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REVIEW

Preparation and Properties of Porous Materials for Biotechnology by the Sol-Gel Process

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1. INTRODUCTION

Most of chemical reactions in biological systems occur in the presence of catalysts called 'enzyme'. All enzymes are characterized by its catalytic power and specificity. Thus enzymes are useful for production and analysis of various materials.

Microbes include many kind of enzyme in their cells and can produce various useful materials with multistep enzyme system. Thus, microbes have been applied for fermentation technology.

Generally, biological catalysts as enzymes and microbes exist in soluble state. In order to apply these biological catalysts for fermentation and analysis, insoluble catalysts are comfortable. Thus, immobilization techniques of biological catalysts on insoluble materials called carrier are required. Organic and inorganic materials are used as the carrier. The porous glass is mechanically and chemically stable and the enzyme covalently coupled on porous glass surface are highly stable. However, the immobilization process on porous glass requires complex surface treatment process.

Therefore, the new immobilization method with sol-gel process has been attempted. The application of sol-gel process for dispersing organic molecules is widely studied[1] and this method is also applicable for entrapment of biological catalysts (enzymes and microbes) in silica gels. The comparison of these processes are shown in Fig. 1.

In this paper, the applications of sol-gel process for immobilization of enzymes, proteins and microbes are reviewed.

Following abbreviations are used.

Tetramethoxysilane: TMOS
Tetraethoxysilane: TEOS
Polyethylene glycol: PEG
Glucose oxidase: GOD
Peroxidase: POD
Bacteriorhodopsin: bR

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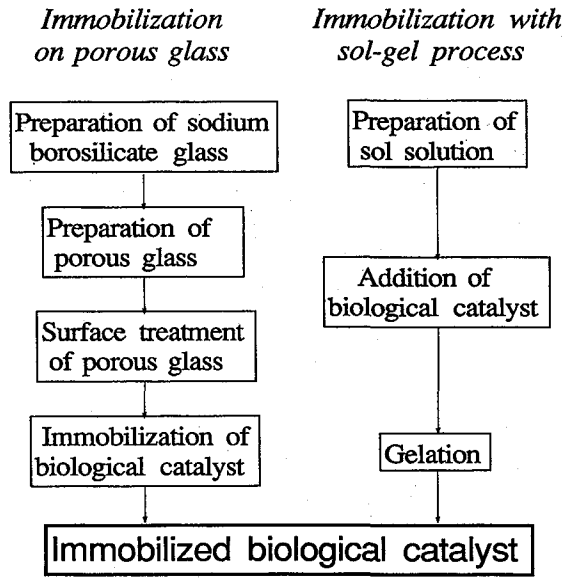

Fig. 1. Immobilization process of biological catalysts on porous glass and in gels with sol-gel process.

2. PREPARATION OF POROUS GELS WITH SOL-GEL PROCESS

On the application the sol-gel process for immobilization of biological catalysts, the porous structure of obtained silica gels may affect on the catalytic property. Generally, the size of enzymes are several nanometers and that of the microbes are several micrometers. The pore diameter of gels should be smaller than the size of entrapped biological catalysts. However, larger pores is favorable for the diffusion of substrates and products through pores. Thus, the porous structures of gels should be controlled to be optimum for entrapped biological catalysts. The gels prepared from general sol-gel method includes micropores smaller than several nanometers and Hench *et al.*[2,3] reported the method to enlarging these micro pores up to 10 nm. Considering the pore size, such gels are suitable for immobilization of enzymes.

The methods to prepare the porous gels contains macro-pores were reported as follows. Kozuka *et al.*[4] reported that the porous gels with macro-pores was obtained by high concentration of HCl containing TMOS mixture. The pore diameter was several micrometers and that was controlled by the composition of starting solution and the kind of silicon alkoxide. Nakanishi *et al.* reported the othermethods to prepare porous silica gel with sol-gel process from silicon alkoxide-organic polymer solution such as TEOS-polyacrylic acid (HPAA),[5] TMOS-poly (sodium stylenesulfonate) (NaPSS),[6] TEOS-polyethyleneglycol (PEG)[7] solution. In these investigations, the porous structures of gels were controlled ranging from micrometer to nanometer by changing the composition of starting solution and the kind of polymers.

Considering the pore size of porous gels and toxicity of starting materials for microbes, silicon alkoxide-organic polymer solution is suitable for the immobilization of microbes with sol-gel process.

3. IMMOBILIZATION OF ENZYMES ON POROUS GELS

The technique of enzyme entrapment in organic gels are widely studied.[8] In this method, enzyme is entrapped by polymerization process of organic gels such as poly (acrylamide). The same method is applicable for enzyme entrapment in silica gel by the sol-gel process.

Table 1. Enzymes immobilized in gels with sol-gel process

Enzymes	Properties and applications	References
Glucose Oxidase (E.C.1.1.3.4)	activity,glucose sensor glucose sensor activity	9 10, 11 12
Peroxidase (E.C.1.11.1.7)	glucose sensor	9, 10, 11
Superoxide dismutase (E.C.1.15.1.1)	optical property	13
Alkaline Phosphatase (E.C.3.1.3.1)	activity	14
Acid Phosphatase (E.C.3.1.3.2)	activity stability	15
Trypsin (E.C.3.4.21.4)	activity	10, 15

Table 1 shows the enzymes have been immobilized by sol-gel process. The first study was carried by Braun *et al.*[14] for alkaline phosphatase (ALP). In this study, aqueous solution of ALP was added into a mixture of TMOS, methanol and NaOH, and the mixture left for polymerization. By this way ALP was successfully immobilized in silica gel and various properties were investigated. The relative activity of immobilized ALP was 28% of soluble ALP and stability at 70°C was slightly increased by the immobilization in gels.

Shtelzer *et al.*[15] reported about the properties of immobilized trypsin and acid phosphatase. The authors added PEG and NaF in a starting solution containing TMOS and the effect of PEG and NaF on enzyme activity was reported. The relative activity of trypsin immobilized in silica gels and PEG containing gels were 21% and 47%, respectively. The activity of trypsin was increased with adding PEG. The stability of immobilized trypsin and acid phosphatase was increased with immobilization and NaF addition was effective to stabilize the immobilized acid phosphatase.

Audebert *et al.*[12] investigated the effect of matrices on activity of immobilized enzyme. The authors immobilized GOD in three type of gels as follows; (1) TMOS derived gel (2) silicon tetraacetate gel (3) gel from colloidal silica. The catalyticactivity of each gels were determined by cyclic voltammetry and activity was detected TMOS gel and colloidal silica.

The transparency of silica gels derived from sol-gel process are suitable forsome optical measurements. Yamanaka *et al.*,[9] Braun *et al.*[10] and Avnir *et al.*[11] prepared the optical glucose sensor. In these investigations, GOD and POD was co-immobilized in gels. The two enzymes catalyze following reactions:

$$\text{GOD: glucose} + O_2 = \text{gluconolactone} + H_2O_2$$
$$\text{POD: donor} + H_2O_2 = \text{oxidized donor} + H_2O$$

The organic dyes (3-methyl-benzothiazolinone or 4-aminoantipyrine) were used as the donor and

their oxidation are easily detected by optical absorption spectrometry. Thus, the glucose concentration could be estimated by the changes in optical absorption by the enzymatic reaction. Braun *et al.*[10] demonstrated the quantitation of glucose ranging from 10 mM to 100 mM by this method.

As the studies about the immobilization of other proteins, Ellerby *et al.*[13] immobilized the superoxide dismutase, cytochrome c and myoglobin in gels and investigated their absorption spectra. These proteins contains metallic ions and their absorption spectra may change by various conditions. The immobilized superoxide dismutase was demetallated and remetallated. The immobilized cytochrome c and myoglobin were reduced and reoxidized. The change in absorption spectra of gels resulting from these reversible reactions were similar to that of each proteins in a solution. This result shows that the proteins were entrapped in gels without structural change from their soluble state.

Wu *et al.*[16] reported about the optical property of immobilized bacteriorhodopsin. Bacteriorhodopsin (bR) is found in the cell membrane of halophilic bacteria and it pumps protons across the membrane by the photocycle with light absorbtion. In their investigation, bR was immobilized in silica gels and lifetimes of intermediates in the photocycle were determined. The authors suggested that the optically transparent and bR containing gels are applicable for optical imaging and optically based ion sensor.

4. IMMOBILIZATION OF MICROBES IN POROUS GELS

The immobilization of microbes in gels is well studied by using the organic gels.[17] The yeast is typical useful microbial for fermentation technology and the immobilized growing yeast cells are usually prepared with organic carriers such as calcium alginate gels[18] or carrageenan.[19] In these cases, yeast cells are mixed with the precursor of gels and yeast cells are contained in carriers after gelation. Although such methods are convenient for immobilization, the mechanical strength and chemical durability of organic carriers are inferior to inorganic carriers such as porous glasses. If the sol-gel process is applicable for immobilization of microbes, mechanically and chemically stable immobilized microbes will be easily prepared.

However, there are some difficulties resulting from the toxicity of raw materials such as silicon alkoxide. Thus, only two studies were carried about this immobilization method.

Carturan *et al.*[20] investigated the method to immobilize yeast cells in silica gel with sol-gel process. In this investigation, suspension of yeast cells *(Saccharomyces cerevisiae)* was added to TEOS-ethanol solution and yeast cells were entrapped in silica thin layer prepared by dip-coating on the glass sheet. Thickness of this silica thin layer was 0.2 mm and this is thinner than the diameter of yeast cells (10 mm). Thus, the authors considered the yeast cells were anchored on the glass surface by gel layer and cells contact with outer solution. In this gel layer, yeast cells were not growing, however invertase in immobilized yeast cells indicated 28% of relative activity compared with that in free yeast cells.

The present authors modified Carturan's process and prepared immobilized growing yeast cells.[21] In this investigation, spores of yeast was used for immobilization because the spores are durable to organic solvents. The yeast spore suspension was added to the mixture of TMOS, PEG, methanol and water. PEG was added to prepare the porous gels. After gelation, gels were crashed and soaked in sterilized water to remove PEG and yeast immobilized gels were

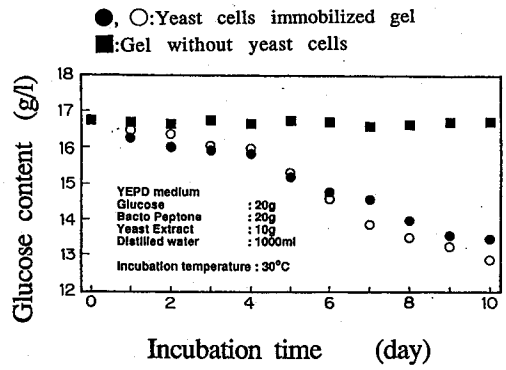

Fig. 2. Glucose consumption of immobilized yeast cells.

obtained. The gels were cultivated in YEPD medium and the activity of immobilized yeast cells was detected by glucose consumption. As shown in Fig. 2, the glucose concentration in YEPD media were changed by the cultivation of immobilized yeast cells and the growth of yeast cells in gels was observed. Thus, the immobilized growing yeast cells were successfully prepared by the sol-gel process.

5. CONCLUSIONS

As mentioned above, various enzymes, proteins and microbes are already successfully immobilized with sol-gel process. By applying the sol-gel process, various bioactive materials are easily immobilized in gels and the obtained gels are optically transparent and mechanically and chemically tough. Thus, development of new type of materials containing bioactive materials are hopeful.

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