Valorisation of vinasses by recovery tartaric acid and bioproduction of lactic acid and emulsifiers by *Lactobacillus pentosus*.

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Abstract

Wineries generate large amounts of solid wastes and effluents. Turning these winery wastes into valuable products as tartaric acid (TA), lactic acid (LA) and bio-emulsifiers (BE) is becoming an essential part of good winemaking practices, further reducing concerns of waste disposal and cutting costs for partly imported wine additives (Boulton et al., 1995).

Frequently, some of these wastes as grape bagasse and lees are distilled in alcohol industries, producing vinasses (V) as the principal liquid waste. In recent years, V have been the subject of a lot of studies to search applications that increase their value, for example by the extraction of tartaric acid or their utilization as nutritional media in biotechnological processes like the production of lactic acid (Salgado et al., 2009; Salgado et al., 2010).

Tartaric acid is an additive used in wineries to acidify wines; whereas lactic acid has a wide range of applications as acidifier, flavouring, pH buffering agent or inhibitor of bacterial spoilage in a wide variety of processed foods (Rivas et al., 2004).

On the other hand during lactic acid fermentation not only lactic acid can be obtained but also bioemulsifiers. So, in previous works it was reported the ability of *Lactobacillus pentosus* to ferment hemicellulosic sugars, from lignocellulosic residues, for obtaining lactic acid and extracellular bioemulsifiers (Portilla et al., 2010). These bio-emulsifiers can have applications in the food, pharmaceutical and petroleum industries.

In this work, we have extracted tartaric acid from vinasses and the residual stream, after tartaric acid extraction, was used as nutritional media for obtaining lactic acid and bioemulsifiers using *L pentosus*.

During fermentation with *L. pentosus* four growth media were tested: solid V after TA recovery (F1); solid V and effluent from the process of TA recovery (F2); solid V without TA recovery (F3) and control consisting on a general media for lactic acid bacteria proposed by Portilla et al. (2010) in previous works (F4). In all cases, commercial sugars in similar concentrations to those found in hemicellulosic hydrolysates were added as carbon source. Assays were carried out in Erlenmeyer flasks of 250 mL with a final volume of 100 mL at 31 ºC and 120 rpm (orbital shakers). Fermentation media also contained 10 g/L of $CaCO_3$ in order to control the pH. Sugar consumption and lactic acid production were analysed by high-performance liquid chromatography system (Salgado et al., 2009).

On the other hand, emulsification studies were performed with mixtures of kerosene and aqueous phase, using non-fermented or fermented media without free-cells as aqueous phase. Emulsification capacity was analysed by the relative emulsion volume (EV, %), emulsion stability (ES, %) and the percentage of emulsified organic phase (EOP, %) calculated after 24 h intervals, up to 72 h (Portilla et al., 2010).

Table 1 shows the concentration of initial sugars and lactic acid produced at the end of fermentations (48 h). The highest LA value was achieved in F4, using commercial nutrients (11.5 g/L), although similar values were achieved in F2 (10.0 g/L), thus confirming that by-products obtained after TA extraction from V can be used efficiently to product LA by biotechnological processes.

Table 1- Lactic acid production and emulsification capacity of non-fermented and fermented media.

	Non-fermented media				*Fermented media*			
	Total sugars (g/L)	ES (%)	EV (%)	EOP (%)	LA_{max} (g/L)	ES (%)	EV (%)	EOP (%)
F1	14.2	84.9	53.9	76.9	6.9	92.5	62.9	84.6
F2	14.1	90.8	55.8	80.8	10.0	93.3	52.6	71.8
F3	13.7	84.5	21.2	26.9	6.6	89.7	43.6	48.7
F4*	12.9	45.0	7.7	7.6	11.5	70.4	24.4	25.6

* 10 g/L of both yeast extract and corn steep liquor, and minerals (0.015 g/L $MnSO_4 \cdot 3H_2O$, 5.068 g/L K_2HPO_4, 0.045 g/L $NaOOCCH_3$, 16.260 g/L $CaSO_4 \cdot 2H_2O$, and 2.194 g/L $MgSO_4 \cdot 7H_2O$).

On the other hand in **Table 1** is also included the EV, ES and EOP of the fermentation media before and after fermentation with *L. pentosus*. It can be observed that the unfermented media already showed some bioemulsifier activity, mainly in those cases supplemented with the residual streams tested in this work. However it is important to underline that after fermentation the percentage of EV increased in all the cases except for the medium formulated with all the residual stream of vinasses after TA recovery (F2).

In conclusion, the recovery of tartaric acid from vinasses allows obtaining an additive used in wine production; meanwhile the stream obtained, after tartaric acid extraction, can be used to formulate effective and economic fermentation media for the biotechnological production of lactic acid and bio-emulsifiers.

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