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Integrated continuous winemaking process involving sequential alcoholic and malolactic fermentations with immobilized cells

Z. Genisheva*, A. Mota, S.I. Mussatto, J.M. Oliveira, J.A. Teixeira

IBB—Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, 4710-057 Braga, Portugal

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ABSTRACT

An integrated winemaking process – including sequential alcoholic and malolactic fermentations operated continuously – was developed. For the continuous alcoholic fermentation, yeast cells (*Saccharomyces cerevisiae*) were immobilized either on grape stems or on grape skins, while bacterial cells (*Oenococcus oeni*) used for conducting continuous malolactic fermentation were immobilized on grape skins only. The produced wines were subjected to chemical analysis by HPLC (ethanol, glycerol, sugars and organic acids) and by gas chromatography (major and minor volatile compounds). The final proposed integrated continuous process permitted the production of 960 mL/d of a dry white wine, with an alcoholic strength of about 13 vol%, by using two 1.5 L tower bed reactors packed with 260 g of grape skins. The produced wines revealed a good physicochemical quality. Moreover, 67% of the malic acid concentration could be reduced in the second reactor. Both fermentative processes proved to be much more efficient than those conducted traditionally with free cells or even with immobilized cells, but in the batch mode of operation.

1. Introduction

The two most important processes in wine production are alcoholic fermentation (AF), conducted by yeasts, and malolactic fermentation (MLF), conducted by bacteria. During the alcoholic fermentation the sugars of grape must are transformed mainly to ethanol and carbon dioxide; additionally, a myriad of by-products are formed. Malolactic fermentation is a secondary fermentation that reduces the acidity and brings biological stability to the wines; moreover, it improves the organoleptic characteristics of the product [1].

In traditional winemaking the fermentation processes are conducted in discontinuous mode, i.e. in batch. The seasonality of the raw-material, the grapes, defines largely the organization of this sector of activity and also the structure of the wine cellars. Nevertheless, continuous processes are known to be advantageous over batch processes. The continuous process is simpler to operate with low energy requirements, allowing almost complete utilization of the substrates and lowering the operating costs. Moreover, capital costs are reduced, with the possibility to obtain higher rates of production by using small bioreactors in the process. Superior productivities may be achieved by employing high concentrations of yeast or bacterial cells within the bioreactor. However, a conventional continuous process has limitations in the maintenance of high cell concentrations in the bioreactor [2]. To overcome this difficulty, immobilized yeast or bacteria cell systems provide high cell density with high flow rates that results in short residence times [3]. Reactors with immobilized cells have shorter fermentation times, higher productivity and operational stability of the cells, as well as easier downstream processing.

When dealing with immobilized cell systems it is of a big importance to choose the proper reactor type. This decision depends on the type of immobilization and type of support used, as well as on mass transfer requirements and conditions of the process. For continuous AF in wine production, multiphase reactors are used, including packed bed reactor, fluidized bed reactor, bubble column and air-lift reactor [3,4]. Packed bed reactor is among the most used for wine production with immobilized cells in continuous mode of operation [5,6]. In this type of reactor the immobilized cells are packed inside the reactor and a current of fermentation media is passed upflow (flooded bed reactor) or downflow (trickle-bed reactor) [7].

Most of the available data published about immobilized cell systems used in winemaking concerns batch processes, and in a less extent continuous alcoholic fermentation. Natural materials such as fruit pieces of apple, quince, pear, guava and watermelon [5,6,8–10], whole grains of corn, wheat and barley [11–13] or residues of the wine industry [14,15] are reported as supports for cell immobilization and further implied in batch winemaking. Most of these immobilized cell systems were found to be of good operational stability. In continuous alcoholic fermentation,

* Corresponding author. Tel.: +351 253 604 411/+351 253 604 400; fax: +351 253 604 429.
E-mail address: zgenisheva@gmail.com (Z. Genisheva).

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for winemaking, the used yeast cells are frequently immobilized on natural organic and inorganic materials. Immobilized cell systems using natural organic materials such as gluten pellets resulted in wines with improved quality [16]. Inorganic materials like kissiris and γ-alumina, are cheap, abundant and can be regenerated and reused, however this materials were considered undesirable as they leave mineral residues in the final product [17,18].

The immobilization methods mostly used for bacteria cell immobilization in malolactic fermentations are entrapment [19] and attachment to natural materials [20,21]. There are few available articles on the continuous malolactic fermentation of wines conducted with immobilized cells [22]. Moreover, according to what we know so far no works were published about an integrated continuous process of winemaking.

The main objective of this study was the integration of both alcoholic fermentation (AF) and malolactic fermentation (MLF) in a sequential continuous winemaking process. To achieve this global goal, both AF and MLF were implemented in distinct packed bed reactors operating with immobilized *Saccharomyces cerevisiae* and *Oenococcus oeni*, respectively.

2. Materials and methods

2.1. Inocula preparation

A commercial *S. cerevisiae* strain (Lalvin QA23®, Lallemand) was used in the alcoholic fermentation experiments. The inoculum was prepared by cultivation of the yeast in 500 mL Erlenmeyer flasks containing 200 mL of YPD medium with the following composition: yeast extract (10 g/L), peptone (20 g/L) and glucose (20 g/L). Cells were cultivated under static conditions, at 30 °C for 24 h, being subsequently recovered by centrifugation (*RCF* = 7000, 20 min), washed with distilled water and resuspended in the fermentation medium to obtain an initial concentration of 1 g/L (dry weight).

A commercial strain of *O. oeni* (Uvaferm® ALPHA, Lallemand) was the bacterial strain used in the malolactic fermentation experiments. The inoculum was prepared by cultivation of the bacteria in 500 mL Erlenmeyer flasks containing 200 mL of MRS Broth medium (Cultimed, Panreac, Barcelona). Cells were cultivated under static conditions, at 28 °C for 48 h, being subsequently recovered by centrifugation (*RCF* = 7000, 10 min), washed with distilled water and resuspended in the fermentation medium to obtain an initial concentration of 1 g/L (dry weight).

2.2. Support materials for cell immobilization

Grape skins and grape stems (from white grape varieties), separately, were used as support materials for cell immobilization. These supports were supplied by a local winemaking company, being washed with distilled water and dried at 60 °C until constant weight. Then, supports were sterilized for 20 min at 121 °C, before use.

2.3. Media composition for fermentation assays

Complex culture medium used in the alcoholic fermentation assays was composed by glucose (120 g/L), yeast extract (4 g/L), $(NH_4)_2SO_4$ (1 g/L), KH_2PO_4 (1 g/L) and $MgSO_4$ (5 g/L). Complex culture medium used in the malolactic fermentation had the following composition: glucose (15 g/L), yeast extract (4.0 g/L), meat extract (8.0 g/L), bacteriological peptone (10.0 g/L), $MgSO_4$ (0.2 g/L), $MnSO_4$ (0.05 g/L), sodium acetate (5.0 g/L), tween 80 (1.0 g/L), dipotassium hydrogen phosphate (2.0 g/L), di-ammonium hydrogen citrate (2.0 g/L) and malic acid (4.0 g/L).

The grape must used for alcoholic fermentations was obtained from a mixture of white grape varieties from the Appellation of Origin *Vinhos Verdes* region, with a total sugar content of ≈200 g/L, quantified by areometry [26]. The used wines for malolactic fermentation were produced in laboratory conditions and had an initial concentration of malic acid around 4 g/L. The grape must and wine were kept at 4 °C, before use. Initially, the studies were conducted with complex medium and later grape must and wine were used for alcoholic and malolactic fermentations, respectively. The choice of complex medium in the initial fermentations avoided difficulties with the supply and storage of grape must. Initially, the glucose content of the complex media (used in the alcoholic fermentation) was 120 g/L for a better and faster immobilization of the yeast cells.

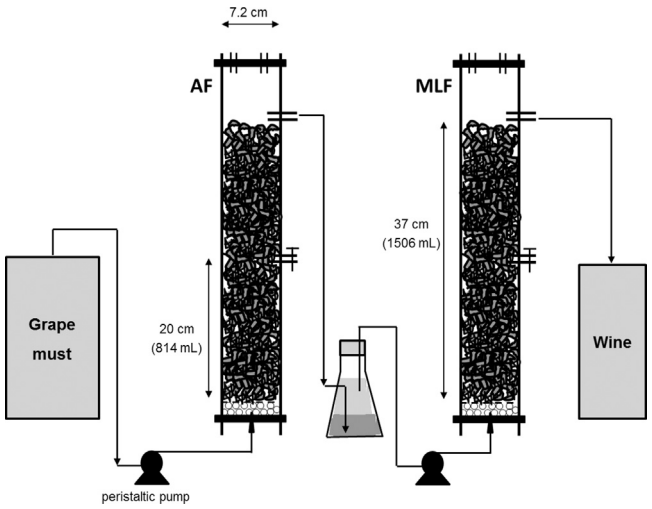

Fig. 1. Schematic representation of the integrated process of continuous winemaking.

2.4. Reactors preparation

Continuous alcoholic and malolactic fermentation assays were performed in distinct cylindrical tower packed bed reactors (7.2 cm inside diameter) with a total volume of about 1750 mL. Two sampling ports were available at 20 cm and 37 cm height, corresponding to working volumes (volume of the empty bed), of 814 mL and 1506 mL, respectively (Fig. 1). Both reactors were operated in upward flow mode.

Before use, the reactors were sterilized with sodium hypochlorite solution (1.5% active chlorine) during at least 4 d prior to fermentations [23]. Then, the reactors were washed with five volumes of sterilized water before filling with the sterilized support. At the bottom of each reactor, 1 cm height of glass beads (6 mm diameter) was placed to allow a regular repartition of the feeding medium in the whole section of the tower. Then, the reactor for continuous alcoholic fermentation was packed with grape stems (60 g or 90 g) or with grape skins (125 g or 260 g) in sterile conditions in the flow chamber. Similarly, the packed bed reactor for continuous malolactic fermentation was filled in with 260 g of grape skins. The assays with 60 g, 90 g and 125 g of material were carried out using the first sampling port, i.e. at 20 cm height; the assays with 260 g of support were performed using the total available volume at 37 cm height. The supports were restricted with an iron nets placed above the glass beads and above the support itself.

2.5. Fermentation assays

A schematic representation of the assays carried out in the present study is depicted in Fig. 2. Initially, for cells immobilization, the reactors were operated in batch mode. The reactors were