ISSN 1330–9862
(FTB-1503)

original scientific paper

Production and Recovery of Aroma Compounds Produced by Solid-State Fermentation Using Different Adsorbents

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Received: April 27, 2005
Accepted: October 24, 2005

Summary

Volatile compounds with fruity characteristics were produced by *Ceratocystis fimbriata* in two different bioreactors: columns (laboratory scale) and horizontal drum (semi-pilot scale). Coffee husk was used as substrate for the production of volatile compounds by solid-state fermentation. The production of volatile compounds was significantly higher when horizontal drum bioreactor was used than when column bioreactors were used. These results showed that this model of bioreactor presents good perspectives for scale-up and application in an industrial production. Headspace analysis of the solid-state culture detected twelve compounds, among them: ethanol, acetaldehyde, ethyl acetate, ethyl propionate, and isoamyl acetate. Ethyl acetate was the predominant product in the headspace (28.55 μmol/L/g of initial dry matter). Activated carbon, Tenax-TA, and Amberlite XAD-2 were tested to perform the recovery of the compounds. The adsorbent columns were connected to the column-type bioreactor. All compounds present in the headspace of the columns were adsorbed in Amberlite XAD-2. With Tenax-TA, acetaldehyde was adsorbed in higher concentrations. However, the recovery found by using the activated carbon was very low.

Key words: aroma production, solid-state fermentation, recovery, *Ceratocystis fimbriata*, coffee husk, adsorption, activated carbon

Introduction

Agro-industrial residues have been used as efficient substrates in several bioprocesses such as the production of organic acids (*1*), production of enzymes and biological detoxification of coffee husks (*2*). The application of agro-industrial residues not only provides alternative substrates for solid-state fermentation (SSF), but it also helps to solve pollution problems (*3*). Cassava bagasse, sugar cane bagasse, apple pomace, giant palm bran, and coffee husk have been used as substrates for aroma production in SSF (*4–7*). It is estimated that around 100 aroma compounds are produced industrially by microbial fermentation (*8*).

Strains of the fungi *Ceratocystis* have been identified as aroma producers. Christen *et al.* (*9*) studied the production of aroma compounds by employing different substrates (wheat bran, cassava bagasse, and sugar cane bagasse complemented with a synthetic medium). It was concluded that the type of aroma depended on two

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different sources (carbon and nitrogen). Fruity aroma was detected in the cultures of *Ceratocystis fimbriata* using coffee husk as substrate. Soares *et al.* (*5*) found that the odour detected in the headspace of the culture depended on the amount of added glucose. High levels of the addition of glucose decreased aroma intensity. According to the authors it seems that glucose concentration had a direct influence on the metabolic pathways and thus on the nature of the volatile compounds. Among the compounds produced ethanol and ethyl acetate were the most abundant.

Product recovery is often a difficult step in bioprocesses, especially for flavour compounds because of their volatility and low solubility. It is also necessary to keep the concentration of volatile compounds in the fermentation medium below a certain level due to its inhibitory effect on microbial growth. There are many on- and off--line technologies that can make the extraction and concentration of flavour compounds (*10*).

One of the most used methods to remove organic compounds from fermentation medium involves solvent extraction, separation on specific membranes and adsorption on activated carbon and porous hydrophobic polymers. The two last ones have been used for the concentration of aroma compounds.

There are numerous reports on the adsorption of flavour compounds. Sorption on activated carbon and porous hydrophobic polymers is a suitable method to extract and concentrate volatile compounds from aqueous medium. Solid sorbents were used in an on-line extraction of γ-decalactone during a bioconversion process (*11*). The sorbents tested were: activated carbon and three porous polystyrene-type polymers (Porapak Q, Chromosorb 105 and Resin SM4). *Sporidiobolus salmonicolor* was cultivated on fermentation broth. Adsorbents were added to culture medium at 20 and 30 g/L. γ-decalactone was extracted from the adsorbents using hexane. The presence of adsorbents in the bioconversion medium allowed a very low concentration of the lactones in liquid medium, as a consequence, it limited the toxicity of the flavour compound to the yeast.

In this work, the volatile compounds produced by *Ceratocystis fimbriata* with coffee husks as substrate by solid-state fermentation in two different bioreactor types, column bioreactor (laboratory scale) and horizontal drum bioreactor (semi-pilot scale), were evaluated. Volatile compounds were recovered by trapping them in different adsorbents. Activated carbon and two porous polymers (Amberlite XAD-2 and Tenax-TA) were tested as adsorbents. The objective of the study was to compare the production of volatile compounds in different scales and to test the efficiency of porous polymers and carbon as adsorbents to recover these aromas.

Material and Methods

Microorganism and inoculum

Ceratocystis fimbriata (CBS 374.83) was used during this work. The strain was maintained on potato dextrose agar (PDA) and stored at 4 °C in agar slants. Inoculum was prepared after 5 days of growth at 30 °C into 250-mL Erlenmeyer flasks with 50 mL of potato dextrose agar. Spores were collected with sterile distilled water containing a few drops of Tween 80 and small glass beads by agitation in shaker (120 rpm, 15 min, 25 °C). The spore suspensions contained 10^8 spores/mL, which were prepared by dilution with sterile distilled water and counted with the Neubauer's chamber.

Preparation of the substrate

Coffee husks were dried at 60 °C in an air oven for 24 h. The dried substrate was milled and sieved to obtain particles of 0.8–2.0 mm size. The material was sterilized in an autoclave at 121 °C for 15 min and enriched with glucose. The initial pH of the medium was adjusted to 6.0 and moisture to 65 %. The medium was subsequently inoculated using $1 \cdot 10^7$ spores/g initial dry matter (IDM).

Fermentation procedure

SSF was carried out in two different bioreactors: columns (Fig. 1) and horizontal drum (Fig. 2) connected with an air distributor.

The glass columns (diameter 4 cm, length 20 cm) were filled with 20 g of coffee husks inoculated with spore suspension. Fig. 1 shows the schematic set-up of this fermentation system. The temperature of the water bath was maintained at 30 °C and the columns were connected with an air distributor. Initial moisture content and pH of the substrate were 65 % and 6.0, respectively. The substrate (coffee husk) was supplemented with 20 % mass fraction of glucose dissolved in water, which was used to humidify the initial substrate. The aeration rate was fixed at 0.6 L/h/column. Fermentation was carried out for 192 h, or until reducing sugars reached low levels. Reducing sugars were measured by Somogyi and Nelson (*12*,*13*).

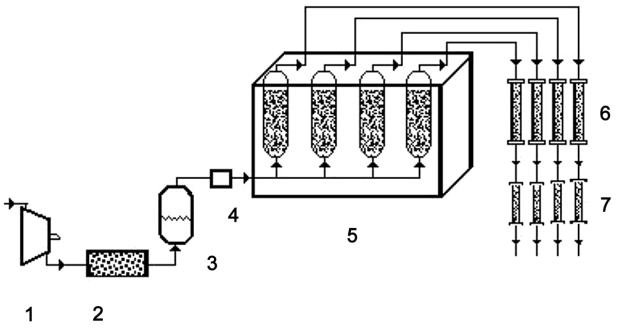

Fig. 1. Schematic set-up of column bioreactor system. **1** air pump, **2** air filter, **3** air moisturizing unit, **4** air distributor, **5** columns in water bath, **6** $CaCl_2$ columns, **7** adsorbent columns

The production of volatile compounds by *Ceratocystis fimbriata* in a stainless steel horizontal drum bioreactor (Fig. 2) was also studied. An air compressor supplied the air required by the growth of fungi inside the bioreactor. Experiments were carried out using 1.5 kg of coffee husks as substrate. The same conditions as used in the column experiments were applied.

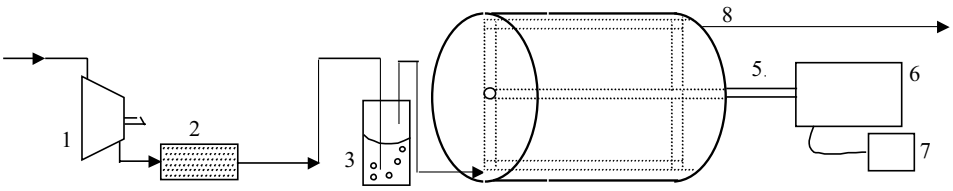

Fig. 2. Schematic set-up of a horizontal drum bioreactor system for aroma production by SSF. **1** air pump, **2** air filter, **3** air moisturizing unit, **4** bioreactor, **5** rotatory agitator, **6** motor, **7** rpm controller, **8** gas outlet

Volatile compounds recovery

Volatile metabolites were collected in adsorbent columns, which were connected at the outlet of the column bioreactor during fermentation. To avoid water interference, pre-columns with $CaCl_2$ were connected before the adsorbent columns. The adsorbent columns were made of glass (length 10 cm, internal diameter 6 mm) and were packed with 320 mg of granular activated carbon (Ultraporous FBC, mesh size 6–8 mm). The same packed quantity of polymeric resins Tenax-TA (60–80 Supelco) and Amberlite XAD-2 were also tested. The columns were fitted between glass wool. The adsorbed volatile compounds were continuously eluted three times with a small volume of solvent (3 mL/adsorbent column). Dichloromethane was the solvent used for activated carbon columns and methanol for two other sorbents (Tenax-TA and Amberlite XAD-2). Column apparatus was constructed in order to desorb the volatile compounds from the adsorption columns according to Janssens *et al.* (*14*). Thus, a concentrated solution of compounds was obtained. A volume of 1 μL of this extract was injected into the capillary column and analyzed by gas chromatography. The conditions used for the GC are given below.

Analytical procedures

Aroma compounds were determined by gas chromatography. A volume of 1 mL of the headspace of the culture was injected directly into a Shimadzu gas chromatograph GC17A, equipped with a flame ionization detector at 230 °C and HP-DB5 capillary column (30 m x 0.32 mm). The temperature program employed was set to start at 40 °C, hold for 5 min, gradually increasing to 150 °C at 20 °C/min rate and holding at 150 °C for 5 min. The injector temperature was maintained at 230 °C under split mode of 1:5 rate. In order to quantify all the compounds, a standard curve of ethanol (Merck) was constructed. Total and individual volatiles were expressed as μmol per liter of headspace.

Results and Discussion

Although the same conditions were applied in column and horizontal drum bioreactor experiments, the results with the horizontal drum bioreactor reached high productivity. The total volatile production was 6 times higher than that obtained in column bioreactors. All the volatile compounds were present in the headspace of both types of bioreactors (columns and horizontal drum).

Fig. 3 presents the evolution of different parameters in the production of volatile compounds in both columns (A) and horizontal drum bioreactor (B). The results show that in glass column bioreactors the maximal production of volatile compounds per g of IDM was 23 μmol/L after 72 h of fermentation. In this case, total productivity was found to be 0.251 μmol/(L·g·h). The horizontal drum bioreactor showed a better performance, which could be observed in the higher concentration of volatiles obtained (144 μmol/(L·g)) after 72 h and total productivity of 1.52 μmol/(L·g·h). These results showed great and promising perspectives for the scale-up of the process for aroma production by solid-state fermentation with agro-industrial residues as fermentation substrates.

A total of twelve compounds were produced, out of which ethyl acetate, ethanol and acetaldehyde were the major compounds. Other compounds, including ethyl

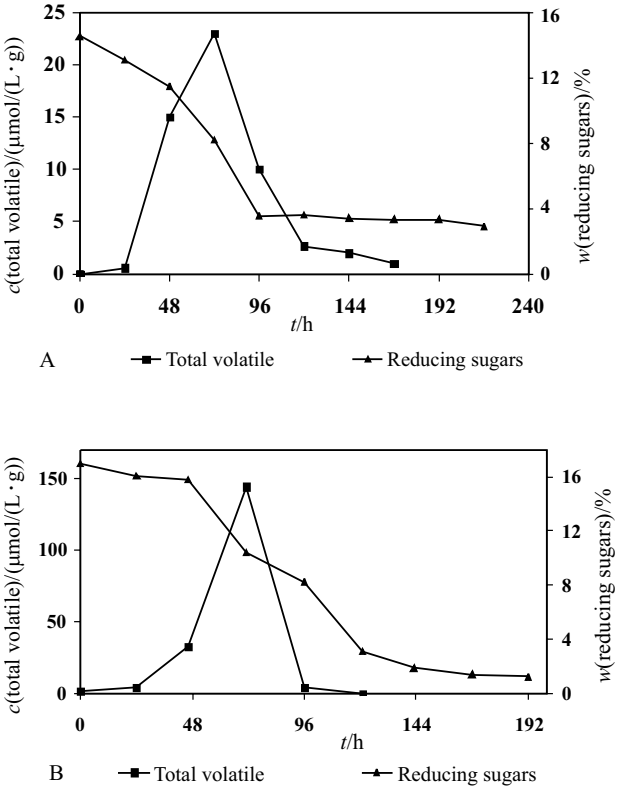

Fig. 3. Evolution of aroma production and reducing sugars during fermentation in column bioreactors (A) and horizontal drum bioreactor (B)

propionate, propyl acetate, ethyl isobutyrate, butyl acetate, were also produced during fermentation. Four compounds remained unidentified. Table 1 shows the concentration of each individual compound produced by *C. fimbriata*, each of them was accumulated in the headspace at their maximum concentration, which was observed on the 3rd day of fermentation. The presence of fruity aroma produced by the culture was attributed to the production of esters. As it is known, alcohols do not contribute to any flavour, although together with other compounds they affect overall flavour quality. Esters of low molecular mass are responsible for fruity odours and consist of acids and their derived compounds such as acetates, propionates, and butyrates. Some examples are ethyl butyrate and isoamyl acetate, which are found in strawberry and banana flavours (*15*).

Table 1. Volatile compounds present in the »headspace« of *C. fimbriata* solid-state cultures in aerated columns (maximum concentration)

Compound	c/(μmol/L)
Acetaldehyde	12.25
A*	8.75
B*	4.25
Ethanol	62.50
Ethyl acetate	472.50
Isopropyl acetate	0
Ethyl propionate	6.50
Ethyl isobutyrate	3.00
Isobutyl acetate	5.25
Ethyl butyrate	2.75
Isoamyl acetate	1.50
D*	0.18

*not identified

It is possible to say that the concentration of volatile compounds in the headspace of the culture is generally affected by several factors, including the nature and concentration of the fermentation medium and its vapor partial pressure. There is the possibility that the compounds, which are less volatile in nature, might not be accurately measured.

Table 2 gives the quantities of the volatile compounds which were recovered by solvent elution of activated carbon, Tenax and Amberlite XAD-2 columns. The recovery of the volatile compounds on activated carbon column was not efficient, probably due to the hydrophilic nature of that support. Only the compounds at higher amounts in the headspace such as acetaldehyde (2.36 μmol), ethanol (24.47 μmol), and ethyl acetate (108.68 μmol) were recovered by solvent elution of the activated carbon column. Porous resins (Tenax and Amberlite XAD-2) showed the best results for the recovery of volatile compounds. In the case of Tenax, acetaldehyde was recovered in significant amounts (649.7 μmol). This compound was easily adsorbed demonstrating that

Table 2. Volatile compounds recovered from different materials during fermentation of coffee husk by C*eratocystis fimbriata*

Compound	n(adsorbed compounds)/μmol		
	Activated carbon	Amberlite XAD-2	Tenax
Acetaldehyde	2.36	519.09	649.70
Ethanol	24.47	107.32	69.37
Ethyl acetate	108.68	610.76	128.19
Propyl acetate	–	0.85	0.06
Ethyl isobutyrate	–	2.38	0.27
Isobutyl acetate	–	3.90	1.47
Ethyl butyrate	–	1.76	–
C*	–	0.31	–
Isoamyl acetate	–	0.06	–
D*	–	0.25	–

*not identified

there is an affinity of the resins for acetaldehyde. Supposing that the volatile concentrations in the headspace were constant during a day, the total volatile quantity produced could be calculated multiplying the concentration by total flow. Thus, the efficiency of the recovery on each adsorbent can be calculated. For example, ethyl acetate was recovered in XAD-2 column with 9 % efficiency. Amberlite XAD-2 and Tenax demonstrate to be more efficient in trapping volatile compounds than activated carbon. Amberlite XAD-2 could adsorb ten of the twelve compounds produced by *Ceratocystis fimbriata* using coffee husk as substrate. Adsorption on activated carbon of aroma compounds in wastewaters from aromatic plants distillation was considered excellent (≥90 %) to moderate (44–77 %) by Edris *et al*. (*16*). The authors verified that some components were more selectively adsorbed. They also observed a moderate recovery (≈70 %) of aroma adsorbed on carbon with diethyl ether. This could explain why few components (ethyl acetate, acetaldehyde and ethanol) were found in the extract of the activated carbon using dichloromethane to recover the volatile compounds. The desorption of the volatile compounds from the adsorbent columns should be improved.

Conclusion

The production of volatile compounds was significantly higher in a horizontal drum bioreactor, showing good prospects for the process scale-up. Twelve compounds were separated by GC headspace analysis of the culture. The predominant compounds were ethyl acetate, ethanol and acetaldehyde. Comparing different types of adsorbents used to recover the aroma compounds, the resin Amberlite XAD-2 adsorbed almost all compounds present in the headspace of the culture when compared with Tenax and activated carbon. The results obtained from the adsorption experiments showed the possibility of using porous resins to recover microbial volatile compounds produced by SSF processes.

Acknowledgements

Adriane B.P. Medeiros and Carlos R. Soccol thank CAPES and CNPq, respectively, for financial support.

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Proizvodnja i izdvajanje aromatskih spojeva dobivenih fermentacijom čvrstog supstrata koristeći razne adsorbente

Sažetak

Hlapljivi spojevi s voćnom aromom dobiveni su s pomoću plijesni *Ceratosystis fimbriata* u dva različita bioreaktora, i to u kolonskom bioreaktoru (u laboratoriju) i horizontalnom bubnju (poluindustrijski). Kao čvrsti supstrat za fermentaciju upotrijebljena je lupina kave. Proizvodnja hlapljivih spojeva bila je kudikamo veća u horizontalnom bubanjskom reaktoru. Stoga ovaj model bioreaktora ima sve preduvjete za uvećanje i primjenu u industrijskoj proizvodnji. Kromatografskom analizom plinske faze iznad fermentiranog supstrata pronađeno je 12 hlapljivih spojeva, a među njima etanol, acetaldehid, etilni acetat, etilni propionat i izoamilni acetat. Etilni acetat bio je glavni hlapljivi proizvod u plinskoj fazi (28,55 μmol/L/g početne suhe tvari). Za izdvajanje hlapljivih spojeva ispitani su aktivni ugljen, Tenax-TA i Amberlite XAD-2. Kolone za adsorpciju bile su povezane s kolonskim bioreaktorom. Sve hlapljive spojeve iz plinskog prostora bioreaktora adsorbirao je Amberlite XAD-2. Tenax-TA adsorbirao je više acetaldehida, a izdvajanje hlapljivih spojeva na aktivnom ugljenu bilo je vrlo slabo.