Development of Poly(vinyl alcohol) Hydrogel for Waste Water Cleaning. II. Treatment of *N,N*-Dimethylformamide in Waste Water with Poly(vinyl alcohol) Gel with Immobilized Microorganisms

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SYNOPSIS

The activated sludge that had been sufficiently cultivated with DMF-containing waste water was entrapped and immobilized in spherical poly(vinyl alcohol) (PVA) hydrogel particles. Features of numbers and distribution of bacteria in the resultant PVA gel were estimated. The DMF-decomposing bacteria named *Bacillus cereus* D-1 was isolated. Several morphological and physiological responses of the bacteria were revealed. Spherical PVA gel with the DMF-decomposing bacteria prepared through of freezing and thawing was tested for treatment of DMF-containing effluent. Test results have shown the ability of stable operation and maintenance at the same capacity of 1 kg/m^3/day by both a basic study and a bench plant test. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

In the Part I of this study,[1] the preparation of a spherical poly(vinyl alcohol) (PVA) hydrogel with immobilized microorganisms by a freezing and thawing method was reported. PVA hydrogel as a carrier for immobilizing microorganisms was proved to be useful for effluent water from the viewpoint of its high tensile and folding strength.

Furthermore, a standard activated sludge process for effluent treatment was compared with a method with PVA hydrogels containing immobilizing microorganisms. As a result, the authors found that the latter method with frozen PVA gel had a treating capacity of two to three times that of the former process. Thus, we studied the features of microorganisms in a frozen PVA gel.

Now, many organic compounds are used as solvents. Amides are frequently used among nitrogen-containing compounds. In particular, *N,N*-dimethylformamide (DMF) is used in many industrial fields thanks to its good water solubility, miscibility with other organic solvents, and solubility for many polymers. With respect to mixed solvents of DMF with water, while ones of high concentration permit DMF to be recovered by distillation or be incinerated, ones of low concentration are difficult to be recovered economically. Moreover, effluents of such solvents deteriorate water quality, i.e., they increase in the content of BOD, COD, and nitrogen.

There have been proposed various processes of using microorganisms to biodegrade DMF. Japanese patent publications and applications disclose the use of microorganisms belonging to the genuses *Micrococcus*,[2] *Mycobacterium*,[3] *Pseudomonas*,[4] and *Paracoccus*,[5] *Xanthobacter*,[6] and photosynthetic bacteria[7] to decompose DMF.

We therefore set our goal to remove at least 80% of DMF from DMF-containing industrial waste wa-

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ter by biological treatment. We isolated pure DMF-decomposing bacteria from cultivated activated sludge at the Kuraray Okayama Plant. The strain *Bacillus cereus* D-1 which belongs to the genus *Bacillus* was found to be capable of assimilating DMF and therefore usable for DMF. In carrying out the biological treatment, we used a new method as a carrier of PVA gel having entrapped and immobilized activated sludge that had been sufficiently cultivated with DMF, and continuous treatment in the case of the basic study (beaker scale) and on the bench plant scale was tested on DMF-containing effluent.

The objects of this study were as follows: (1) the preparation of spherical PVA hydrogel particles with microorganisms, (2) the characterizations of microorganisms in the frozen PVA hydrogel, (3) the isolation of DMF-decomposing bacteria, and (4) the continuous treatment test for DMF-containing effluent.

EXPERIMENTAL

Preparation of Spherical PVA Hydrogel Particles with Microorganisms

A mixed solution of 16% aqueous PVA (PVA-HC: degree of polymerization, 1700, and degree of saponification, 99.98 mol %) solution, 4% aqueous sodium alginate (named Duck algin-ND made by Kibun Food Chemifa Co.) solution, and activated sludge (MLSS is 20,000 mg/L used for waste water treatment at the Kuraray Okayama Plant that had been sufficiently cultivated with DMF) with microorganisms was added dropwise to an 0.4 mol/L aqueous calcium chloride solution for a coagulating bath, whereby spherical particles are formed. The resulting spherical gel particles were frozen overnight at −20°C and thawed at room temperature, and the cycle was repeated three times. The gel then was washed thoroughly with distilled water. The composition of the spherical PVA gel was as follows: PVA 4%, sodium alginate 1%, microorganisms 1%, and water 94%. Figures 1 and 2 show the spherical PVA gel particles and gel structure taken with an optical and scanning electron microscope, respectively.

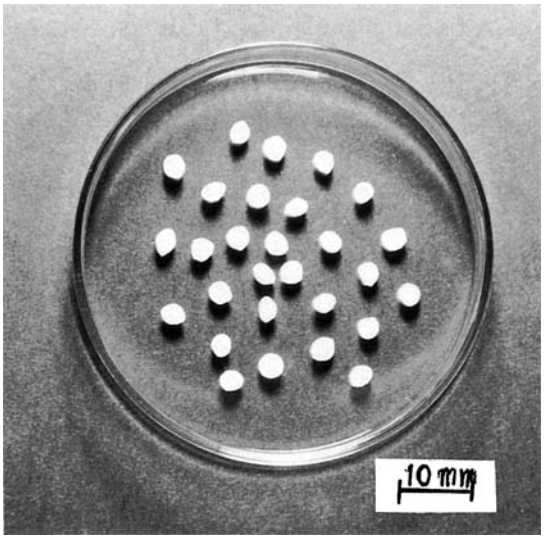

Figure 1 Spherical particles of frozen PVA gels containing calcium alginate and activated sludge that has been sufficiently cultivated with DMF.

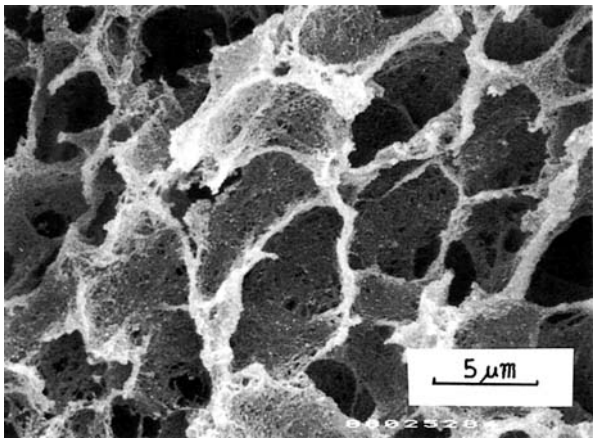

Figure 2 SEM observation of cross section of spherical particle of frozen PVA gel.

Features of Microorganisms in Frozen PVA Gel

Number of Bacteria in Frozen PVA Gel

The activated sludge used at the Kuraray Okayama Plant was condensed, filtered, and then entrapped and immobilized on various polymers to prepare the gels. A designated amount was weighed from each gel and dispersed in water with a homogenizer at 15,000 rpm for 10 min. Each of the dispersions was cultivated on agar culture, after which the number of colonies was counted. The investigation of the polyacrylamide (PAAm) gel reported previously[1] was carried out for comparison.

Bacteria Distribution in Frozen PVA Gel

A PVA gel particle with a diameter of 3 mm was separated into a surface part and central part with

a size of 1 mm by cutting. They were then dispersed in water with a homogenizer under the above conditions. The numbers of colonies on the agar culture were counted in the same manner as above.

Isolation of DMF Decomposing Bacteria in Cultivated Activated Sludge at the Kuraray Okayama Plant

The strain *B. cereus* D-1 was isolated from a DMF-acclimated activated sludge and is deposited under deposition No. FERM P-13911 at the Biotechnology Industrialization Research Laboratory of Agency of Industrial Science and Technology.

From the bacteriological properties shown in Tables II–V and based on retrieval of *Bergey's Manual of Determinative Bacteriology,* 8th edition,[8] the above strain was identified as one belonging to the species cereus of the genus *Bacillus,* and Kusudo named this the strain *B. cereus* D-1.

Continuous Treatment Test for DMF-containing Effluent

Testing Apparatus and Conditions for Basic Study

Figure 3 shows an outline of the testing apparatus used. A 3 L aeration tank equipped with an air diffuser at the bottom was charged with 600 mL (20 vol %) of PVA gel with entrapped and immobilized activated sludge (containing *B. cereus* D-1) of the Kuraray Okayama Plant, sufficiently cultivated with DMF. A continuous treating test was carried out at a flow-in rate of 9 L per day (retention time: 8 h). Overflow of the aeration tank was introduced into a sedimentation tank and the discharged water was regarded as treated water. The aeration tank had been provided with a sulfuric acid addition tank for controlling pH and a phosphoric acid addition tank.

Treatment Test with Bench Plant

The process of the bench plant is as follows: DMF flow-in water was stored in a storage tank (7 m^3), in which the total organic carbon (TOC) concentration was adjusted to a range of 2000 to 10,000 mg/L by addition of water. The aeration tank (volume: 1300 L; diameter: 115 cm; height: 125 cm) was equipped with a donut-type diffuser on the bottom and aeration agitation of the raw water and the microorganism-immobilized PVA gel was conducted by forced-air blowing. The microorganism-entrapped-and-immobilized-PVA gel had been prepared by the process shown in the previous section and was added to the aeration tank in an amount of 20 vol %. The pH control and phosphoric acid addition in the tank were carried out in the same manner as shown in Figure 3. The treated water was discharged through a sedimentation tank (400 L).

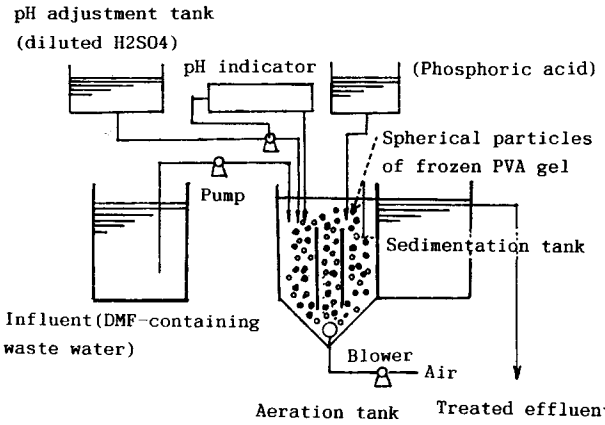

Figure 3 Continuous treatment testing apparatus for DMF-containing waste water.

RESULTS AND DISCUSSION

Spherical PVA Hydrogel Particles with Microorganisms

As we previously reported, spherical PVA particles and the gel structure was observed with an optical and scanning electron microscope (SEM). Figure 1 shows the PVA gel particles of an average diameter of 3.0 mm, and Figure 2 shows the SEM observation of cross section of frozen PVA gel of the particles. This reveals that they had a sponge structure with voids of size 1–10 μm, each surrounded by a thin membrane 0.02–0.1 μm thick. A specific gravity of 1.03 and a water content of 94.0% were estimated in the same manner as the previously reported method on the physicochemical properties.

Features of Microorganisms in Frozen PVA Gel

Number of Bacteria in Frozen PVA Gel

To count the number of microorganisms in frozen and thawed PVA gel, dispersed and powdered gel with microorganisms was cultivated on agar culture, after which the number of colonies was counted. Table I shows the number of bacteria and the survival ratio based on the raw bacteria. Entrapped and immobilized microorganisms in PVA gel were hardly damaged by freezing and thawing, but those in poly-

Table I Bacteria Present in Entrapped and Immobilized Gels

Gel	No. Bacteria (MLSS per g)	Survival Ratio (%)
Activated sludge bacteria (raw)	1.0 × 10 exp (+8)	100
PVA frozen gel	0.6 × 10 exp (+8)	60
Polyacrylamide gel	3.0 × 10 exp (+3)	0.003

acrylamide gel were damaged and sharply decreased the number of bacteria. This is due to the initiator used for the polymerization.

Bacteria Distribution in Frozen PVA Gel Particles

To count the number of bacteria of the surface part or central part of the spherical frozen and thawed PVA gel particles, they were dispersed with a homogenizer. For each dispersion, the number of colonies on the agar culture was counted. The numbers were 2.2 × 10 exp (+7) and 0.9 × 10 exp (+7) pieces/g-gel for the surface part and central part, respectively.

While a large number of bacteria were found on the surface part, a considerable number were present also at the central part. Since oxygen and nourishments (BOD, COD) were penetrated into the PVA gel and were resolved into substances by microorganisms, they were spread out from the PVA gel. The leads to a changing of place of some substances through the membrane, making a sponge structure of the PVA gel.

Isolation of DMF Decomposing Bacteria in Cultivated Activated Sludge at the Kuraray Okayama Plant

Tables II–V show the morphological characteristics, state of growth on each culture, physiological responses on various reactions, and physiological responses on the utilization of carbon sources, due to bacteriological activities of the strain *B. cereus* D-1, respectively.

Cultivation of the bacterium that belongs to the genus *Bacillus* and can assimilate and decompose DMF can be carried out in the same manner as for

Table II Morphological Characteristics

(1) Size of bacterium (length × width, μm)	1.0 × 3.0 to 1.2 × 5.0
(2) Shape of bacterium	Bacillus
(3) Movability	Yes
(4) Flagellum	Yes
(5) Spore	Yes
(6) Gram's strain	Positive

Table III State of Growth on Each Culture

(1) Bouillon agar plate culture	
a. Color of colonies	Blue-white
b. Transparency of colonies	Opaque
c. Luster of colonies	Sharp luster
d. Surface condition	Smooth
e. Shape of bulging	Conical
(2) Bouillon agar liquid culture	
a. State of growth on surface	Good
b. Turbidity	White turbid
(3) Bouillon agar stab culture	
a. State of growth	Upper part better than lower part
(4) Bouillon gelation stab culture	
a. Liquefaction	Not liquefied
b. State of growth	No growth
(5) Lithmus milk	
a. Reaction	Acid
b. Coagulation	Minimum

Table IV Physiological Responses on Various Reactions

(1) Nitrate reduction	+
(2) Denitrification	−
(3) Methy red test	−
(4) Voges–Proskauer test	+
(5) Indol production	−
(6) Hydrogen sulfide production	−
(7) Starch hydrolysis	−
(8) Citrate utilization	−
(9) Inorganic nitrogen sources utilization	+
(10) Pigment production	−
(11) Urease	−
(12) Oxidase	+
(13) Catalase	+
(14) Ranges for growth pH temp	6–8
optimum	30–37°C
(15) Oxygen requirement	Aerobic
(16) Oxidation/fermentation test	Fermentative

+: The bacterium has the designated property or can produce the designated substance. −: The bacterium does not have the designated property or cannot produce the designated substance.

Table V Physiological Responses on Utilization of Carbon Sources

Utilization of Carbon Source	Acid	Gas Generation
(1) L-Arabinose	−	−
(2) D-Xylose	−	−
(3) D-Glucose	+	−
(4) D-Mannose	−	−
(5) D-Lactose	+	+
(6) D-Maltose	+	+
(7) Maltose	−	−
(8) Sucrose	−	−
(9) Lactose	−	−
(10) Trehalose	−	−
(11) D-Sorbitol	−	−
(12) D-Mannitol	−	−
(13) Inositol	−	−
(14) Glycerine	+	+

+: Produces −: does not produce.

the usual microorganisms and is generally conducted by shake culture or aeration and agitation culture.

In carrying out cultivation, other nutrient sources that the above bacterium can assimilate may be added, as required, to the treated liquid that is used as a culture medium. The DMF in the treated liquid is also used as a carbon source. Examples of other usable carbon sources are glucose, saccharose, maltose, glycerine, peptone, meat extract, and yeast extract. These carbon sources may be used either singly or in combination, in a concentration of 0.1–5% by weight.

Examples of usable nitrogen sources are inorganic nitrogen sources, e.g., ammonium sulfate, ammonium chloride, and ammonium nitrate, and organic ones, e.g., peptone and meat extract. In general, the concentration of nitrogen sources in the treated liquid is adjusted at 0.1–0.5% by weight. Since the DMF in the treated liquid is utilized as a nitrogen source, it is not always necessary to add these inorganic or organic nitrogen sources.

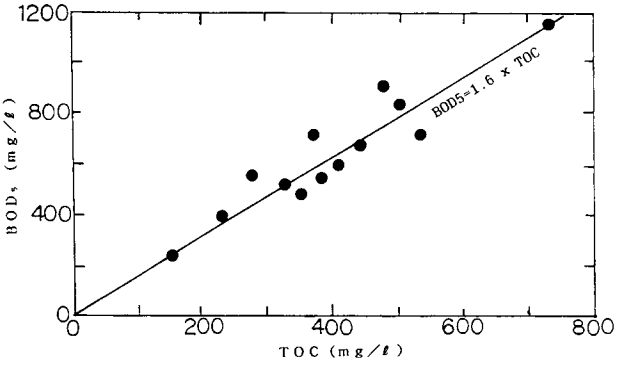

Figure 4 Relationship between BOD5 and TOC.

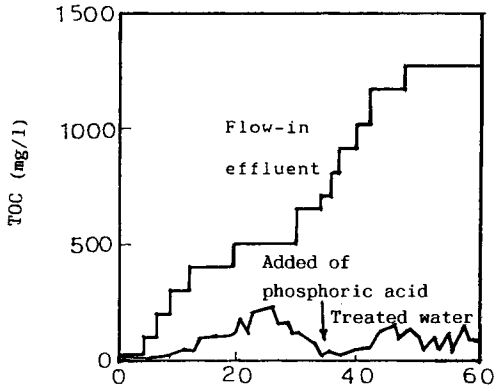

Figure 5 Relationship between DMF effluent treating test with elapsed time (days) and TOC of flow-in effluent and TOC of treated water.

To the treating liquid, in addition to the above carbon sources and nitrogen sources, phosphates such as dipotassium hydrogenphosphate, disodium hydrogenphosphate, and potassium dihydrogenphosphate may be added if necessary. These phosphates are generally added in a concentration in the treated liquid of 0.01–0.5% by weight. Also, inorganic salts such as magnesium salts and iron salts and, as

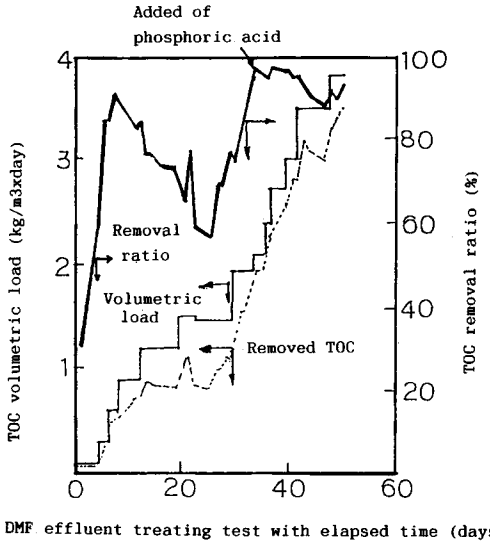

Figure 6 Relationship between DMF effluent treating test with elapsed time (days) and TOC volumetric load and TOC removal ratio.

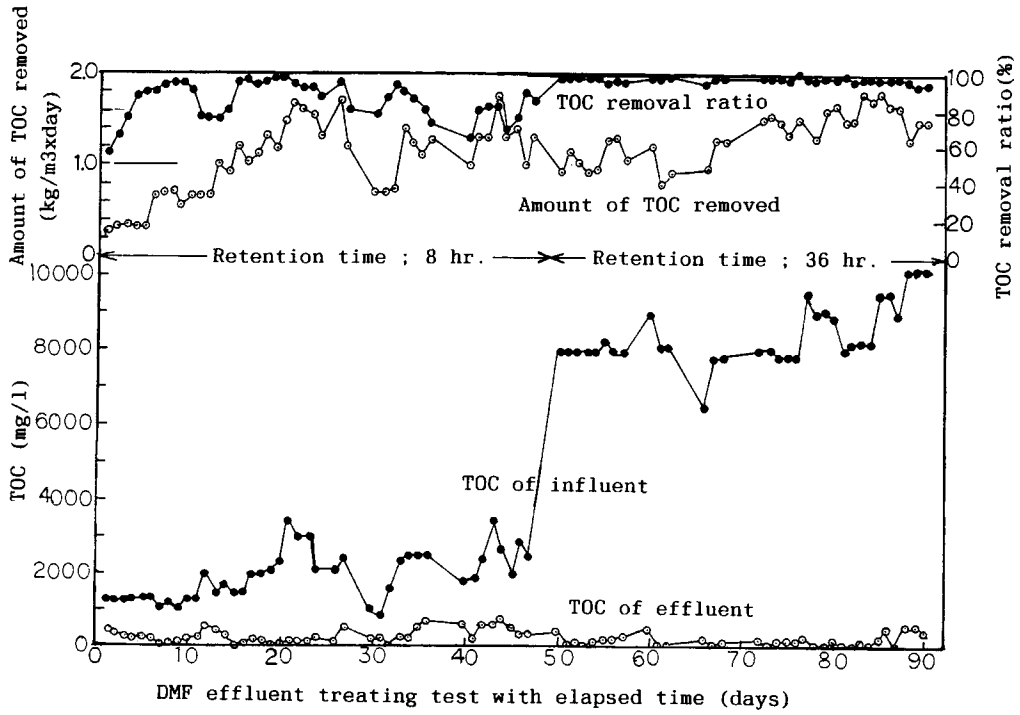

Figure 7 Relationship between DMF effluent treating test with elapsed time (days) and TOC influent and effluent, amount of TOC removed, and TOC removal ratio by bench plant.

necessary, vitamins may be added to the treated water.

Cultivation conditions are not critical. However, the cultivation is preferably carried out by shake culture or submerged culture with aeration and agitation at a temperature of 25–30°C and pH of 6.5–7.5. The cultivation time is generally 6–72 h.

The concentration of the dissolved oxygen in the treated liquid is adjusted such that the above bacterium grows and propagates and is generally about 0.5–5.0 mg/L. To achieve this dissolved oxygen concentration, various methods may be employed, such as adjustment of the rate of aeration or agitation, use of oxygen or a mixed gas of oxygen and air for the aeration, and elevation of the pressure in the cultivation vessel.

Continuous Treatment Test for DMF-containing Effluent: Results for Basic Study and Bench Test

For quick analysis, the relationship between BOD5 and TOC was preliminarily studied. As a result, it was found that, as shown in Figure 4, BOD5 = 1.6 × TOC. An effluent treatment test was continued for about 50 days, during which time no change of the spherical PVA gel, such as dislocation, was observed through the basic study (beaker scale). The test results are shown Figures 5 and 6. Figure 5 shows how raw effluent flowed in and the TOC (mg/L) of treated water was changed. Figure 6 shows the volumetric load of DMF (TOC) and TOC removal ratio with time. During the operation, the pH gradually increased up to 8.3 with a decreased amount treated. It was found that an amount of the COD volumetric load removed was at least 1–3 kg/m^3/day. Figure 7 shows the results of the bench plant test. It was found that stable treatment at a capacity of 1 kg/m^3/day, the same as that of the basic test, was possible within a TOC concentration range of 1500–10,000 mg/L. As the mechanism on decomposition of DMF had not been studied yet, we will have to investigate further.

CONCLUSIONS

1. Entrapping immobilization of microorganisms in spherical PVA hydrogel was carried out, and the features, properties, and the number and distribution of bacteria of the resultant gel were determined.
2. The DMF-decomposing bacteria was isolated,

which was found to be as *Bacillus cereus* D-1. It was trapped and immobilized in frozen PVA gel.

3. The trapped and immobilized bacteria, the DMF-decomposing bacteria, in spherical frozen PVA gel particles was tested for treatment of DMF-containing effluent.

Test results have shown the ability of stable treatment at a capacity of 1 $kg/m^3/day$, by both the basic study (beaker test) and the bench plant test.

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