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Comparative study of batch and continuous multi-stage fixed-bed tower (MFBT) bioreactor during wine-making using freeze-dried immobilized cells

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ABSTRACT

A freeze-dried immobilized biocatalyst produced by immobilization of *Saccharomyces cerevisiae* AXAZ-1 yeast cells on gluten pellets and subsequent freeze-drying was used in a multistage fixed-bed tower (MFBT) bioreactor for batch and continuous wine-making. The MFBT bioreactor resulted in higher alcohol productivity compared to fermentations carried out in a packed bed (PB) bioreactor and showed an important operational stability and no decrease in activity, even at low fermentation temperature (5 °C) and after storage for 6 months at 4 °C. The production of amyl alcohols proved to be temperature dependent and was significantly reduced at low temperatures. Re-activation of the freeze-dried immobilized cells after storage for 6 months resulted in further decreased content of amyl alcohols. The SPME GC/MS analysis of volatile compounds revealed no significant differences in the wines produced by MFBT and PB bioreactors, while the preliminary sensory evaluation ascertained the overall improved quality of the produced wines. Potential industrial application of MFBT bioreactor is also assessed and discussed.

1. Introduction

In the past few years there has been an upsurge of interest in immobilized cells due to attractive technical and economic advantages compared to the conventional free cell system (Margaritis and Merchant, 1984; Stewart and Russell, 1986). Many immobilization supports have been proposed for use in wine-making, such as sodium alginate (Fumi et al., 1987), Ca-alginate (Ferraro et al., 2000; Yajima and Yokotsuka, 2001), kissiris (Bakoyianis et al., 1992, 1993), γ-aloumina pellets (Kana et al., 1989; Loukatos et al., 2000), gluten pellets (Bardi et al., 1996, 1997a,b), delignified cellulosic materials (Bardi and Koutinas, 1994), DEAE-cellulose (Maicas et al., 2001), fruit pieces (Kourkoutas et al., 2001, 2002a,b, 2003; Mallios et al., 2004) and dried raisin berries (Tsakiris et al., 2004). However, industrial application of the technology is still uncertain. Immobilization supports suitable for the wine industry should accomplish additional prerequisites, such as food-grade purity, low cost, abundance, suitability for low-temperature fermentation and ability for long-term storage.

A wide range of fermentors varying shape, size and technical design are nowadays available. Although in many industrial fermentations continuous systems are usually used, such an application rarely exists in wine industry, especially in the production of high quality wines which show subtle and complex associations of hundreds of compounds. Thus, the majority of fermentors usually used in wine industry are of batch type. Continuous fermentation offers several advantages compared to the traditional fermentation, such as increase in productivity and reduction in production costs (Jackson, 1994). Although a relatively high number of studies reporting continuous wine-making is available in literature (Bakoyianis et al., 1992; Nedovic et al., 2000; Maicas et al., 2001; Kourkoutas et al., 2002a,b), there is a lack concerning use of freeze-dried immobilized yeast cells in a continuous process, according to the authors knowledge. As wine is a product with seasonal character, continuous fermentor designated for wine production, should be also designed for the production of other product(s) (e.g. alcohol production from molasses), in order to achieve economic feasibility of the industrial investment.

A Multi-Stage bioreactor was previously proposed for continuous wine fermentation using calcium alginate as yeast carrier consisted of a horizontal fermentor with five replaceable immobilized plates (Ogbonna et al., 1989). Likewise, a catalytic multi-stage fixed bed tower (MFBT) bioreactor was used for potable alcohol production from molasses in industrial scale (Bakoyianis and Koutinas,

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1996; Koutinas et al., 1997). It consisted of a vertical stainless steel cylindrical tank with five packed sections containing kissiris as promoter. Such a bioreactor design is considered advantageous, as high pressures at low levels, which may result in support destruction, are avoided.

The aim of the present study, hence, was to investigate batch and continuous wine production in a vertical MFBT bioreactor in comparison to a packed bed bioreactor, as regards fermentation kinetics and wine quality.

2. Materials and methods

2.1. Yeast strain

AXAZ-1, an alcohol resistant and psychrofilic *Saccharomyces cerevisiae* yeast strain isolated (Argiriou et al., 1992) from the Greek agricultural area, was used in the present study. It was grown aerobically on complete culture medium consisting of yeast extract, 0.4%; $(NH_4)_2SO_4$, 0.1%; KH_2PO_4, 0.1%; $MgSO_4 \cdot 7H_2O$, 0.5% and glucose, 4% at 30 °C. The synthetic medium was sterilized at 130°C for 15 min prior use. Sterilized air (4.0 vvm) was continuously supplied into the bioreactor using an air compressor (Compressori D' Aria LT 50 HP 1.5). Cells were harvested at 4000 rpm for 10 min and used in the present study.

2.2. Grape must

Concentrated grape must was diluted with water to a final °Be density range of 11.2–12.2. No nutrient addition was carried out. Musts were sterilized at 121 °C for 15 min before employed in the fermentations.

2.3. Supports and immobilization of cells

Gluten pellets (GP) were used as immobilization supports for yeast cells. Preparation of the support and immobilization of yeast cells was carried out as described previously (Bardi et al., 1996).

2.4. Preparation of freeze-dried immobilized biocatalyst

Wet immobilized cells on gluten pellets were frozen to −45 °C with a cooling rate of 3 °C/min in a controlled rate freezer (BioCool, FTS Systems, NY, USA), while no cryoprotecting media was used. The frozen samples were freeze-dried overnight at 5×10^{-3} bar and at −45 °C in a Freeze Dry System, Freezone 4.5 (Labconco, Kansas City, Missouri, USA) according to Iconomopoulou et al. (2002).

2.5. Batch fermentations

Batch fermentations of grape must were carried out in a packed bed (PB) and in a catalytic multi-stage fixed bed tower (MFBT) bioreactor.

2.6. Packed bed (PB) bioreactor

Hundred grams of freeze-dried immobilized yeast cells on GP and 1250 mL grape must, of 11.2–12.0 °Be density were placed in a 1.5 L glass cylinder bioreactor (29.5 cm height and 9.0 cm internal diameter) and 15 repeated fermentation batches were carried out at 30, 20, 10 and 5 °C. All fermentations were carried out without agitation. When the fermentation was completed, the liquid was decanted and the support was washed twice with 400 mL of must and then the biocatalyst was used for the next fermentation batch.

2.7. MFBT batch (MFBT-b) bioreactor

Batch fermentations of grape must were also carried out in a 1.5 L Multi-Stage Fixed Bed Tower (MFBT-b) bioreactor (29.5 cm height and 9.0 cm internal diameter). It consisted of a glass cylinder containing four packed sections abstained 6 cm each other, thus separating the bioreactor in five compartments. 25 g of freeze-dried immobilized cells on GP were charged to each section. 1250 mL grape must of 11.2–12.0 °Be were added into the bioreactor and a series of 15 repeated fermentation batches were carried out at 30, 20, 10 and 5 °C. All fermentations were carried out without agitation. When the fermentation was completed, the liquid was decanted and the support was washed twice with 400 mL of must, as described above. Then, the biocatalyst was used for the next fermentation batch.

At the end of every batch in both PB and MFBT bioreactors, samples were collected and analyzed for ethanol, residual sugar, wet free cell concentration, total and volatile acidity and volatile by-products.

2.8. Continuous fermentation

Continuous fermentation of grape must at ambient and low temperatures was carried out in a 5 L (9 cm internal diameter and 80 cm height) catalytic MFBT (MFBT-c) bioreactor similar to that used in batch fermentations. The bioreactor contained 100 g of freeze-dried immobilized cells on GP and 4.5 L of grape must. Grape must of 11.6–12.2 °Be was continuously supplied into the bioreactor in an upstream flow by the aid of a high accuracy peristaltic pump (Cole Parmer Instruments Co., Chicago, Illinois, USA) at various dilution rates. The flow rate was reduced as the temperature was decreased from an initial 2000 to 500 mL/day. The reactor was operated continuously for 55 days and the fermentation temperature was decreased gradually from 30 to 10 °C. Temperature was decreased at the rate of 2–3 °C/day to avoid inactivation of yeast cells. Samples were collected daily at different flow rates and temperatures after at least seven days of pumping to achieve steady conditions in the bioreactor and analyzed for °Be density, residual sugar, ethanol concentration, free cell concentration, total and volatile acidity and volatile by-products.

2.9. Recovering of stored freeze-dried immobilized biocatalyst

In order to study the effect of storage on metabolic activity, freeze-dried immobilized biocatalyst stored at 4 °C for 6 months was tested for continuous wine-making in the MFBT-c bioreactor. Grape must of 11.6–12.2 °Be was continuously supplied into the bioreactor, with a daily flow rate of 1000 mL at 20 °C. The reactor was operated continuously for 10 days. Samples were collected at various intervals and analyzed as described above.

2.10. Analyses

Ethanol and residual sugar were determined by high performance liquid chromatography, using a Shimadzu chromatograph with a SCR-101N stainless steel column, a LC-9A pump, a CTO-10A oven at 60°C and a RID-6A refractive index detector. Three times distilled water was used as mobile phase with a flow rate of 0.8 mL/min and 1-butanol was used as an internal standard. Samples of 0.5 mL and 2.5 mL of a 1% (v/v) solution of 1-butanol were diluted to 50 mL and 40 μL were injected directly to the column. Ethanol and residual sugar concentrations were calculated using standard curves and expressed in terms of % (v/v) and grams of residual sugar/L, respectively.

Determination of ethanol enabled calculation of ethanol productivity, which was defined as grams of ethanol per liter liquid