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A mycelium with polyelectrolyte complex-bunched hyphae: Preparation and fermentation performance

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Abstract

We studied the immobilization of a mycelium (*Aspergillus niger*) using the working hypothesis as follows: (a) when polycation was added to the cell suspension, a few parts of it would bind on the surface of a hypha, allowing to gather the hyphae in part but not all; (b) upon further addition of polyanion, such a gathering of the hyphae is tightly bunched by the polyelectrolyte complex (PEC) which is resulted from the remaining polycation; (c) as a result, a mycelium with partially bunched hyphae can be obtained. Potassium poly(vinyl alcohol) sulfate and trimethylammonium glycol chitosan iodide [6-O-(2-hydroxyethyl-2-(trimethylamonio)-chitosan iodide) were used as the polyanion and the polycation, respectively. The optical and electron microscopic analyses showed that our immobilized cell contains many of PEC-bunched hyphae. The sedimentation rate increased with the weight ratio of PEC to dry cells and leveled off at the weight ratio larger than 0.5. The gluconic acid production from glucose was studied by a semi-large scale (1 l) cultivation of the imobilized and free cells using a jar fermentor. It was found that an apparent specific activity of the immobilized cells for glucose oxidation becomes 1.44 times that of the free cells even at a high cell density of 40 g/l.

Keywords: Polyelectrolyte complex; Cell immobilization; Filamentous fungi; High cell density fermentation; Gluconic acid production

1. Introduction

One of the most serious problems in submerged mycelial processes is the very high non-Newtonian viscosity of fermentation broths. This is associated with the growth of mycelial organisms in a highly extended and branched filamentous form. There have been a lot of attempts to solve this problem, which fall into two categories: improvements in the fermentation device [1–3] and in the morphology of mycelial cells [4–8]. For the latter, a rational approach involves immobilization of mycelial hyphae using porous beads of celite [4] and cellulose [5], gels of *K*-carrageenan [6] and calcium alginate [6,7] and polyurethane foams [8].

A systematic study made in 1980s by Kokufuta et al. has demonstrated that polyelectrolytes (PEs) or polyelectrolyte complexes (PECs) are very useful for the immobilization of enzymes and microbial cells [9–21]. In the case of a PEC-immobilized mycelial organism (*Aspergillus terreus*) [18], a marked improvement in the sedimentation was observed, although there was little difference in itaconic acid production between the immobilized and free cells in a small scale (100 ml) cultivation with an Erlenmeyer flask on a rotary shaker. From this result [19] and also from the results [9–11,13,14,18] of other PEC-immobilized cells, we set up the following working hypothesis for the immobilization of filamentous fungi through mixing of hyphae with the same equivalent of both polyanion and polycation (see Fig. 1): (a) a few parts of polycations would bind on the surface of a hypha, the process of which should allow to gather the hyphae in part but not all; (b) such a gathering of the hyphae is tightly bunched with the PEC resulting from the remaining polycation when a polyanion was further added; (c) as a result, we may obtain filamentous fungi with partially bunched hyphae, the part of which is very stable and does not fall in pieces during the cultivation.

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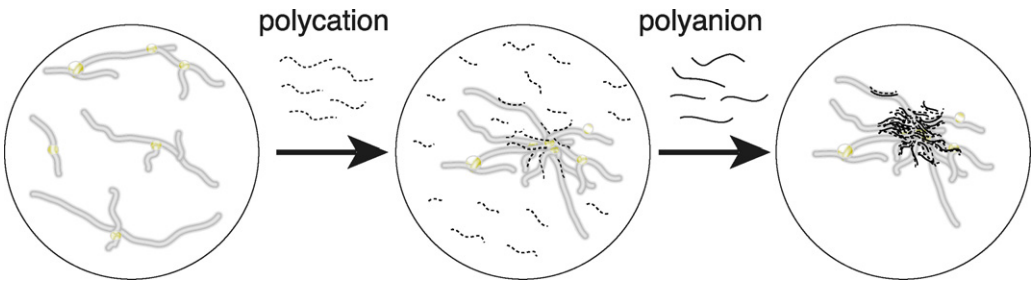

Fig. 1. Schematic illustration for the immobilization of filamentous fungi using polyelectrolyte complex based on our working hypothesis. Broken and solid line denote polycation and polyanion, respectively.

Taking into account this working hypothesis, here we attempted to study the gluconic acid production via a semi-large scale (1 l) cultivation of PEC-immobilized *Aspergillus niger* cells using a jar fermentor. The present paper reports the excellent sedimentation property and the fermentation performance under a high cell density, together with the morphology of the PEC-immobilized *A. niger* cells.

2. Experimental methods

2.1. Chemicals

Potassium poly(vinyl alcohol) sulfate (KPVS) and trimethylammonium glycol chitosan iodide (TGCI) were commercially obtained from Wako Pure Chemical Industries (Osaka, Japan). The physical quantities (as nominal data) of both polyelectrolytes are as follows. Equivalent weight (E_w) ∼162 for KPVS and ∼375 for TGCI; the degree of polymerization (DP) ∼1500 for KPVS and ∼400 for TGCI. Both polymers were dissolved in distilled water and their concentrations were given in equivalent per volume (eq/ml).

2.2. Medium

We used two media; a basal medium for growth of the mycelial organism and a fermentation medium for gluconic acid production. The composition (in g/l) of the former was: glucose, 30; yeast extract, 9; polypeptone, 15; $CaCO_3$, 4; pH 7. The latter composition (in g/l) was: glucose, 100, $MgSO_4{\cdot}7H_2O$, 0.15; KH_2PO_4, 0.2; Na_2HPO_4, 0.4; pH 6. Note that the nitrogen source was not added to the fermentation medium to suppress the cell growth during the gluconic acid production.

2.3. Strain and cultivation

A. niger IFO 31012 was used in this study. The sporulated culture on a malt agar slant in a 500 ml Erlenmeyer flask was mixed with 200 ml of the basal medium and carefully scraped off by a glass rod. The inoculum was transferred to a 500 ml Erlenmeyer flask and incubated at 30 °C for 2 days on a rotary shaker. The mycelial organism obtained by incubation was harvested by suction filtration and washed well with sterile distilled water.

2.4. Immobilization

The harvested microorganism (10 g by dry weight) was suspended into 600 ml of sterile distilled water. The immobilization was performed by addition of aqueous TGCI solution (100 ml; 130 meq/l), soon followed by the KPVS solution of the same volume and the same concentration to the mycelial suspension under stirring (500 rpm). These procedures were completed within 10 min because of a rapid formation rate of PEC. The PEC-immobilized *A. niger* cells were then separated by suction filtration and washed well with sterile distilled water. In the preparation of the immobilized samples for sedimentation measurements, different volumes of the above polyelectrolyte solutions were added to the same cell suspension as used above.

2.5. Morphological observations and sedimentation measurements

Morphology was studied by optical and electron microscopes. The specimens were prepared according to the same procedures as those described in our previous papers [10,11,19].

The sedimentation rate was determined as a function of the weight ratio of PEC to dry cells. The measurements were carried out using a graduated test tube fulfilled with distilled water. A few grams of the immobilized cell were dropped from the top of the test tube, and then the drop rate was measured between 5 and 15 cm from the top to the bottom. The measurements were repeated 10 times separately, and the eight data were averaged after the removal of the most fastest and slowest data.

2.6. Gluconic acid production

Different weights of the PEC-immobilized *A. niger* cells were incubated in 1 l of the fermentation medium at 30 °C under stirring (1000 rpm), using a computer-controlled jar fermentor as shown in Fig. 2. During the incubation, the pH of the fermentation broth was adjusted to ca. 6 with 8 N NaOH. Also adjusted by the flow of O_2 was the level (35 ± 3 mg/l) of dissolved oxygen (DO). The concentration of gluconic acid was determined by a HPLC apparatus (Shimazu model HIC-6A) equipped with a UV monitor (Shimazu model SPD-6AV). A column packed with TSK gel SCX(H+) (Tosoh Co., Tokyo, Japan) was employed. The glucose concentration was measured using a Wako Glucose CII-Test kit (Wako Pure Chemical Industries).