PL-1

Recovery of Intracellular Biomaterials from the Suspension of Lysed or Disintegrated Yeast by Membranes

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1. Introduction

Many useful biomaterials like enzymes are contained in yeast cells. However, the release of these intracellular biomaterials from the cells is required to recover them with hot water, solvent[1] or various cell breakage methods of mechanical[2] or non mechanical ones. The cell lysis or breakage of yeast is usually made by solvent like ethyl acetate and mechanical disintegration with high pressure homogenizer[3] or agitating beads mill[2] . The separation of cell debris (i.e. solid liquid separation) is done by centrifuge or membrane depending on the recovery conditions. The features of both separation methods are shown in Tables 1 and 2 [4] As it is often difficult to obtain a clear supernatant by centrifuge from the suspension containing cell debris, the membrane separation is also often used to get a clear supernatant.

In this report we introduce the several applications of membrane separation to separate the cell debris of yeast disintegrated chemically or mechanically and to recover the intracellular biomaterials.

Table 1 Advantage disadvantage of centrifuges

Advantages
· compact design · capability of continuous operation · good biological containment · short retention time · ease of separation efficiency control · relative economy at large scale
Disadvantages
· high capital and maintenance costs · harsh treatment of biological molecules and cells · significant rise in temperature at low flow rates · often less than 90% solids recovery · cleaning and sterilisation problems · unsuitability for separations where solids form compact sediments

Table 2 Advantage disadvantage of crossflow filtration

Advantages
· potentially 100% recovery of solids · product washing is simple · biological containment is good · temperature control is simple · batch, single pass, feed/bleed or multistage modes are possible · capacity can easily be increased by adding further modules
Disadvantage
· permeate flux is time dependant · high membrane replacement and pumping costs · high retentate residence time · limited control over separation performance · uneconomic at large scale · often unacceptably low solute transmission · possible loss of biological activity of product

2. Recovery of ADH from the lysed yeast by lytic chemicals.

Most of enzymes in yeast cells and tasty ingredient like yeast extract are obtained by cell lysis with ethyl acetate. The recovery of alcohol dehydrogenase (ADH, EC 1.1.1.1, MW 148000, pI-5.6) with microfiltration(MF) membrane was tried from the suspension containing yeast cells lysed with ethyl acetate. The lytic method is shown in Fig.1. In Fig.2 is shown the apparatus of membrane separation system with the device of backwashing. The membrane module is a thin channel flow type of cross flow filtration (CFF). The washing of membrane was performed by the following three methods:[5]

(1) Stop of feed pump(abbreviated as SP): The feed pump was stopped periodically.

(2) External pressure(filtrate)(EPF): The membrane was backwashed periodically with the filtrate, which was forced into the membrane by compressed N_2 gas of 0.1MPa.

(3) Reverse motion of feed pump(RMP): The membrane was backwashed periodically by feeding the filtrate into the feed side by making the motion of pump reverse.

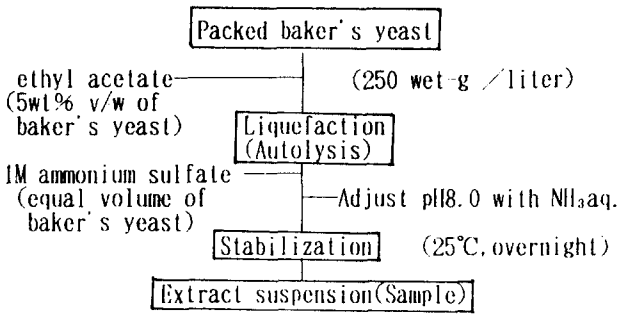
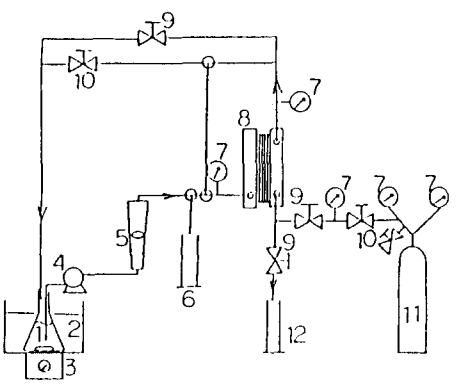


Fig.1 Extraction method of intercellular enzymes in yeast cell



1. Sample (Suspension of autolysed yeast)
2. Constant temperature bath
3. Magnetic stirrer
4. Feed pump (peristalic pump)
5. Flowmater
6. Sampling cylinder
7. Pressure guage
8. Membrane module
9. Solenoide valve
10. Pressure control valve
11. Nitorogen gas
12. Filtrate receiver

Fig.2 Schematic view of experimental apparatus for membrane separation system with backwashing

The time course of permeate flux is shown in Fig.3 using the MF membrane (regenerated cellulose)of 0.4μm pore size. The filtration mode is total recirculation one. This fig. indicates that the backwashing methods of EPF and RMP are effective to keep a high permeate flux. Fig.4 shows the influence of pH of suspension on the permeate flux. The flux in the membrane of 0.4μm pore size hardly depended on the pH of suspension. On the other hand the flux in the membranes having above 0.7 μm of pore size decreased with the increase in the pH value. The reason why the influence of pH on flux depends on the membrane pore size is not clear.

washing mode	operation condition (washing / filtration)	key
no washing	——	○
EPF(backwashing)	5 s / 5 min	◑
	5 s / 2 min	●
RMP(backwashing)	5 s / 2 min	○
SP(stop of pump)	5 s / 2 min	●

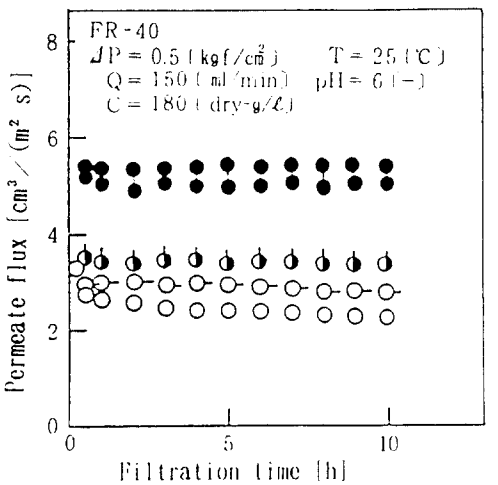


Fig. 3 Time coure of permeate flux depending on membrane washing methods

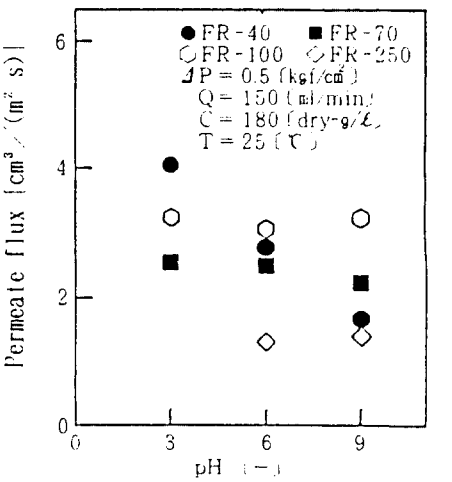


Fig. 4 Effect of pH of suspension copntaining autolysed yeast cells on permeate flux

3. Recovery of ADH from the disintegrated yeast by combining centrifuge with MF membrane.

It is well known that the high M.W. materials like protein or even low M.W. one like saccharides were retained by MF membrane when the suspension contains high M.W. materials and colloidal materials like cell debris owing to the formation of dynamic(secondary) membrane. Then, we investigated the effect of pretreatment of suspension containing cell debris by centrifuge on the performance of membrane filtration (flux and transmissivity of ADH).

The baker's yeast suspended in RO water(the conc. of yeast = 25 wet wt%) was disintegrated with Dyno mill(agitating beads mill). The suspension was at first separated by centrifuge, and then the supernatant was filtrated with the MF membrane(cellulose triacatate) of a 0.22 μm pore size(FM22).

The filtration mode was continuous concentration by CFF. The time course of flux ratio ($=J/J_0$) and volume concentration factor(X) is shown in Fig. 5. And the relationship between apparant transmissivity of ADH ($=C_P/C_{F0}$)and filtration time is also shown in Fig. 6. Wher J_0, C_P, C_{F0} are initial flux of virgin membrane, ADH activity in permeate and that in feed suspension, respectively. The activity of ADH and amount of disolved protein in feed suspension was about 270 IU/$m\ell$ and 20 mg/$m\ell$, respectively.

It is seen in Fig. 5 that the flux ratio gradually decreased with the increase in filtration time(or in solid concentration) and that the effect of pretreatment by centrifuge was not observed when the rotating speed of rotor of centrifuge N was below 12000. However, we can say from the results in Fig. 6 that the effect of pretreatment on the transmissivity of ADH was considerably confirmed. Fig. 7 shows the relationship between N and amount of total solid including disolved materials(TS) and suspended solid without disolved mate

rials(SS). It is concluded from these figures that the flux is not determined only by the amount of SS, but by percolation of dissolved material of high M.W. with deposited cake besides the amount of cake. The transmissivity of enzyme is mainly determined by the amount of SS.

Pretreatment condition with centrifuge

key	N[rpm]	acceleration[G]
○	0	0
△	2000	300
□	12000	11000
◇	24000	45000

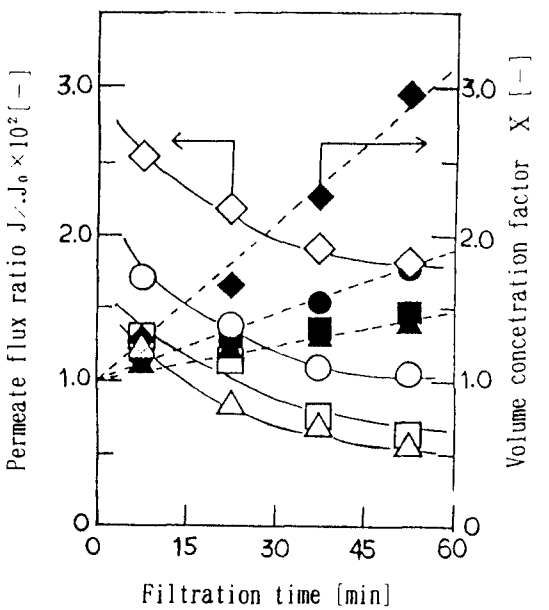


Fig. 5 Relationship among filtration time, premeate flux ratio and volume concentration factor

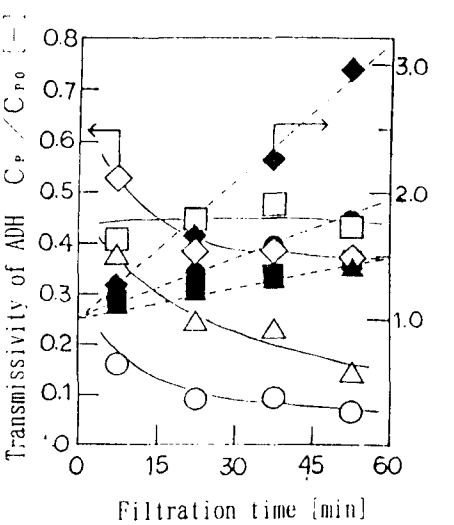


Fig. 6 Relationship between the filtration time and the transmissivity of ADH.

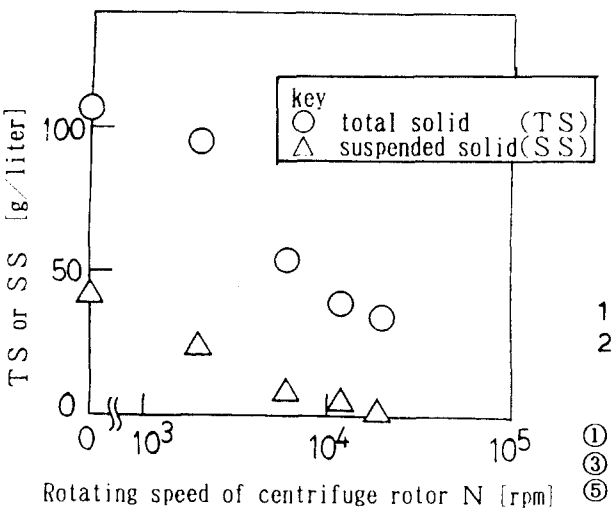


Fig. 7 Effect of rotating speed of centrifuge rotor on the amount of total solid (TS) and suspended solid(SS)

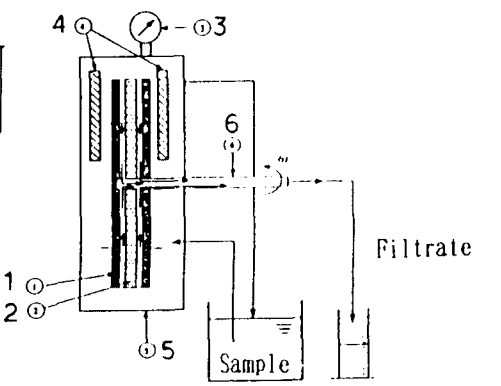


Fig. 8 Schematic view of rotating membrane system

4. Recovery of glutathione from disintegrated yeast with rotating UF and MF membranes.[1]

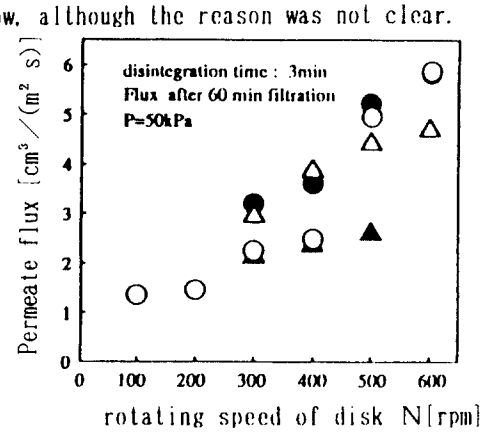
Glutathione(M.W. 307)has conventionally been prepared from the yeast by hot water extraction. However, hot water extraction has the following problems:①the complete recovery from the yeast is not attained, ②the utilization of residual cell is difficult owing to an inactivation of enzymes in the cell. These problems could be improved by releasing the glutathione from the yeast by disintegration with agitating beads mill.[2] The cell debris was tried to separate with rotating UF and MF membrane module shown in Fig. 8(Hitachi Plant Construction Co., Ltd). The effect of operation factors like rotating speed of membrane disk on the permeate flux and rejection of glutathione and co existing protein were investigated. The glutathione was assayed by DTNB reagent.

The suspension containing of yeast of which concentration was 250 wet g / liter was disintegrated by Dyno mill. Although the filtration performance depends on the disintegration time, the following results were obtained under the condition that the disintegration time was fixed as 3 minutes. Fig. 9 summarized the relationship between rotating speed of disk of UF and MF membranes and flux. In any membrane the flux increased with the increase in rotating speed of disk. However, the increasing tendency depended on membrane type. Fig. 10 shows the time courses of flux, yeast concentration and viscosity of slurry when UF membrane was used, and that there is a good correlation among these values. That is, the flux decreased with the increase in yeast concentration, that is, in viscosity of suspension. In Table 3 is shown the average rejection of glutathione and and protein calculated from Eq. (1)

$$(C_0 V_0 - C_P V_P) / C_0 V_F = X^R \qquad (1)$$

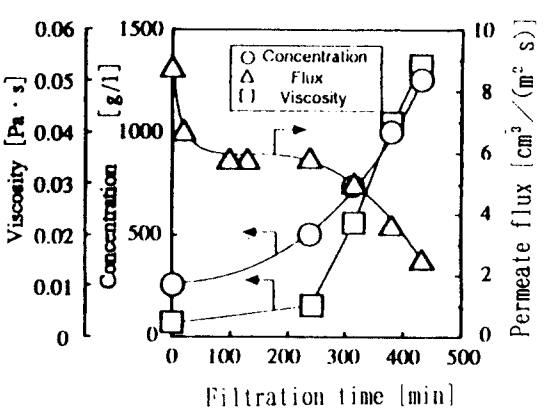
where C_0 : initial concentration
C_P : permeate concentration
V_0 : initial volume of suspension
V_P : volume of permeate
V_F : volume of concentrate
R : rejection
X : volume concentration factor

The rejection of glutathione and protein in Teflon MF membrane of 0.1 μm was especially low, although the reason was not clear.



●UF (M.W.C.O. = 50 000, Polysulfone memb.)
○UF (M.W.C.O. =750 000, Polysulfone memb.)
△MF (pore size= 0.2μm Alumina memb.)
▲MF (pore size= 0.1μm Teflon memb.)

Fig. 9 Relationship between rotating speed of disk and flux.



disintegration time : 3min
filtration pressure : 50kPa
rotating speed : 500rpm
UF memb. (M.W.C.O. =750 000, Polysulfone)

Fig. 10 Relationship among flux, yeast conc. and viscosity of suspension

Table 2 Rejection of glutathione and protein

Membrane type	Rejection of glutathione[-]	Rejection of protein[-]
UF[Polysulfone] (M.W.C.O.= 50 000)	0.25	0.99
UF[Polysulfone] (M.W.C.O.=750 000)	0.30	0.96
MF[Alumina] (pore size=0.2μm)	0.27	0.98
MF[Teflon] (pore size=0.1μm)	0.11	0.68

5. Future prospect

Application of membrane for bioseparation would be in future spreaded and expanded to wider field. Many good books on the membrane application to bioprocessing have been published.[7,8] The bioprocessing where a lot of MF or UF membranes would be employed are the separation of cell or cell debris and the sterilisation. In many field the centrifugal separation is employed. However, the separation of cell in viscous suspension and cell debris containing colloidal particles by centrifuge is said to be difficult. Recently ceramic membranes are used for these bioprocessing, especially in pharmaceuticals. There are many problems such as fauling which should be solved to replace the centrifuge by membranes. So we should make an effect to solve these problems, produce new membrane material and develop the sophisticated membrane modules, devices and separation systems.

(Acknowledgement)

I appreciate Prof. Dr. Tae-moon Tak to invite me and have a chance to give my lecture in the Annual Meeting of The Membrane Society of Korea.

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