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Characterization of vinasses from five certified brands of origin (CBO) and use as economic nutrient for the xylitol production by *Debaryomyces hansenii*

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ABSTRACT

Vinasses coming from the five CBOs of Galicia, north-western Spain, were characterized, and successfully employed as economic nutritional supplements for xylitol production by *Debaryomyces hansenii*. All fermentations can be modelled showing kinetic patterns fairly described by the mathematical models. No negative effect of the phenolic compounds in the liquid phase on the initial volumetric rate of product formation (r_{P0}) was observed. Multiple linear regression analysis was used to describe the effect of metals and initial xylose acting on P_{max} and $Y_{P/S}$. Zn was the most influential variable. Besides, partial least-squares regression models show a clear separation, based on the first two principal components, between the whole vinasses and the liquid fractions, which provided the higher P_{max}, with the exception of CBO 4, where P_{max} = 40.4 g/L, was achieved using the solid and liquid fraction.

1. Introduction

Viticulture is an important subsector in the agricultural structure of Galicia, north-western Spain, cultivating in the 2007 campaign 9504 ha in five certified brands of origin (CBO) (see Table 1). As a result, this activity produced more than 41 million kilograms of grapes and almost 36 million litres of wine, as well as a huge amount of microbial biomass (lees), bagasse and other residues that usually are processed in distilleries to recover alcohol and other aromatic volatile compounds. The definition of wine lees given by the European Council Regulation (EC) No. 337/79 states that "wine lees is the residue that forms at the bottom of recipients containing wine, after fermentation, during storage or after authorized treatments, as well as the residue obtained following the filtration or centrifugation of this product" (Pérez-Serradilla and Luque de Castro, 2008). According to the EC 1493/1999 on the common organisation of the wine market, grape marc and wine lees must be sent to alcohol distilleries, producing exhausted grape marc and a liquid waste (vinasse) (Bustamante et al., 2008). Consequently, vinasses are the main liquid wastes from the distillation process, which are acidic effluents with high organic content, including acids, carbohydrates, phenols, and unsaturated compounds with high Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD_5), and solid concentrations (Beltrán et al., 1999). Their discharge into public watercourses promotes a high and toxic contamination of the medium, resulting in significant environmental problems. The extreme toxicity of some of these effluents arises from their high BOD_5, resulting in local fish kill and damage to other aquatic biota (Niranjan and Shilton, 1994). The solution does not lie in impeding industrial development, but in taking adequate steps to avoid or counteract pollution at origin (Sales et al., 1986).

Wastewaters from agro-industrial processes, especially those from distilleries are characterized generally by low concentrations of suspended organic materials (which can be removed partly by sedimentation, filtration or flotation); very high concentrations of dissolved organic materials (i.e. carbohydrates, proteins, lipids and cellulosic or lignocellulosic materials), which are generally biodegradable, and considerable concentrations of nitrogen, phosphate and potassium compounds (Weiland, 1987), as well as recalcitrant compounds (including phenols, pesticides, and heavy metals) that cannot be removed and may create problems in the operation of a standard sewage treatment plant (Mena et al., 1997). In order to treat vinasses, and depending on the distillery type, several studies have been conducted to reduce the COD, BOD_5, and solids in suspension. Physical and chemical treatments have not been very successful until now. They are used to adapt wastewaters from subsequent biological depuration and reduce their high contamination levels. The high organic content of these residues make them well suitable for biological treatment, especially for aerobic biological treatment (Beltrán et al., 1999). Nevertheless, capital and operating costs of the available physicochemical and biological treatment processes of this waste streams

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Table 1
Technical data (2007) of the five certified brands of origin.

	CBO 1	CBO 2	CBO 3	CBO 4	CBO 5	Total
Cultivated area (ha)	1352	2732	3496	1222	702	9504
Vine-growers	2024	6022	6511	2870	617	18,044
Industries	41	116	194	100	20	471
Grape production (kg)	48,53,730	116,79,000	187,21,990	45,42,000	13,03,720	411,00,440
Qualified wine (L)	35,39,444	107,24,960	178,00,407	30,81,591	623,200	357,69,602
Vinasses estimated (kg)	97,000	233,000	375,000	90,000	26,000	821,000

CBO 1, Valdeorras; CBO 2, Ribeiro; CBO 3, Rías Baixas; CBO 4, Ribeira Sacra; and CBO 5, Monterrei.
http://mediorural.xunta.es/alimentacion/produtos/index.php.

are inevitably high (Kim et al., 1997). Recently, other treatments have been developed, for example ozonation, because of its ability to convert biorefractory compounds into less toxic and more readily biodegradable compounds (Beltrán et al., 1999). Alternatively, these wastewaters could be revalued when seeking alternative treatments, which can provide additional benefits. In this sense, their use as a source of low-cost nutrients for fermentation technologies could represent an attractive solution.

The search for alternative, financially competitive nutrient sources to carry out biotechnological procedures is particularly interesting, taking into account that the nutrients used traditionally in most of the fermentative media, particularly yeast extract and peptone, turn out to be very expensive being able to account for almost 30% of the total cost of the process (Miller and Churchill, 1986). Corn steep liquor is an inexpensive source of essential microbial nutrients already used for the xylitol production by *Debaryomyces hansenii* (Carvalheiro et al., 2007); meanwhile viticulture lees, an inexpensive and easily available source of biomass (Chassagne et al., 2005), have been used for the lactic acid production using *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus pentosus*, *Lactobacillus casei*, and *Lactobacillus coryniformis* subsp. *torquens* (Bustos et al., 2004a,b); but no studies have been reported for the biotechnological production of xylitol by *D. hansenii*.

Xylitol, a pentitol ($C_5H_{12}O_5$) with increasing interest owing to its dietetic and clinical properties, has a comparatively high added-value and growing market, the world-wide production being estimated about 30,000 tons/year (Rivas et al., 2006). This low caloric sweetener has negative heat of solution, anticariogenic properties, does not undergo the Maillard reaction, it is suitable as sugar substitute for diabetics, and it limits the tendency to obesity when it is continuously supplied in diet, fostering its utilization in the food industry as a food ingredient (Parajó et al., 1997). Among yeasts, *Debaryomyces hanenii* is a promising alternative for industrial xylitol bioproduction. Thus, Sampaio et al. (2008) screening 270 isolated yeasts, found *D. hansenii* the most appropriated for xylitol production. The supplementation requirements for optimal biotechnological xylitol production vary considerably according to the process. Most of the works have been successfully studied using commercial nutrients such as yeast extract, malt extract or peptone (Domínguez et al., 1997), although some attempts have been carried out to employ other chipper nutrients, for example, Carvalheiro et al. (2007) found the best results for xylitol production using 3 g yeast extract/L and 5 g corn steep liquor/L.

In the present work, vinasses from five certified brands of origin (CBO) have been comprehensively studied to develop a profitable technology for their benefit. Without autolysis treatments, vinasses were used as cheap nutrients to carry out the xylose to xylitol bioconversion by *D. hansenii*. Chemometric methods (multiple linear regression analysis and partial least-squares regression) based on pattern recognition have been used for the classification and comparison of different samples.

2. Methods

2.1. Vinasses sampling and storage

Vinasses, obtained after the distillation of lees, from the campaign of 2007 were kindly supplied by the five certified brands of origin of Galicia (Spain): Valdeorras (CBO 1), Ribeiro (CBO 2), Rías Baixas (CBO 3), Ribeira Sacra (CBO 4), and Monterrei (CBO 5), and stored at 4 °C. Vinasses were used directly as nutrient or after centrifugation to separate both solid and liquid phases. Fig. 1 shows the map of Spain indicating the region of Galicia and its viticulture areas registered as certified brands of origin (CBO).

2.2. Vinasses characterization

Vinasses were oven-dried to constant weight at 102 °C in order to determine the percentage of solids. Ashes in vinasses were oven-dried to constant weight at 550 °C.

N, C and metals were analyzed in vinasses or in the liquid fraction obtained after filtration. Nitrogen and carbon percentages were analyzed using a Thermo Finningan Flash Elemental Analyzer 1112 series, San Jose, CA (USA), meanwhile Fe, Mn, Zn, Ca, Mg, Al, and Cu were analyzed in ashes using an Atomic Absorption Spectrometer 220 Fast Sequential, VARIAN, Palo Alto, CA (USA). Previously, 0.15 g ashes were digested with 5 mL HNO_3 65%, 1 mL H_2O_2 30% and 0.5 mL HF 40% in a Microwave Labstation MLS 1200 MEGA, MILESTONE, Bergamo (Italy).

pH and electrical conductivity (EC) were analyzed directly in vinsasses using a pHmeter, Crison Instruments (Barcelona, Spain) and conductivity meter HI 8633, Hanna instruments (Woonsocket, RI, USA).

Finally, total phenol content in the liquid fraction was determined according to the Folin–Ciocalteu method using gallic acid as the standard (Vázquez et al., 1974).

2.3. Microorganism

D. hansenii NRRL Y-7426 was kindly provided by the National Center for Agricultural Utilization Research (Peoria, Illinois, USA). Freeze-dried cells were grown on a basal medium containing 30 g commercial xylose/L, 3 g yeast extract/L, 3 g malt extract/L, and 5 g peptone/L. The microorganism was maintained in agar slant tubes containing a medium formulated with the same components and concentrations as the previous one plus 20 g agar/L. Inocula were prepared by solubilization of cells with sterile water and underwent growth during 24 h in the previous medium without agar. Biomass in inocula was measured by optical density at 600 nm and adjusted by dilution with water to reach a final concentration in the inocula of 2.3 g/L. Inoculum was added to fermentation broth to reach a final concentration of 0.12 g/L.