**Enhancing high-density cultivation of *Bifidobacterium bifidum* with Calcium Carbonate-Alginate Immobilized Beads**

**Ngoc Dang-Huu-Bao**, **Vy Nguyen-Cat-Khanh**, Huy Bui-Quoc, Thai Nguyen-Minh

Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam.

**Background and aims.** *B. bifidum* has received significant attention due to its ability to promote host health. However, strict anaerobes such as *Bifidobacteria* are difficult to cultivate at high density since they produce lactic acid, which adversely affects cultivation efficiency. Calcium carbonate-alginate (CaCO₃-alginate) immobilized gel beads have been evaluated as an effective and low-cost pH-buffering system. This study aims to examine the pH-buffering capacity of CaCO₃-alginate gel beads in high-density cultivation of *B. bifidum*.

**Methods.** Gel beads were prepared by dropping CaCO₃-alginate suspension into 100 mM CaCl₂ solution. *B. bifidum* was cultivated under anaerobic conditions in MRS broth supplemented with 20% (v/v) gel beads at 37 °C for 24, 48, and 72 hours. A control experiment was performed in parallel without gel beads. Cell density was examined for changes via the plate count method and Flow Cytometry (FC).

**Results.**  After 72 hours of cultivation, the spent media from gel beads-based cultivation exhibited a significantly higher pH compared to the control. Microscopic observation revealed that *B. bifidum* cells grown in the media with gel beads appeared larger and more elongated than those in the control. Cell density after 24 and 48 hours of cultivation showed no significant difference with or without gel beads, but after 72 hours of cultivation, the cell density with gel beads was higher than the control. Microscopic analysis revealed morphological changes in the presence of gel beads.

**Conclusion.** This study demonstrates the potential of CaCO₃-alginate gel beads as a pH buffer to enhance the growth and viability of *B. bifidum* in high-density cultivation.

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