Rapid Communication

(Received November 19, 1986)

**IMMOBILIZATION OF MICROORGANISMS
ON POLY(VINYL ALCOHOL) SUPER FINE FIBERS**

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Abstract

Aminated poly(vinyl alcohol) (PVA) super fine fibers (SFF) immobilized a lot of baker's yeast, but sulfonated SFF did not immobilize it at all. It was observed by scanning electron microscope that the yeast cells immobilized on SFF proliferated enormously, after it was immersed in glucose solution. The yeast-SFF complex charged into a glass column converted glucose to ethanol continuously.

1. Introduction

Immobilization of microorganisms and/or cells has been attracting special interest recently. This is partly because animal cell culture requires the attachment of the cell on a solid surface. Another reason is that a high productivity is expected by the immobilization because immobilized microorganisms are really localized in a limited space which leads to extremely high loading density of microorganism.

Microorganisms not only produce useful substances but also biodegrade various substances which lead to environmental protection. According to the report published by OECD¹⁾, biotechnology is expected to play a great role in wastewater treatment. Immobilized microorganisms are being applied to this field²⁻⁵⁾.

Poly(vinyl alcohol) super fine fibers (SFF) developed by us have been so far applied only for enzyme immobilization. Therefore, a first attempt to immobilize yeast on the SFF was made. Yeast has been used for alcohol fermentation for a long time and the properties of it have been well studied. Moreover, alcohol production with immobilized yeast cells is being widely studied in the world⁶⁻¹⁸⁾. But, in most cases, yeast cells

were entrapped in polymer gel such as calcium alginate, κ -carrageenan *etc.*

In this study, baker's yeast was ionically adsorbed on the SFF and the continuous alcohol production was attempted.

2. Experimental

Wet baker's yeast was dispersed in distilled water. The liquid was diluted to various concentration. The liquids with various concentration were dried in a vacuum drying oven and weighed. A linear relationship does exist between the dilution ratio and the weight of dried yeast. SFF was put in the liquid under agitation. A part of the liquid was dried and weighed before and after adsorption. The SFF sample immobilizing yeast was freeze-dried and then weighed. The same sample was observed by scanning electron microscope (Hitachi S-500A). The adsorbed yeast was determined from the weight difference.

The glass column (inner diameter; 1 cm, length; 10 cm) of Amicon Co. was used for continuous alcohol production. A dimethylaminated SFF (D-SFF) sample immobilizing about 150 mg of dry yeast was charged into the column and 20 wt% of glucose solution with extracted yeast, which was adjusted to pH 4.5 by phosphorous acid, was

continuously supplied to it at about 0.5 ml/min. The column and the glucose solution were kept at 37°C. The effluent was analyzed by high performance liquid chromatography (HPLC). The HPLC consisted of a refractometer (Shodex RI SE-51) of Showa Denko Co., a column (#2618) of Hitachi Seisakusyo Co. and a pump (655 liquid chromatograph) of Hitachi Seisakusyo Co.

3. Results and discussion

The amounts of yeast adsorbed on three kinds of SFF, dimethylaminated SFF (D-SFF), trimethylaminated SFF (T-SFF) and sulfonated SFF (S-SFF), were compared. Amount of adsorbed yeast was determined by weighing both dried SFF samples and dried yeast solutions before and after an adsorption experiment. The results are listed in Table 1. It was found that baker's yeast was adsorbed on D- and T-SFF. Fig. 1 shows the

scanning electron micrographs of three kinds of SFF samples after an adsorption experiment. No yeast is found on S-SFF surface, while a lot of yeast cells are observed on D- and T-SFF surfaces. Most of microorganisms dispersed in water have a net negative charge on their surfaces. The charge is thought to be produced by the dissociation of carboxylic and phosphonic acids in cell membranes¹⁹⁾. Morisaki *et al.*¹⁹⁾ stated that strongly basic anionic resins had positive charges on their surfaces and were able to adsorb cells more than 10^{10} by electrostatic force of attraction. Krug *et al.*¹²⁾ also examined the ability of various ion exchange resins for adsorbing the cells of *Zymomonas mobilis* and concluded that macroreticular, anionic resins were the good materials for adsorbing the microorganism. Therefore, the repulsion between negative charges on the yeast cells and S-SFF would be a main reason why yeast was not bound on S-SFF.

Fig. 2 shows the adsorption isotherm for baker's yeast and T-SFF. One gram of the SFF sample immobilized about 200 mg of dry yeast and was expected to immobilize more yeast. Daugulis *et al.* stated that yeast cells less than 130 mg and *Z. mobilis* cells less than 50 mg were adsorbed on a gram of ion exchange resin^{6,12)}. Compared with their results, T-SFF has a higher capability to ionically adsorb microorganisms on its surface.

Table 1 Amount of baker's yeast adsorbed on three kinds of SFF samples

	D-SFF	T-SFF	S-SFF
Weight of SFF [mg]	114	153	115
Adsorbed yeast ¹⁾ [mg]	23	31	—
Adsorbed yeast ²⁾ [mg]	21	33	—

1) The values in this column were determined by the weight of dried SFF samples.

2) The values in this column were determined by the weight of dried yeast in solution.

Fig. 1 Scanning electron micrographs of baker's yeast immobilized on three kinds of PVA super fine fibers (SFF)

- (a) dimethylaminated SFF
- (b) trimethylaminated SFF
- (c) sulfonated SFF

Fig. 2 Adsorption isotherm for baker's yeast

Fig. 3 Scanning electron micrographs of immobilized baker's yeast
(a) before proliferation
(b) after proliferation

After a small amount of yeast was immobilized on D-SFF, a part of the sample was observed through a scanning electron microscope (see (a) of Fig. 3). Another part of the sample was incubated in glucose solution at 37°C for 2 d and was then observed in the same way as described above (see (b) of Fig. 3). The growth of the immobilized yeast is recognized. That is a very encouraging result because SFF may have a potential to be applied to other cell culture.

The D-SFF-yeast complex was charged in a glass column and glucose solution was continuously fed in it. Fig. 4 shows the time course of alcohol production. About 1 wt% of ethanol flowed out of the column, while flow rate was kept at about 0.5 ml/min during the whole period. The productivity was over 0.3 g-ethanol/l·min at 3.82 h⁻¹ of the space velocity, although the value itself was not so high.

From these results it is concluded that aminated SFF is a really promising support for immobilizing baker's yeast ionically.

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Fig. 4 Variation of ethanol concentration and flow rate with elapsed time
○ ethanol concentration
● flow rate

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ポリビニルアルコール極細繊維(SFF)による微生物の固定化

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秤量法及び電子顕微鏡を用いた観察より、アミノ化ポリビニルアルコール極細繊維(SFF)は多量のパン酵母を吸着固定するが、スルホ化 SFF は全く吸着しない事がわかった。この結果は、多くの微生物が負の電荷を帯びているという説とも一致する。また、アミノ化 SFF

に吸着されたパン酵母をグルコース溶液に浸漬したところ、酵母が大量に増殖する事も観察された。

更に、アミノ化 SFF 固定化酵母をガラスカラムに充填してアルコールの連続発酵を試みたところ約1wt%エタノール溶液が流出した。