Immobilized yeast cells in double-layer hydrogel carriers for beer production

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Introduction

The use of immobilized cell systems is an attractive and rapidly expanding research area because of the technical and economical advantages compared to the free cell systems. The selection of a proper support matrix is very important for successful performance of an immobilized cell system. It is desirable that the cell carrier possesses large surface area, permeability, chemical, mechanical and thermal stability, insolubility and suitable shape. In that respect, we tried to investigate synthetic polymer carriers for cell immobilization and to use these systems for beer fermentation process. Within this project, two different fermentor configurations have been used. One is stirred tank bioreactor, convenient to test mechanical stability and resistance to abrasion of the biocatalyst. The second reactor type used for the fermentation process is the barbotage bioreactor with low to moderate local mixing.

Materials and methods

Preparation of double-layered cryogels

Cryogels of hydroxyethylcellulose (HEC) were prepared, using the same equipment, by u.v. irradiation of 2% moderately frozen aqueous solutions for 2 minutes at both sides (total time was 4 min) in the presence of the same photoinitiator used for the synthesis of hydrogels. The biocatalyst (single pellet) used for the stirred tank reactor contained 0.03 g of yeast cells, while the mass of cells in pellets aimed for barbotage reactor was 0.15 g. After their preparation HEC cryogels were covered (coated) with layer of poly(ethylene oxide) that was u.v. curried for 2 minutes on both sides by "Dymax 5000-EC" u.v. equipment with 400 W metal halide flood lamp.

Repeated batch culture

The performance of the immobilized cells was investigated by carrying out repeated batch fermentations in two different types of reactors (Figure 2) containing 8 peaces or gel pellets (25 g) with immobilized cells as biocatalyst (solid phase). 400 of sterilized culture media or wort (liquid phase) with initial sugar concentration of 92 g/L was introduced into the fermentor with gas barbotage, while nitrogen was inserted (gas phase) to provide liquid circulation around the gel peaces. The nitrogen flow rate was 4 min-1. Stirred tank reactor (600 ml) consisted of a glass vessel with a magnetic stirrer (400 r/min) and an outer jacket to maintain the temperature (22 or 15 ºC).

Analytical methods

Flavor-active compounds were analyzed by gas chromatograph, Perkin-Elmer 900.

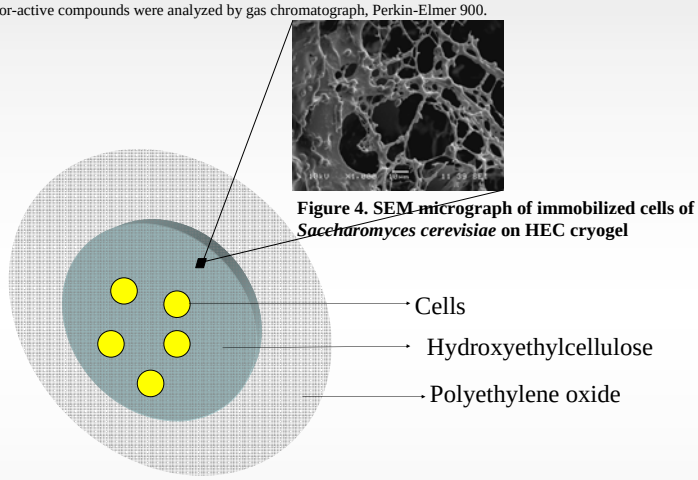


Figure 4. SEM micrograph of immobilized cells of *Saccharomyces cerevisiae* on HEC cryogel

Figure 1. Double-layer polymer hydrogel with immobilized cells

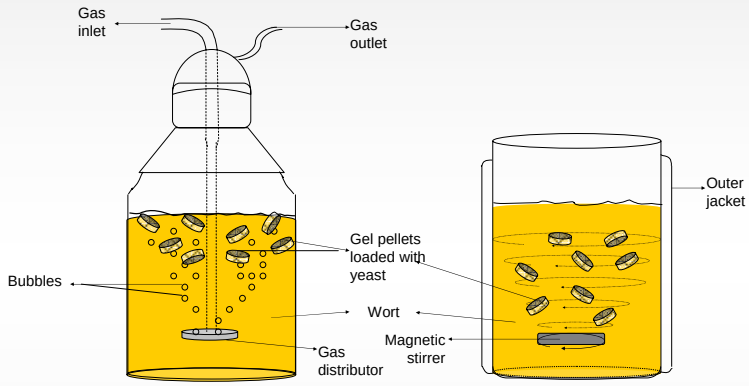


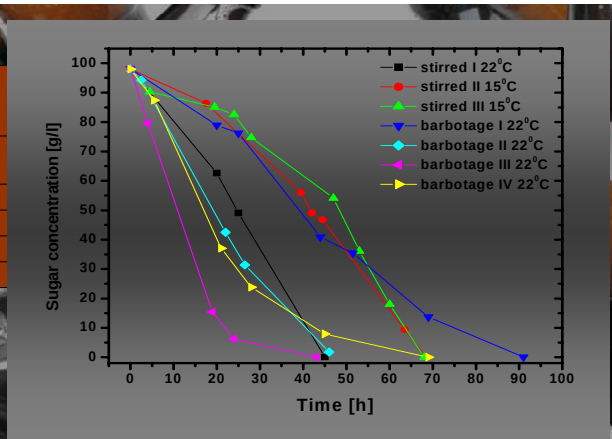
Figure 2. Fermentors

Results and discussion

Figures 3 shows gradually decrease of sugar content during consecutive batch fermentations. Fermentations may be assessed as rapid, considering the fact that the content of immobilised biocatalyst per mass unit of liquid phase was very low (6.2 w/w% and 4.2 w/w% for the barbotage and stirred reactor, respectively), where the achieved cell number per gram of biocatalyst was very high, 9×10^9. The achieved average specific productivities were 8.6 g/g/L/day for the barbotage reactor at 20 °C and 8.2 g/g/L/day for the stirred tank fermentor at 15 °C. The concentration of suspended cells amounted to more than 50% of an overall cells number. High free cells concentration implies the poor protection expected from the poly(ethylene oxide) layer. This, in addition to structure of large open porous (Figure 4) of hydroxyethylcellulose, facilitated cell leakage from the gel biocatalyst. Moreover, the biomass washout was assisted by intensive fluid circulation caused by gas flow or mechanical mixing in the barbotage and stirred tank reactor, respectively. Another disturbing result is the disintegration of gel pellets in case of stirred tank fermentor. In order to assess beer quality, samples were collected and analyzed on flavor-active compounds (Table 1). Amounts of isobutanol were in the optimal range, while concentrations of isoamyl alcohol were significantly increased. Enhanced formation of fusel alcohols is, most likely, a result of rapid yeast growth due to high mass transfer rates in the fermentor, leading to an increased amino acid uptake. The concentration of acetaldehyde was up to 5 times higher than the threshold, causing a green leaf-like flavor. The amount of esters, very important compounds in beer, methyl acetate, ethyl acetate and isoamyl acetate were evaluated as low and under the ranges considered as optimal. The appropriate ester concentrations are necessary for the nicely balanced fruity/flowery aroma profile in beers. The depleted ester synthesis possible may be a result of the altered cell metabolism after immobilization step.

Table 1. Volatile compound in beers produced by double-layer gel in barbotage and stirred tank fermentors.

Ferm.	Temp. (°C)	Reactor	Dimethyl-sulfide (µg/l)	Acetaldehyde (mg/l)	Methyl acetate (mg/l)	Ethyl acetate (mg/l)	Isoamyl acetate (mg/l)	Isobutanol (mg/l)	Isoamyl alcohol (mg/l)
			(60-90 µg/l)	(5-10 mg/l)	(0-1.5 mg/l)	(10-25 mg/l)	(0.5-1.5 mg/l)	(6-15 mg/l)	(30-60 mg/l)
I	20	Barbotage	33.6	11.6	0.09	4.26	0.16	8.8	102.4
II	20	Barbotage	37.1	41.9	0.26	2.8	0.12	13.8	96.4
I	20	Stirred	44.6	20.2	0.20	8.9	0.61	8.6	147.1
II	15	Stirred	32.9	49.7	0.24	4.4	-	14.9	104.2



Conclusion

To assess the suitability of this cryogel-hydrogel matrix for beer fermentation process, extensive investigations are required. They should enclose optimization of the system biocatalyst-reactor. Except the structure, the geometry of the biocatalyst is another feature, closely related to the reactor design, which should be considered in prospect. In addition, the future experiments should focus on continuous fermentation with double-layered cryogels under different operating conditions.