**Poly(2-hydroxyethyl methacrylate) cryogels as the entrapment cell carriers for high-cell-density bioproduction**

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**Highlights**

* PHEMA cryogels were prepared *via* cryopolymerization
* PHEMA cryogels were used as the cell entrapment carriers during *L. paracasei* fermentation
* Higher cell concentration using cryogels was observed than that without cryogels
* Dried cell concentration of 5.32 g·L-1 was achieved

**1. Introduction**

The bioproduction of high-value organic acids like phenyllactic acid (PLA) *via* microbial fermentation or biotransformation has received increasing attentions in biological and biotechnology fields in recent years. [1-4] During these processes, the culture of cells at high-cell-density to increase the total biomass concentration in the broth is of great important to the yield, the conversion rate and the productivity. In this work, the poly(2-hydroxyethyl methacrylate) (pHEMA) cryogels were prepared by cryo-polymerization under freezing conditions. The obtained cryogels were used the cell entrapment carriers during *L. paracasei* fermentation towards the production of PLA. The morphology and the cell concentration during the fermentation process were investigated experimentally.

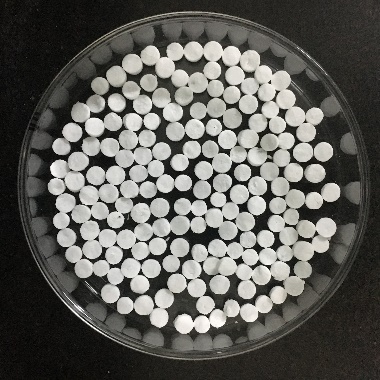
**2. Methods**

Monolithic pHEMA cryogels were prepared by cryo-polymerization. Typically, the reactive solution containing HEMA (11.6%, w/w) and poly(ethylene glycol) diacrylate (PEGDA, 3.5%, w/w) initiated by TEMED and APS (both with the mass ratio of 4% to the total mass of HEMA and PEGDA) was poured into glass columns with inner diameter of 4-6 mm and height of 100 mm. Free radical cryo-polymerization was conducted under freezing condition for 48 h to get the cryogel.

The cryogels were cut into pieces with height of about 3-5 mm and used as the cell carriers. *L. paracasei* 16C3 strain was cultured in MRS medium with and without cryogel pieces and the cell mass was measured at given time intervals. The pore morphology of the cryogel was investigated by scanning electron microscope (SEM, S-4700, Hitachi, Japan). The samples were cut from the middle of the sample columns, directly dehydrated using an ethanol-water solution (10-30-50-70-80-90-99.5%), dried at critical point, coated with gold and then observed by SEM.

**3. Results and discussion**

The results showed that pHEMA cryogels have supermacropores with the sizes from several to 200 μm. These pores provided enough void space for the growth, entrapment and deposition of microbial cells during fermentation processes. The cryogel carriers permit more surfaces for the attachment and deposition of cells and also contributed to the reduction of the substrate inhibition during cell growth. The cell concentrations by using these cryogels as the carriers were higher than those without cryogels for the growth of *L. paracasei* 16C3 strain in MRS medium. The maximum cell concentration of about 5.32 g·L-1 in the fermentation broth was achieved, as shown in Figure 1(a), indicating that the cryogels could be used as potential and interesting cell carriers for high-cell-density fermentation and bioconversion.

(a) (b) (c)

**Figure 1.** (a) Morphology of pHEMA cryogel carriers, (b) microstructure within the cryogels an (c) cell concentrations with and without cryogels during the fermentation of *L. paracasei* 16C3 in MRS broth for the production of PLA.

**4. Conclusions**

PHEMA cryogels were interesting cell entrapment carriers and can be used for the high-cell-density culture of microbial strains like *L. paracasei* 16C3 for the production of bio-based organic acids. The concentration of cells using cryogels as the cell carriers can be increased to twice the biomass in traditional fermentation without cryogels.

**References**

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**Acknowledgments**

The authors gratefully acknowledge the financial supports partially by the National Natural Science Foundation of China (Nos. 21576240, 21777143, 21106132) and the Zhejiang Provincial Natural Science Foundation of China (Nos. LY14B060005, LZ14B060001).