Continuous production of wine in a tower fermentor using entrapped yeast cells in double layer alginate – chitosan beads

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Abstract

Continuous production of wine in a tower fermentor by entrapped yeast cells of Saccharomyces cerevisiae in double layer alginate – chitosan beads (DAC beads) has been studied. The wine was produced from the grape variety of Muscat of Alexandria. Continuous fermentation was carried out for 53 days and the composition of the wines produced in different flow rates of the medium was determined. Also suspended yeast cells in a free state from the same strain were used under similar conditions for the production of wine by batch fermentation.

The composition of the wines produced by the entrapped cells in the continuous process in the different flow rates of the medium was similar. The maximum productivity of ethanol and the maximum daily production of wine achieved during the continuous process were 3 - 4 times higher than those achieved during the batch process. The composition of the wines produced from the two different processes was similar. In the continuous fermentation the concentrations of higher alcohols were at lower level while the concentrations of volatile esters were at higher level compared to batch fermentations.

Keywords: Continuous wine production, alginate-chitosan complex, double layer beads, tower fermentor

Introduction

Continuous fermentation is a process that offers important advantages over batch fermentation such as higher productivities, yield improvement, higher conversion rates, uniform and constant composition of fermentation products, reduction in the production cost, the ability of using mixed cultures (Guidoboni 1984, Verbelen et al. 2006). Continuous fermentation based on immobilized yeast technology provides high cell densities in the bioreactor and ensures that there is no biomass washout when the critical dilution rate is exceeded.

One of the most common techniques of yeast immobilization is entrapment within porous matrices. Several natural materials such as carrageenans (Thomas 1997), chitosan (Park et al. 2004) and alginates (Smidsrød and Skjåk-Bræk 1990) and synthetic polymeric matrices such as polyvinylalcohol (Shindo and Kamimura 1990) polystyrene (Yoshida et al. 2003) and polyurethane (Ramakrishna and Prakasham 1999) have being used for this purpose.

Alginate and chitosan are two of the above polymers with a numerous applications in the immobilization technology due to their non-toxic, biocompatible, biodegradable and antimicrobial properties (George and Abraham 2006, Espevik et al. 1993, Hirano and Nagano 1989, Rhoades and Roller 2000).

Alginate is an anionic polymer which has the ability to form a gel when it comes in contact with bivalent cations such as Ca^{2+} (Grant et al. 1973). This gel has a typical pore size distribution of 5 to 200 nm which ensures the entrapment of yeast cells in the structure of the gel (Smidsrød and Skjåk-Bræk 1990). Entrapment can be achieved when dripping a mixture of cells and water soluble sodium alginate into a solution containing calcium cations. The gels prepared following this procedure are usually in the form of beads.

The most common problems occurred using entrapped yeast cells in calcium alginate beads for the process of alcoholic fermentation is the cell leakage, the destabilization of the alginate gel and the rupture of the beads. Cell leakage is occurred by the ability of the cells located on the periphery of the beads to multiply and be released into the medium. The presence of chelators such as phosphate, lactate or citrate and non-gelling cations such as K^+, Na^+ or Mg^{2+} which have the ability to replace the calcium ions in the alginate gel causes the destabilization of the gel structure (Smidsrød and Skjåk-Bræk 1990). The rupture of the beads can be occurred

by the growth of the cells and the CO_2 formation and accumulation within the beads (Liouni et al. 2007, Yu et al. 2007, Martins dos Santos et al. 1997).

Double layer alginate – chitosan beads (DAC) have been constructed for the entrapment of yeast cells with the aim of using them in alcoholic fermentations (Liouni et al. 2007). The presence of the outer layer prevents cell leakage from the beads into the medium while the coating of the beads with chitosan improves the mechanical and the chemical stability of the beads during fermentation.

In this work yeast cells entrapped in DAC beads were used for the continuous production of wine in a tower fermentor. The composition of the wines produced in the different flow rates of the must was determined and compared with the composition of the wines produced by "free" yeast cells during batch fermentations. Also the productivities of these two types of fermentation (continuous and batch) were compared.

Materials and Methods

Microorganisms

Oenological yeast from ANCHOR (South Africa) *Saccharomyces cerevisiae* strain NT 45.

Grape Must

The wine was produced from Muscat of Alexandria must with 217 g/L of total sugars concentration, pH 3.69 and total acidity 4.20 g/L as tartaric acid.

Immobilization polymers

Alginic acid sodium salt from brown algae was purchased from Fluka 71238 (Fluka Chemie AG). Chitosan was purchased from Aldrich with a molecular weight lower than 5 kDa and the degree of deacetylation was 85%. All others were analytical reagents.

Entrapment of yeast cells

Yeast cells were entrapped in DAC beads as described by Liouni et al. 2007. The beads were constructed by 2.5 % (w/v) of sodium alginate solution for the inner layer and 2.0 % (w/v) of sodium alginate solution for the outer layer. The initial cell loading

was 1.8×10^9 cells/mL in the inner gel solution. The gelling solution contained 0.18 M $CaCl_2$ and the coating solution contained 1.0 % (w/v) of chitosan and 0.18 M $CaCl_2$.

Description of the fermentation system for the continuous production of wine

The fermentor was constructed by Pyrex - glass column with a diameter of 8 cm which had an expansion (14 cm diameter) on the top. The working volume of the fermentor was 2.8 L. Continuous fermentation was carried out by feeding the fermentor with the must from the feed tank in an upstream flow using a peristaltic pump (Watson – Marlow 101 U/R). The effluent from the fermentor was collected in the product tank. The temperature of the fermentation inside the fermentor was controlled with a glass tube in the shape of U which was connected with a thermo circulator in a water bath (Figure 1). The fermentor had an exit on the top connected with a condenser for the exhaust of CO_2 and the condensation of volatile products.

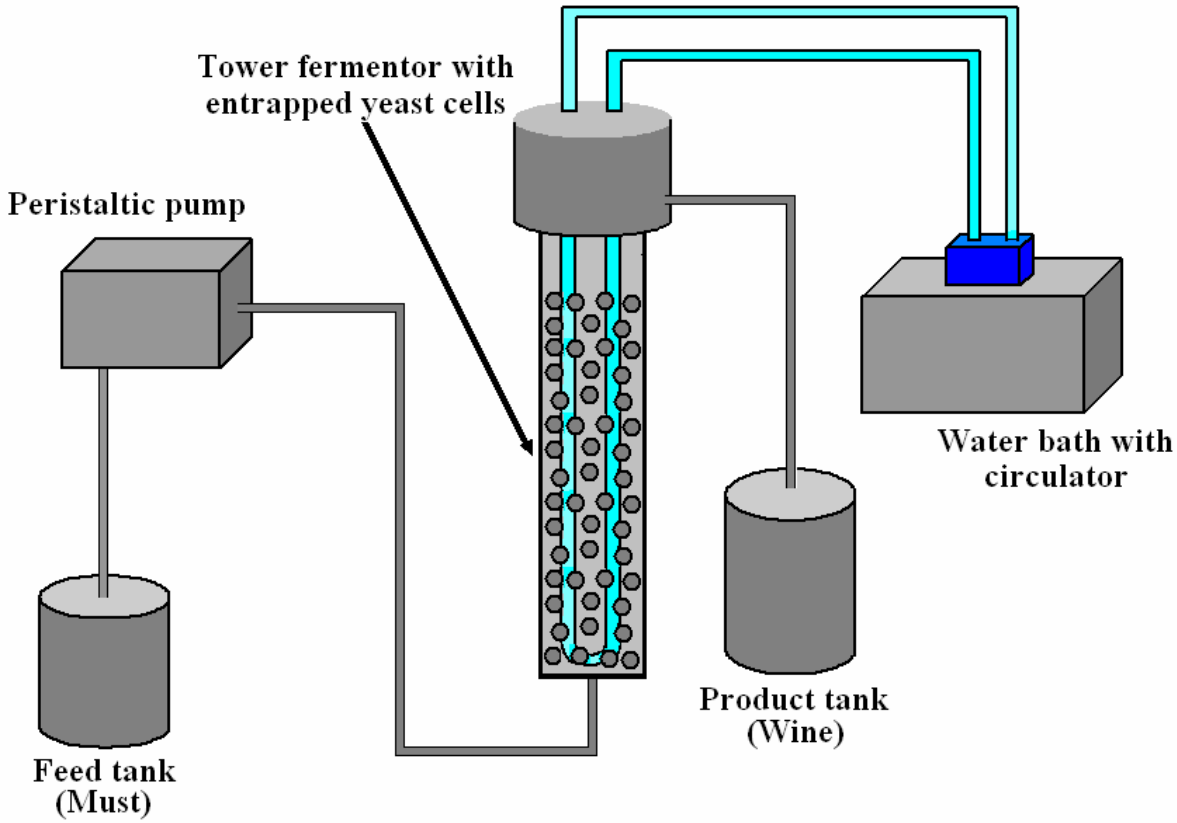

Figure 1 Schematic diagram for the continuous production of wine.

Analytical Methods

Reducing sugars were determined by the method of DNS (Miller 1959). The concentration of ethanol, higher alcohols, total volatile esters and total acidity, volatile acidity and pH of the samples were determined according to Amerine and Ough (1988).

Results and discussion

Continuous fermentation

About 80 % of the working volume of the fermentor was packed with DAC beads and 12 g of dry yeast cells were entrapped in the fermentor. The temperature of the fermentation was adjusted at 20 °C. The must was pumped into the fermentor in different flow rates. During the fermentation, samples were taken periodically from the exit for analysis. The flow rate of the must increased until the biomass in the fermentor couldn't consume all the sugars, therefore the concentration of sugars in the products started to increase. The bioreactor was operated continuously for 53 days.

The composition of the wines produced in the different flow rates of the must is given in Table 1. Ethanol productivity was calculated by multiplying the dilution rate by ethanol concentration.

As can be seen in Table 1, higher dilution rate resulted in higher daily production of wine and ethanol productivity. The composition of the wines was not affected significantly by the changes in the flow rate of the medium. The concentrations of ethanol, residual sugars and the values of total acidity and pH were about the same. Higher alcohols and volatile esters had variations in their concentration which were low. Similar results were obtained for volatile acidity.

Batch fermentation

Wine was produced by batch fermentations with freely suspended yeast cells from the same strain. The temperature of the fermentations and the concentration of the yeast cells in the must were the same as in the continuous process.

The composition of the wines produced by the batch processes is given in Table 2.

Comparison between continuous and batch fermentation

The type of fermentation affected significantly the ethanol productivity and the daily production of wine. The maximum ethanol productivity and daily production of wine monitored in the continuous fermentation using entrapped yeast cells, were 3 – 4 times higher than those monitored during batch fermentations using "free" yeast cells (Table 1 and 2).

The wines produced by these two procedures had similarities in their chemical composition. The main characteristics of these wines such as ethanol concentration, residual sugars, total acidity, volatile acidity and pH were nearly the same. The only difference was that for the wines produced by the continuous fermentation the concentrations of volatile esters were higher (about 20 to 40 mg/L) while the concentrations of higher alcohols were lower (about 70 to 100 mg/L).

Conclusions

The continuous production of wine by entrapped yeast cells offers significant economical advantages in comparison with the traditional method of winemaking such as, the increase of the productivity and the higher duration in using the biomass. Most of the characteristics of the wines produced by the continuous fermentation were similar with these of the wines produced by the batch fermentations.

Table 1 The composition of the wines produced by the continuous fermentation.

DILUTION RATE (1/h)	WINE PRODUCED (ml/day)	RESIDUAL SUGARS (g/L)	ETHANOL CONCENTRATION (% v/v)	ETHANOL PRODUCTIVITY (g/L·h)	TOTAL ACIDITY (g/L tartaric acid)	VOLATILE ACIDITY (g/L acetic acid)	HIGHER ALCOHOLS (mg/L)	VOLATILE ESTERS (mg/L)	pH
0.007	463	1.0 ± 0.2	12.70 ± 0.04	0.691±0.006	5.75 ± 0.09	0.635±0.042	189.5 ± 25.5	175.8±12.5	3.62 ± 0.05
0.009	593	1.4 ± 0.2	12.69 ± 0.06	0.906 ± 0.012	5.80 ± 0.06	0.712±0.055	184.6 ± 29.6	180.4±15.4	3.63 ± 0.04
0.011	700	1.3 ± 0.3	12.69 ± 0.04	1.051 ± 0.009	5.70 ± 0.12	0.615±0.072	201.8 ± 20.3	181.5±12.9	3.60 ± 0.05
0.013	864	1.7 ± 0.1	12.66 ± 0.05	1.301 ± 0.014	5.85 ± 0.04	0.686±0.058	172.2 ± 31.3	171.2±14.1	3.62 ± 0.03
0.015	950	1.4 ± 0.2	12.68 ± 0.04	1.448 ± 0.013	5.80 ± 0.06	0.595±0.064	174.7 ± 27.5	168.3±12.7	3.61 ± 0.03
0.016	1037	1.6 ± 0.2	12.66 ± 0.05	1.554 ± 0.017	5.80 ± 0.04	0.653±0.081	187.5 ± 23.2	177.7±16.7	3.59 ± 0.05
0.017	1123	1.8 ± 0.2	12.65 ± 0.03	1.697 ± 0.011	5.75 ± 0.08	0.732±0.059	195.3 ± 22.4	185.4±13.6	3.61 ± 0.03
0.019	1181	2.0 ± 0.2	12.64 ± 0.04	1.798 ± 0.016	5.80 ± 0.06	0.615±0.077	204.3 ± 25.3	173.5±15.3	3.63 ± 0.04
0.021	1255	1.9 ± 0.3	12.65 ± 0.03	1.897 ± 0.012	5.75 ± 0.06	0.633±0.039	187.9 ± 24.6	167.8±11.3	3.61 ± 0.04

Results are shown as mean value ± S.D. (n = 4)

Table 2 The composition of the wines produced by the batch fermentations

CHARACTERISTICS OF THE WINES	CONCENTRATION
WINE PRODUCED (ml/day)	339
RESIDUAL SUGARS (g/L)	1.5 ± 0.2
ETHANOL CONCENTRATION (% v/v)	12.67 ± 0.04
ETHANOL PRODUCTIVITY (g/L·h)	0.709 ± 0.015
TOTAL ACIDITY (g/L tartaric acid)	5.50 ± 0.07
VOLATILE ACIDITY (g/L acetic acid)	0.717 ± 0.044
HIGHER ALCOHOLS (mg/L)	276.7 ± 22.1
VOLATILE ESTERS (mg/L)	143.0 ± 12.4
pH	3.67 ± 0.03

Results are shown as mean value ± S.D. (n = 4)

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