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Enhancing the bioproduction of value-added aroma compounds via solid-state fermentation of sugarcane bagasse and sugar beet molasses: Operational strategies and scaling-up of the process

Oscar Martínez, Antoni Sánchez*, Xavier Font, Raquel Barrena

Composting Research Group, Department of Chemical, Biological and Environmental Engineering, Escola d'Enginyeria, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193 Barcelona, Spain

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ABSTRACT

Bioproduction of generally recognized as safe (GRAS) products starting with low-cost raw materials has become significant in the biorefinery concept. Thus, the solid-state fermentation (SSF) of agro-industrial residues using GRAS strains appears as alternative to obtain aroma compounds. Here, the SSF of the mixture sugarcane bagasse/sugar beet molasses was used for producing a mixture of value-added fruit-like compounds. The study aimed to enhance the production and ester selectivity evaluating three operational strategies at three scales (0.5, 4.5 and 22 L) using non-sterilized residues. While the average total volatile production was 120 mg_{Vol} per gram of dry substrate (g_{iTS}^{-1}), fed-batch operation promoted the highest increases in the ester content up to 57 $mg_{Est}\ g_{iTS}^{-1}$, an 88 and 59% more than in the static-batch and intermittent mixing modes respectively. Alternative operational strategies have compensated the scale-up adverse effects in the bioproduction, moving towards a sustainable large-scale application in a circular economy scheme.

1. Introduction

Aroma compounds are constituted by several volatile and non-volatile components with specific physicochemical properties such that they produce odor perceptions in our brain (Berger, 2015). Natural aromas can be found in food, spices or essential oils, but also in flowers or plants (Sarma et al., 2014). Nowadays, their use as additives in food, cosmetic and fragrance industries is extensive due to their effect on the products, enhancing their organoleptic properties, and therefore, positively influencing the final consumer's perception and acceptance (Ziegler, 2007). Natural aromas are typically extracted from the matrices containing these compounds, but the low concentration of these species makes their recovery intricate and costly (Longo and Sanroman, 2006). Despite its cost, consumers prefer natural aromas than synthetic ones, since the latter tend to generate undesirable by-products imparting off-odors that change the organoleptic profiles of these (Etschmann et al., 2002). Consequently, the search for alternative routes for obtaining these compounds has become of major interest during the recent years (Dastager, 2009; de Oliveira Felipe et al., 2017). In this context, biotechnological routes appear as promising substitutes given the ability of some microorganisms to transform some raw materials into aroma compounds (Longo and Sanroman, 2006; Medeiros et al., 2010). In fact, these processes are encouraged by the current European and American legislation due to the qualification of Generally Recognized As Safe (GRAS), determining that products obtained by biotechnological routes are considered natural when the substrate used for this purpose also comes from a natural source (Dubal et al., 2008).

One of the biochemical process to obtain aroma compounds is the solid-state fermentation (SSF), which has been explored to produce value-added aromas like vanillin (dos Santos et al., 2008), 2-phenethyl alcohol (Martínez et al., 2018), coconut-like aroma (Fadel et al., 2015) or fruit-like aromas (Martínez et al., 2017). Also, since the intrinsic characteristics of SSF made of it a suitable process to valorize solid organic waste (Castilho et al., 2009; Yazid et al., 2017), coupling SSF with agro-industrial residues becomes a good way for obtaining natural aroma compounds from a low-cost raw material (Sarma et al., 2014). Furthermore, since SSF is easy to operate and it requires relatively low capital investment (Idris et al., 2017), integration of wastes with SSF results in more sustainable, environmentally friendly and economic processes (de Oliveira Felipe et al., 2017). Obtaining aroma compounds via SSF of organic residues has been tested using different microorganisms: *Trichoderma* strains for producing coconut-like aroma (de Souza et al., 2008), *Saccharomyces cerevisiae* for aroma volatiles (Aggelopoulos et al., 2014), *Ceratocystis fimbriata* and *Kluyveromyces*

* Corresponding author.
E-mail address: Antoni.Sanchez@uab.cat (A. Sánchez).

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marxianus for fruit-like compounds (Martínez et al., 2017; Rossi et al., 2009). *K. marxianus* characteristics (versatility, adaptability to grow under extreme conditions and in different solid media) make of this strain a promising volatiles producer (Lane and Morrissey, 2010; Morrisey et al., 2015).

Nevertheless, the efforts made to verify the feasibility of these bioprocesses are typically limited by constraints like the process scale, the sterilization of the substrates or the use of pure reagents as precursors, which in turn, restrict their use in large-scale applications (Aggelopoulos et al., 2014; Sarma et al., 2014). Besides, previous studies (Martínez et al., 2017) have shown the importance of the operating conditions in the selectivity of volatile compounds produced in SSF but, to our knowledge, there is no information about the effect of the operational strategies (operating modes) on the volatile compounds generation via SSF. In general, reported systems are limited to those in which substrates are completely loaded at the beginning of the process and considering that the solid media remains static during the fermentation time (a static-batch). Since the nutrients availability is also a crucial parameter to the products selectivity in SSF processes (Raimbault, 1998), the way the fermentation is performed, could serve as a tool to modify this availability along the process. In this sense, the intermittent mixing of the solid bed could improve process yield (Jiménez-Peñalver et al., 2016) and fed-batch operation could enhance the microbial growth since the nutrients are not entirely available at the beginning of the fermentation (Astolfi et al., 2011). In the same way, the scale-up effect is a typical disadvantage of SSF process due to the heat and mass transfer phenomena presented in the solid-liquid-gas interphases (Cerda et al., 2017b; Soccol et al., 2017), hampering the development of the technology at industrial scale. Since the behavior of the fermentation changes because of these effects, productivity and selectivity are prone to be affected as well, so the implementation of operating approaches able to minimize these negative effects (Astolfi et al., 2011) becomes of major importance. Particularly, the bioproduction of aroma compounds via SSF has been limited to lab and bench scales (Martínez et al., 2018, 2017), so the understanding of the process at higher scales is still very limited. As described by some authors (de Oliveira Felipe et al., 2017; Sánchez et al., 2015), by integrating more efficient strategies in SSF, it is also expected to contribute in the improvement of the process sustainability, as well as in the industrial development of the technology in the framework of the circular economy.

The aim of the study was to assess some operational strategies such as static-batch, intermittent mixing and fed-batch to enhance the production of the aroma compounds (fruit-like characteristics) in the SSF of the mixture sugarcane bagasse (SCB)/sugar beet molasses (SBM) by means of *K. marxianus*. Also, it was investigated how these strategies affect the selectivity of the produced ester species (those most valuable for their high fruit profile). With this purpose, the selected strategies were tested using the non-sterilized substrate at three different scales (0.5, 4.5 and 22 L). Each scale was characterized by the potential effect in the fermentation temperature profile, produced due to the heat removal strategy of the process. Thus, at 0.5 L temperature was kept constant as a reference condition, at 4.5 L reactors worked at a near-isolated condition and at 22 L reactor was neither temperature-controlled nor isolated.

2. Materials and methods

2.1. Inoculum

Kluyveromyces marxianus (ATCC 10022) was purchased from Colección Española de Cultivos Tipo (CECT, Valencia, Spain). The strain was grown at sterile conditions (materials and media have been previously sterilized by autoclaving at 121 °C for 30 min) at 30 °C during 20 h on agar slants containing: glucose ($40\,g\,L^{-1}$), yeast extract ($5\,g\,L^{-1}$), soy peptone ($5\,g\,L^{-1}$) and agar ($20\,g\,L^{-1}$). *K. marxianus* was maintained in cryovials containing impregnated pearls with the strain at −80 °C. Preparation of the inoculum consisted of adding one pearl into a 150 mL Erlenmeyer flask filled with 80 mL of a liquid medium consisting of glucose ($40\,g\,L^{-1}$), yeast extract ($5\,g\,L^{-1}$) and soy peptone ($5\,g\,L^{-1}$). Then, using a rotary shaker, the culture was incubated for 20 h at 30 °C and 180 rpm. Once grown, it was used to inoculate the non-sterilized substrate.

2.2. Substrate preparation

Sugarcane bagasse was supplied by Ingenio Ntra. Sra. del Carmen (Málaga, Spain) and it has been dried at 60 °C in an air oven during 24 h. Then, the substrate was ground to achieve a particle size distribution in the range 0.5–32 mm by means of a granulator mill. The dried and grounded substrate was stored at −20 °C until it was used. Sugar beet molasses were provided by the sugar company AB Azucarera Iberia S.A. (Madrid, Spain) keeping them at 4 °C until their addition to the substrate mixture. The substrate mixture was prepared with sugarcane bagasse, adjusting the pH, moisture content, and molasses content. This process was performed using a 1:1 (v:v) mixture of a phosphate buffer pH 7 (0.1 M) and a nutrient solution containing $3.0\,g\,L^{-1}$ $MgSO_4{\cdot}7H_2O$, $0.4\,g\,L^{-1}$ $MnSO_4.4H_20$, $0.8\,g\,L^{-1}$ $ZnSO_4{\cdot}7H_2O$, $1.9\,g\,L^{-1}$ $(NH_4)_2SO_4$ and $1.5\,g\,L^{-1}$ $Fe(NO_3)_3{\cdot}9H_2O$ Following, molasses were added to the mixture above. Once they were dissolved, they were mixed with the dried sugarcane bagasse. After preparation of the substrate, it was inoculated using approximately 10^8 colony forming units (CFU) of *K. marxianus* per gram of initial total solids content (ITS) of substrate (g_{ITS}).

2.3. SSF operational modes

Table 1 contains the main description of the performed experiments.

Table 1
Characteristics of the evaluated operational strategies performed at 0.5, 4.5 and 22 L scale.

Experiment	Corresponding Strategy	Characteristic	Substrate load*
Static-batch	Static substrate (Reference condition)	Substrate remains static along the fermentation	100% at t_0
Mixing	Intermittent mixing	Improve interaction nutrients-strain 12 h fixed mixing interval	100% at t_0
FB-50[a]	Fed-batch operation + Intermittent mixing (at selected points[b])	Limiting nutrient availability + temperature control by means of a partial feeding	50% at t_0 50% at t_1
FB-33			33% at t_0 33% at t_1 33% at t_2

* t_0: At the beginning of the process; t_1: Point of maximum activity achieved after t_0; t_2: Point of maximum activity achieved after t_1. FB-50: fed-batch with a split of two; FB-33: fed-batch with a split of three. The reference for 100% load is the maximum amount of substrate used in the static-batch (*i.e.*, 96 g for 0.5 L, 860 g for 4.5 L and 2.5 kg for 22L).

[a] Fed-batch 50% was performed only at 0.5 L scale.

[b] Mixing points in these strategies were defined based on the temperature profile; once the maximum temperature was achieved a mixing was performed.