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# Zirconia microbial hollow fibre bioreactor for *Escherichia coli* culture

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**Abstract**

Porous ceramic hollow fibre membranes can be used as bioreactors for microbe immobilization and growth. In this work, zirconia ceramic hollow fibre membranes were synthesised from 80 nm zirconia particles using a combined phase inversion and sintering technique The resulting membranes were characterised using SEM and XRD and tested for *Escherichia coli* immobilization. The membrane structural evolution with controlled *E. coli* growth was investigated. The well-maintained *E. coli* growth clearly shows that not only the hollow fibre lumen but also these micro-channels inside the fibre wall can be used as micro-bioreactors. Due to the unique structure properties, the prepared $ZrO_2$ hollow fibre membranes can find wide applications in microbe immobilization system.


*Keywords:* Hollow fibre; Zirconia; *E. coli*; Bio-ceramic membrane; Macro-encapsulation; Protein delivery

## 1. Introduction

Gram-negative *Escherichia coli* (*E. coli*) is one of the most commonly used prokaryotic hosts for recombinant DNA due to its capability for rapid growth, well documented genetic background and high yield of recombinant proteins [1]. The culture of genetically modified *E. coli* has also been actively studied for the production of heterologous proteins, microbial electricity and biomass energy [2–4]. There are several methods to cultivate *E. coli* in bulk. Conventional suspension systems generally consist of chemostat or other suspended growth-based biotreatment, but require subsequent separation steps. Alternatively, to increase cell reutilization and eliminate the need for expensive product recovery and purification processes a cell immobilization method is preferred. By avoiding the interference caused by fluid motion, this method provides an improved environment for maintained high cell activity. Cell immobilization can occur in two ways: surface immobilization or entrapment within specially designed microspaces. These immobilized microbial cells can be used extensively in both industrial applications and various scientific studies [5].

Among the different technologies for cell entrapping, membrane bioreactors have generated particular interest [6–11]. The membranes can be designed into a number of different geometries including flat plates, tubular or annular configurations [12]. Compared to other geometries, hollow fibre membranes provide many advantages including the highest surface area to volume ratios, the ability to act as a barrier against particle contamination and their high efficiency at retaining targeted microorganisms with reduced levels of resistance or by-product generation [13]. Bio-membrane reactors have been reported to provide a 40-fold increase in ethanol production by *Saccharomyces cerevisiae* (budding yeast) in comparison with batch operation [14].

In biological applications, mechanical stress from continued growth of bacteria and thermal stress caused during the fermentation process are usually encountered. Traditional polymeric hollow fibres suffer from problems of low solvent resistance and membrane rupture/distortion due to their poor mechanical strength [15–18]. Ceramics are promising alternatives owing to their increased structural, thermal and chemical stability, providing a material with high mechanical strength, toughness and unlimited autoclavability.

Based on the polymer phase inversion technique, inorganic hollow fibre precursors containing inorganic powder and the organic binder (polymer) can be prepared from the inorganic–organic mixture at room temperature using less expensive

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extruding equipments [19]. Depending on the final application, these fibre precursors can be sintered into fully densified or porous structure by adjusting the sintering programme. Zirconia hollow fibres have recently been developed with excellent mechanical strength reducing the problems of membrane rupture in cell immunoisolation [20–22]. However, different cell system poses different requirements on the membrane being employed as cell size and shapes are quite varied depending on the species.

In this work, we report the development of zirconia ceramic hollow fibres with unique properties to be applied as immobilized microbial cell reactors. The microstructure evolution of hollow fibre membrane bioreactor with controlled microbe (*E. coli*) growth was carefully investigated. This work is the preliminary results of our project with the ultimate goal of realizing protein or biofuel production from microbes in ceramic hollow fibre membrane reactors.

## 2. Experimental

### 2.1. Chemicals

$Y_2O_3$ (3 mol%) partially stabilized $ZrO_2$ powder with particle size of 80 nm [Shandong, China], N-methyl-2-pyrrolidone (NMP) [Synthesis Grade, Merck] and polyethersulfone (PESf) [Radel A-300, Ameco Performance, USA] were used as the raw materials to prepare the green hollow fibres. Untreated tap water and distilled water were used as the internal and external coagulants, respectively. Standard formulations of Bacto™ Tryptic Soy Broth (DIFCO, 17.0 g/l pancreatic digest of casein, 3.0 g/l enzymatic digest of soybean meal, 5.0 g/l sodium chloride, 2.5 g/l dipotassium phosphate, 2.5 g/l dextrose), both in analytical and biochemical grade, were used as the culture media and cell system, respectively. *E. coli* (ATCC25922) was stored at −80 °C after growth and subsequently transferred to fresh TSB before preparation of inoculum.

### 2.2. Preparation of $ZrO_2$ hollow fibre membranes

The $ZrO_2$–organic mixture was produced using 40% (wt) NMP, 10% PESf and 50% $ZrO_2$ and was thoroughly stirred to ensure uniform distribution of the $ZrO_2$ particles. The ceramic hollow fibre precursor containing $ZrO_2$ and PESf was extruded through a tube-in-orifice spinneret using a procedure described elsewhere [21]. The dried hollow fibre precursors were subsequently sintered at 1200 and 1400 °C for 10 h, respectively, in air atmosphere to form porous $ZrO_2$ ceramic hollow fibre membranes with respective bending strength of 49 and 220 MPa [21].

### 2.3. Bioreactor experiment

Short hollow fibres with length of 20 mm were sterilized at 120 °C and sealed on one end using zinc phosphate cement. The sterilized hollow fibres were immersed inside the autoclaved distilled water and Tryptic Soy Broth (TSB) both for 1 day. Ten microlitre cultures (produced fresh overnight) of *E. coli* were inoculated into 10 ml TSB and cultured until reaching mid-logarithmic-phase, ready for the hollow fibre encapsulation test. 100 μL *E. coli* (with approximately 0.1 optical density, reading at 600 nm) was injected inside the hollow fibre lumen and sealed on one side using zinc phosphate cement. $ZrO_2$ hollow fibres with embedded *E. coli* were then vertically suspended inside the soy broth with the sealed end just above the surface. Finally, the fibre sample was incubated at 37 °C with shaking (100 rpm) for 2, 6 and 16 h. *E. coli* viability and population inside the hollow fibres was carefully observed at various culture times.

### 2.4. Characterisation

Structures of $ZrO_2$ hollow fibre structures were examined using a scanning electron microscope (SEM, JEOL JSM-7400F and LEO 1550 VP field emission). After *E. coli* growth, the cells and hollow fibres were washed with 1× PBS three times then fixed in Formalin solution (10% neutral buffered, contains formaldehyde 4% w/v, Sigma–Aldrich) overnight. They were further washed with distilled water before being dehydrated using a series of ethanol washes and dried in a critical point dryer (Autosamdri-815, Tousimis Research Corporation, USA) and mounted onto aluminum stubs. The samples were coated with platinum prior to SEM observation.

## 3. Results and discussion

### 3.1. Preparation of zirconia ceramic hollow fibre membranes

Preparation of the appropriate hollow fibre precursor is the prerequisite for the successful application of this technology. Our experience indicates that the weight ratio of the inorganic particles to polymer for the fibre precursor should be arranged from 3 to 12 depending on the particle size and individual material density. Usually, the fibre precursor is more easily extruded using inorganic particles of higher density and larger particle sizes (0.1–50 μm) due to the improved flow characteristics. It is extremely difficult to prepare fibre precursors using pure nano-sized particles as the starting powder. For example, based on an alumina particle size of 10 nm, the hollow fibre precursor could not be made as it was impossible to achieve a homogenous alumina/polymer solution. This was in part due to the increase in slurry viscosity at above 10% (by weight). By contrast, the homogenous zirconia slurry appropriate for spinning can be prepared as zirconia has a higher density (6.0 g/cm$^3$) and a larger particle size (80 nm as observed from SEM in Fig. 1a) compared to that of alumina (density of 4.0 g/cm$^3$ particle size of 10 nm). Inorganic nano-particles with a particle size less of than 10 nm can be applied for many other advanced applications. As such nano-particles cannot be used in the fields of green ceramic extruding or casting because of its low particle loading density unless the addition of these nano-particles is limited to a few percent only.