Development of Poly(vinyl alcohol) Hydrogel for Waste Water Cleaning. I. Study of Poly(vinyl alcohol) Gel as a Carrier for Immobilizing Microorganisms

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SYNOPSIS

The structures and properties of poly(vinyl alcohol) (PVA) hydrogel prepared by the freezing and thawing method and poly(ethylene glycol) and polyacrylamide hydrogels prepared by polymerization were compared in view of immobilizing microorganisms. Observation in a scanning electron microscope and measurement of the physicochemical properties revealed that the frozen PVA gel is superior in water content and oxygen permeability than the other gels. The PVA hydrogel has a high tensile and folding strength and good handleability and processability. The durability against abrasion and chemical resistance of PVA gel were also studied. Activated sludge was immobilized and fixed on PVA gel particles and incubated. This gel had a treating capacity for synthetic sewage of about two to three times that with the standard activated sludge process. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

Poly(vinyl alcohol) (PVA) is known to give a rubberlike, elastic material upon gelation of its solution through freezing,[1-4] called PVA hydrogel. The gel has excellent mechanical strength and hence has been studied as a material comparable to biopolymers and used for medical purposes. The hydrogel was supposed to be useful as a carrier for immobilizing microorganisms for waste water treatment.[5]

The structure and properties of poly(ethylene glycol) (PEG) and polyacrylamide (PAAm) gels used for waste water treatment were studied in comparison with those of PVA gel.[6] This article first reports the properties of a PVA hydrogel prepared with freezing PVA solution. Second, it discusses preparation of stable spherical particles containing activated sludge through (1) freezing the PVA solution containing it and (2) the availability of the hydrogel for waste water treatment. PVA hydrogels can be prepared by crosslinking PVA with chemical reagents. However, since microorganisms are damaged during the reaction, the preparation methods are not useful. The biochemical oxygen demand (BOD) removing ability with immobilized microorganisms in PVA frozen gel is compared with that of the standard activated sludge process.

EXPERIMENTAL

Preparation of Hydrogels without Microorganisms

PVA Gel Sheets

A PVA with a degree of polymerization of 1700 and degree of saponification of 99.98 mol % (PVA–HC) was used. An aqueous PVA solution with a concentration of 8% was poured in a vat and frozen overnight at −20°C and thawed at room temperature. This procedure was repeated three times, and the obtained gel was washed thoroughly with distilled water.

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Poly(ethylene glycol) Gel

An aqueous solution with 20% polyethylene glycol dimethacrylate supplied by Shin–Nakamura Chemistry Inc. and 0.5% *N,N,N′, N′*-tetramethylethylenediamine/0.25% K2S08 as an initiator system was cast on a vat. Then a PEG gel sheet was obtained by polymerization at 20°C.

Polyacrylamide Gel

An aqueous solution with 18% acrylamide and 3% methylenebisacrylamide and 0.5% *N,N,N′,N′*-tetramethylethylenediamine/0.25% K2S08 as an initiator system was cast on a vat. Then a PAAm gel sheet was obtained by polymerization at 20°C.

Observation of Gel Structures in Scanning Electron Microscopy

Since scanning electron microscopy (SEM) observation in the presence of water was difficult to conduct, it was carried out on samples dried without destroying the structure. A procedure similar to that for microorganism observation was followed. Each gel sample was cast on a plate. Figures 1 through 3

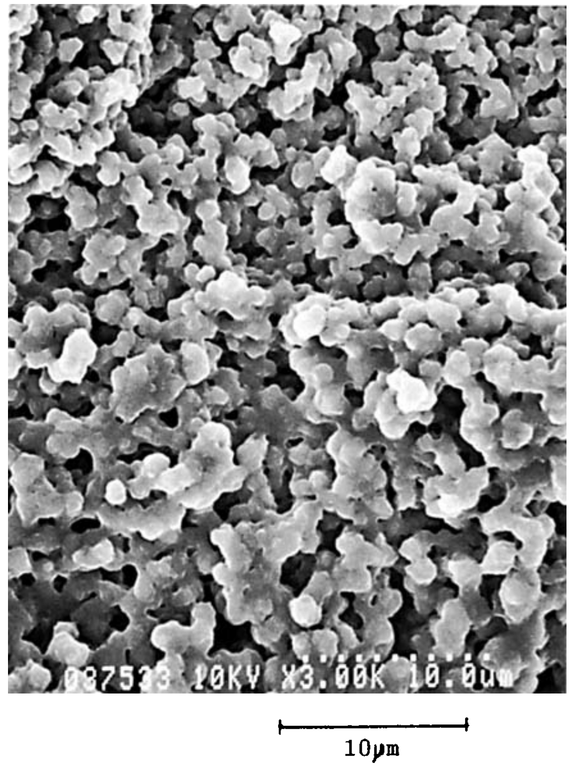

Figure 2 Cross section of PEG gel.

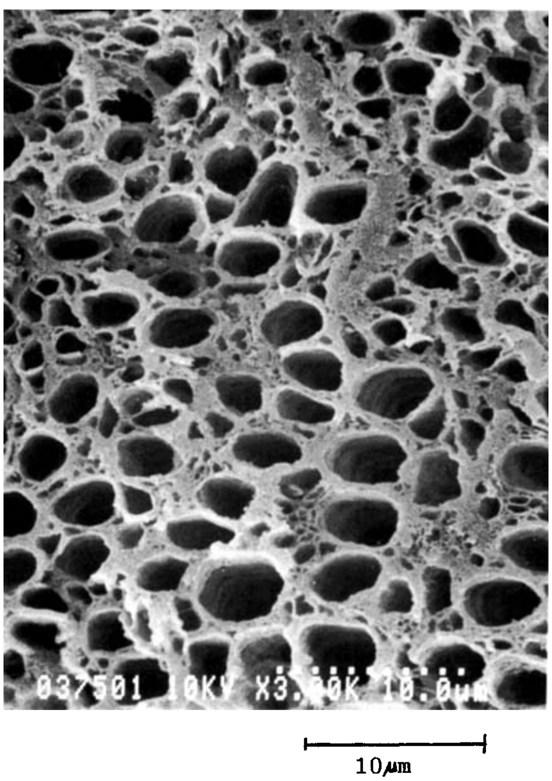

Figure 1 Cross section of frozen PVA gel.

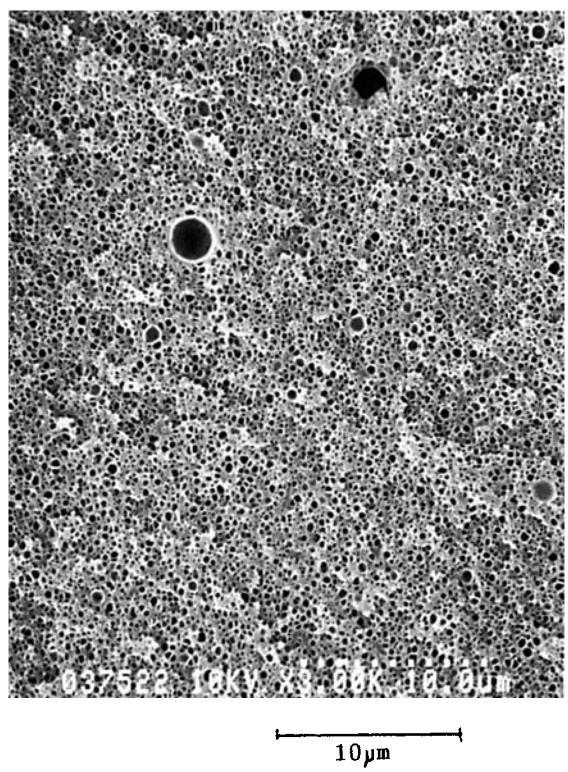

Figure 3 Cross section of PAAm gel.

show the cross sections of PVA frozen gel, PEG gel, and PAAm gel, respectively.

Physicochemical Properties of the Gels

The properties of the frozen PVA, PEG, and PAAm gels are appraised by the following methods:

1. *Specific gravity:* An amount of each gel was weighed in air (W_a) and in water (W_o). The specific gravity of each gel was calculated by $W_a/(W_a - W_o)$.
2. *Water content:* An amount of each gel was weighed in air (W_a), dried at 120°C for 12 h in an oven, and then weighed (W_d). The water content (%) of each gel was calculated by $(W_a - W_d) \times 100/W_a$.
3. *Free water content:* The freezing enthalpy (ΔH, Cal/g) was measured on each gel cooled from 50°C to −30°C by differencial scanning calorimetry (DSC). The free water content (%) was calculated by $\Delta H \times 100/70.4$. (70.4 Cal/g is the freezing enthalpy of pure water.[7]) Free water content is the percentage of free water to total water. The DSC equipment used was Model DSC-2C of Perkin–Elmer Inc.
4. *Oxygen transmission coefficient (D_k value):* A cast gel sheet with a thickness of 1 mm was vertically mounted in an apparatus of Seikaken type made by Rikaseiki Inc.,[8] which is used for measuring the oxygen transmission rate for film. A 0.9% aqueous NaCl solution was poured into one side of this sheet, and water saturated with oxygen was poured into the opposite side of the sheet. D_k values were obtained from the change of electric current running between electrodes set on both sides of this sheet. D_k values were evaluated by D_k value $= (C \times L)/(N \times F \times A \times PO_2)$, where C is the constant electric current at measurement (μA), L is the thickness of the sheet (cm), N is the number of electrons on one molecule of oxygen (4), F is Faraday's constant ($96{,}500 \times 10 \exp(+6)/22{,}400\ \mu q/cm^3$), A is the area of Pt electrode (cm^2), and PO_2 is the partial pressure of oxygen gas at measurement (mm Hg).
5. *Tensile strength:* Breaking tensile load was measured with a load cell at breaking elongation by Instron Inc. Type-1130. Tensile strength was estimated from the breaking load devided by the cross-section area of this sheet.
6. *Folding test:* A gel sheet sample of 1 mm thickness was gradually folded down and the growth of crack was observed.
7. *Chemical resistance of the frozen PVA gel:* The changes in shape, volume, and weight were observed in aqueous solutions with a pH of 0 to 14 and containing several salts, such as NaCl, Na_2SO_4, and KCl, in 10 g/L for 6 h. These were observed under stirring with a magnetic mixer at room temperature.
8. *Durability against abrasion of frozen PVA gel:* Samples (1.0 kg each) were placed in stainless wire net boxes with openings of 1 mm to occupy 40% of the space. The boxes were immersed in an aeration tank of the effluent treating system at Kuraray Okayama Plant. The weight loss of each of the samples was measured at intervals of several months.

Preparation of Spherical PVA Hydrogel Particles with Microorganisms and Application for Waste Water Treatment

Equal amounts of 16% PVA solution and 4% aqueous sodium alginate solution were mixed to prepare solutions of PVA of 8% and sodium alginate of 2%. Sodium alginate was used as an auxiliary agent to form stable spherical PVA gel particles because sodium alginate reacts with calcium ions to form a calcium alginate gel containing PVA solution.

An activated sludge (mixed liquor with suspended solids, MLSS 20,000 mg/L, used for waste water treatment at Kuraray Okayama Plant) was added to the mixture in a ratio of 1 : 1. These mixed dopes were extruded through a nozzle with a metering pump and dropped into 0.4 mol/L aqueous calcium chloride coagulation bath. The gelation of spherical particles was carried out in the same manner as pure PVA gel: frozen overnight at −20°C and thawed at room temperature. After three repeated cycles of freezing and thawing, the gel was washed thoroughly with distilled water. Particles with a diameter of 3 mm were prepared. Sodium alginate named Duck algin ND was supplied by Kibun Food Chemifa Co., Ltd. The viscosity of 0.5% sodium alginate aqueous solution measured by a B-type rotary viscometer was 1500 centipoise at 25°C.

The composition of the spherical PVA gel was as follows: PVA, 4%; sodium alginate, 1%; microorganisms, 1%; and water, 94%. Figure 4 shows the schematic process for the preparation of spherical particles of PVA hydrogels used for waste water treatment.

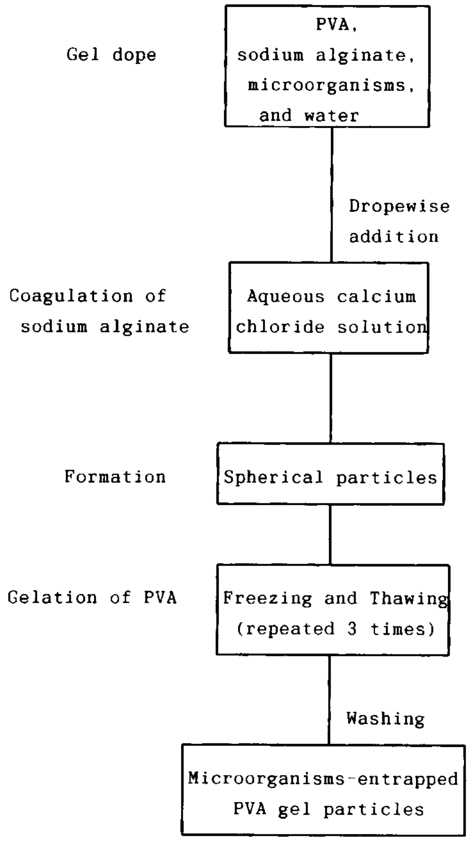

Figure 4 Process for preparation of spherical PVA gel particles.

A simple effluent treatment test was carried out with these spherical frozen PVA gel particles after they were incubated on synthetic sewage. A continuous effluent treating test apparatus replaced by an activated sludge process is shown in Figure 7. Table II shows the composition of synthetic sewage for this test. A mother liquid with the composition is used in different overall concentrations.

The standard activated sludge (floating) method was employed. Activated sludge of Kuraray Okayama Plant (MLSS: 1000 mg/L) was placed in an aeration tank. The rate of flow by water was 9 L/day, and the mean cell residence time was 8 h. The sidestreams of 3 L/day were returned to the aeration tank.

A new method was used for these spherical PVA gel particles. Another aeration tank was charged with 300 g of the PVA gel particles containing microorganisms (MLSS: 1000 mg/L gel). The flow rate by water was 9 L/day, and the mean cell residence time was 8 h. A part of treated water was not returned to the aeration tank. Wire net was mounted at the exit of the tank to prevent the gel from flowing out.

RESULTS AND DISCUSSION

Observation of Gel Structure in SEM

Frozen PVA gel (Fig. 1) is distinguished by a spongelike structure with voids. The voids are vestiges of ice pieces grown like frost columns from water isolated upon syneresis of the aqueous PVA solution during freezing. The formation of ice excludes PVA and thereafter gelation occurs, whereby voids remain. The PVA phase from which water has been removed upon syneresis becomes a high concentration phase and forms a strong gel, as described later. The strong gel is formed from only several percent aqueous PVA solution by phase separation due to freezing and thawing. Then the network structure consists of a high-concentration PVA membrane. When aqueous PVA solution forms a gel, the microorganisms will be removed from high-concentration PVA entrapped in the PVA voids. The sodium alginate mixed with PVA solution is present in the resulting spherical gel particle as a forming additive agent.

PEG gel (Fig. 2) contains no voids. PAAm gel (Fig. 3) has a spongelike structure with isolated holes of a size less than 1 μm. Of the aforementioned three

Table I Physicochemical Properties of Three Kinds of Gels

Item	PVA Frozen Gel	PEG Gel	PAAm Gel
(1) Specific gravity	1.025	1.040	1.050
(2) Water content (%)	95	87	82
(3) Amount of free water (%)	80	66	61
(4) Oxygen transmission coefficient (D_k value)	70	58	54
(5) Tensile strength (kg/cm^2)	1.7	0.4	0.3
(6) Folding test	Normal	Broke	Broke

Units of D_k values: in $\times$ 10 exp (-11) cm^3 $\times$ cm/cm^2 $\times$ s mm Hg.

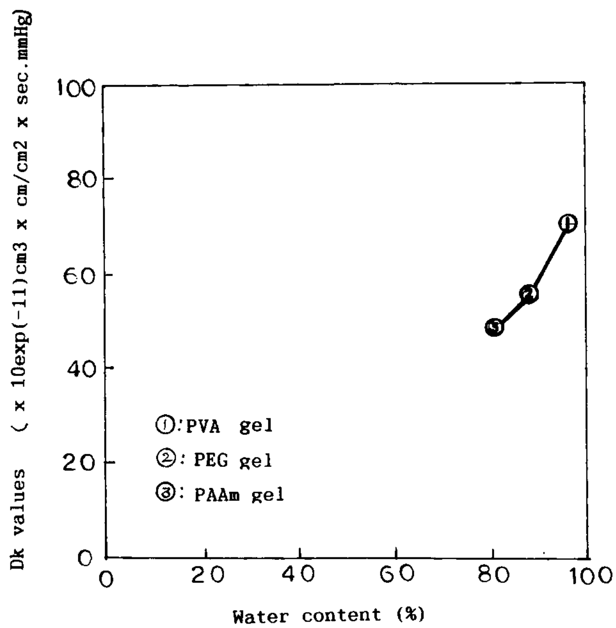

Figure 5 Relationship between water content and D_k value.

gels, only freezing and thawing PVA gel produces voids of several micron size and a network structure with high strength even in the gel from a low-concentration solution.

The PVA gels obtained by repeated freezing and thawing have physical junctions and are considered to be less harmful to microorganisms. The mechanism involved in this gelation is that intermolecular force generated by fluctuation of local concentrations of polymer molecules forms microcrystals, which are more stabilized from the viewpoint of energy level and constitute crosslinking points to form a three-dimensionally crosslinked gel. While high-concentration aqueous PVA solutions can gel at temperatures above freezing, low-concentration ones are more difficult to gel.

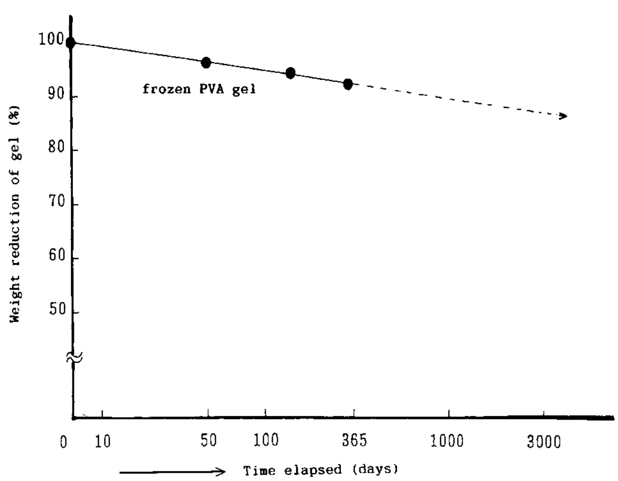

Figure 6 Durability against abrasion of spherical PVA gel particles.

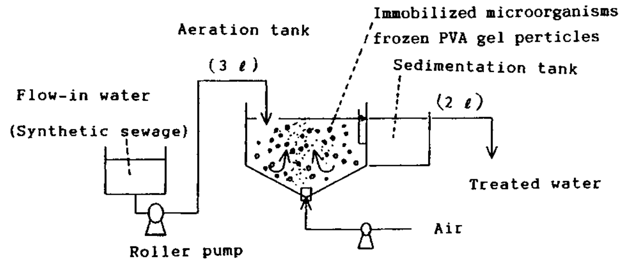

Figure 7 Effluent treating test apparatus.

Physicochemical Properties of Various Carrier Gels

Table I shows the physicochemical properties of three kinds of carrier gels: specific gravity (1), water content (2), free water content (3), tensile strength (5), and the results of folding test (6). Oxygen transmission coefficients (D_k values) (4) are also shown. Figure 5 shows the relationship between water content and D_k values. The specific gravity of frozen PVA gel is closer to 1.00, and therefore less energy is required for carrier flowing than with PEG and PAAm gels. In addition, the water content of PVA gel is higher than other gels. The higher water content means better living conditions for microorganisms since PVA gels as carriers have high permeabilities of dissolved oxygen and substrates. Furthermore, the amount of free water is much higher than other gels, so this water is not bonded to polymers and is available for microorganisms. Higher D_k values allow microorganisms to live more inside the gel. Finally, the frozen PVA gel has good chemical resistance; no change in shape, volume, and weight was observed.

The durability against abrasion of frozen PVA gel is shown in Figure 6. The ordinate represents the percentage of weight reduction based on the initial weight. The weight loss was as low as 5% in a year. The gel is highly resistant to wear. No change

Table II Composition of Synthetic Sewage (g/L)

Meat extract	120	$MgSO_4$ $7H_2O$	6.2
Peptone	160	KCl	4.2
Na_2HPO_4 $12H_2O$	25.2	NaCl	90
$CaCL_2$ $2H_2O$	5.56	$NaHCO_3$	250

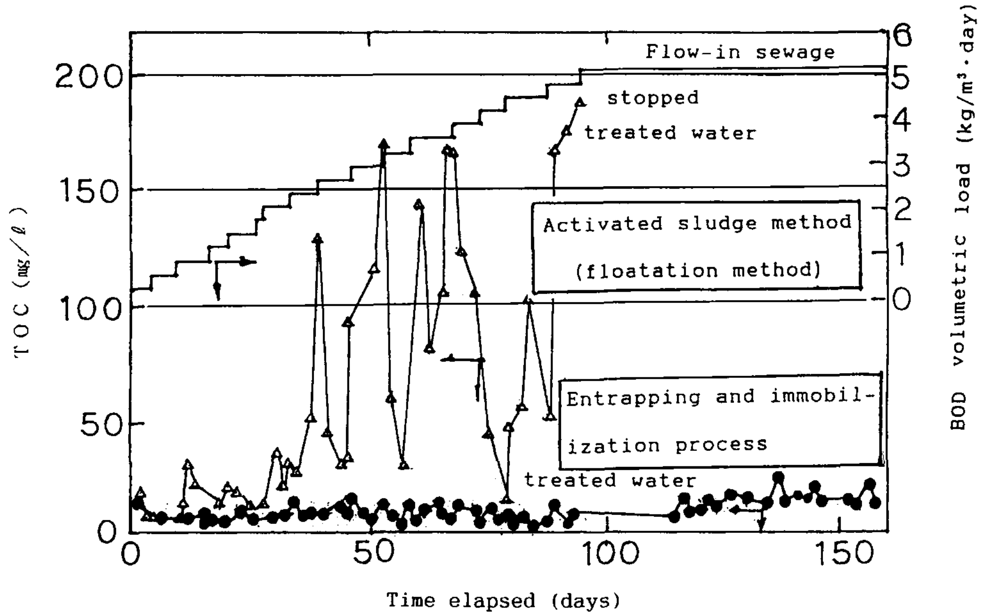

Figure 8 Comparison between activated sludge method and entrapping and immobilization process.

in shape or weight occurred in water at temperatures below 45°C. When the water temperature exceeded 50°C, PVA gel softened. PEG and PAAm gels were stable at temperatures over 100°C.

PEG and PAAm gels had lower tensile strength than that of the PVA gel, although the water contents of the former were lower than that of the latter. The strength of the PVA gel is maintained by the PVA membrane due to freezing, whereas that of the former is maintained by the chemical crosslinking points distributed homogeneously in gels. The further reduction in water content in the gels is necessary to prepare the higher strength.

Repeated freezing and thawing produces a gel that has both high water content (i.e., from a low-concentration aqueous PVA solution) and high strength, two properties that are generally considered contradictory to each other. Due to mechanical properties of tensile strength and folding test, frozen PVA gel is distinguished by high strength and elongation, is resistant to deformation, and is not fragile but is rubberlike.

Waste Water Treatment Using Microorganisms Immobilized in Frozen PVA Gel

Figure 8 shows the testing flow diagram of the relation among effluent treating test with elapsed time (days), TOC (total organic carbon), and BOD volumetric load. Figure 9 shows the relation between BOD volumetric load and amount of BOD removed. In the activated sludge process, the capacity of treated water fluctuates in a range of BOD volumetric load of at least 1-2 kg/m^3 × day. According to this PVA gel method, the complete treatment is possible up to a BOD load of 5 kg/m^3 × day. The capacity of waste water treatment using microorganisms immobilized in frozen PVA gel was esti-

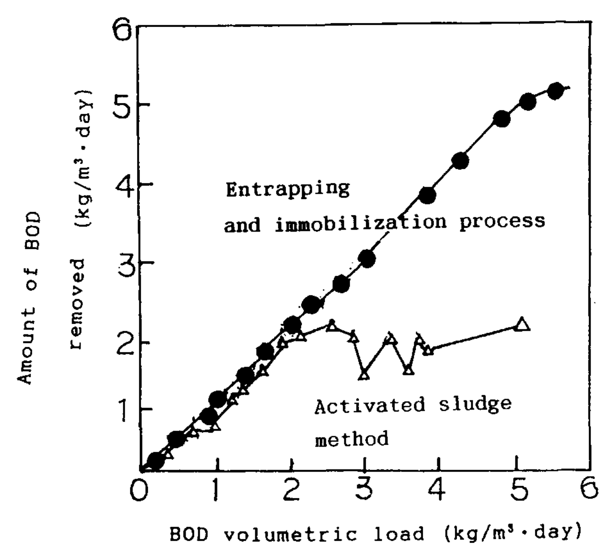

Figure 9 Relationship between BOD volumetric load and amount of BOD load removed.

mated about two to three times that with the standard activated sludge process. Microorganisms are trapped in the voids of the frozen PVA hydrogel.

CONCLUSIONS

1. The PVA frozen gel, different from the hydrogels formed from PEG or PAAm networks, is distinguished by the presence of voids with a size of several microns, which are formed upon freezing. Strong membranes constituting the walls of the voids are interconnected in a network to form a spongelike structure.
2. Due to the aforementioned structure, the frozen PVA gel has both a large water content and a large volume of free water; therefore, it has good oxygen permeability and offers good living conditions for microorganisms. The gels, formed by freezing and thawing, produce little adverse effect on microorganisms. The chemical resistance of frozen PVA gel is superior to acids, alkalines, and salts. Durability against abrasion of frozen PVA gel is good.
3. A simple effluent treatment was tested using microorganisms immobilized in frozen PVA gel. The treating capacity for synthetic sewage was about two to three times larger than that with the standard activated sludge process.

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