústria açucareira e alcooleira), In: Paranhos S.B. (Ed.), Sugar cane: cultivation and use (Cana-de-açúcar:cultivo e utilização), Campinas, Fundação Cargill, 2, 435-504.

Sarubbo L., Luna J., Rufino R., Farias C., Santos V., 2012, Production of biossurfactants for application in the removal of hydrophobic contaminats originated by the petroleum industry, Chemical Engineering Transactions, 27, 7-12, DOI: 10.3303/CET 122702.

Silva C.F., Arcuri S.L., Campos C.R., Danielle M.V., Alves J.G.L.I., Schwan R.F., 2011, Using the residue of spirit production and bio-ethanol for protein production by yeasts, Waste Management, 31, 108-144.

Silva M. A. S., Griebeler N. P., Borges L. C., 2007, Use of vinasse and impacts on soil properties and groundwater (Uso de vinhaça e impactos nas propriedades do solo e lençol freático), Revista Brasileira de Engenharia Agrícola e Ambiental, 11, 1, 108-114.