MATERIALS AND INTERFACES

Synthesis and Characterization of Hydrogels Based on Poly(vinyl alcohol)-*g*-Poly(styrene) Copolymers

**M. Velazco-Díaz,[†,‡] F. A. Ruiz-Treviño,*[,†] M. C. Doria-Serrano,[†]
A. González-Montiel,[§] and Mikhail Zolotukin[‖]**

*Departamento de Ingenierías, Universidad Iberoamericana, Prolongación Paseo de la Reforma No. 880,
México D. F. C. P. 01210, Facultad de Química, Universidad Autónoma del Estado de México,
Paseo Colón intersección Paseo Tollocan S/N, Toluca, Estado de México C. P. 50120, CID Centro de
Investigación y Desarrollo Tecnológico, Avenida de los Sauces No. 87, M6 Parque Industrial Lerma,
Estado de México C. P. 52000, and Instituto de Investigación en Materiales, Universidad Nacional Autónoma
de México, Apartado Postal 70-360, CU, Coyoacan, México D. F. C. P. 04510*

A useful hydrogel for supporting and/or entrapping living microorganisms has been developed by the syntheses of graft copolymers based on vinyl alcohol and styrene, PVOH-*g*-PS, and through the formation of macro- and microporous structures formed under the slow evaporation of a toluene/dimethyl sulfoxide, DMSO, mixture of solvents. The PVOH-*g*-PS copolymer was obtained by the methanolysis of the vinyl acetate part of a previously bulk-polymerized poly(vinyl acetate)-*g*-poly(styrene) copolymer, PVAc-*g*-PS. To produce the so-called PVOH-*g*-PS hydrogel, polymer films formed by the evaporation of a toluene/DMSO mixture were subjected to different freeze/thaw cycles in order to crystallize the PVOH part of the copolymer. Calorimetric studies carried out on these types of hydrogels show melting endothermic transitions that correspond to fusion of the PVOH, and the crystallization areas increase with the number of freeze/thaw cycles. The low-vacuum scanning electron microscopy and atomic force microscopy images reveal that the PVOH-*g*-PS copolymer can form films with homogeneous macro- and micropore size distributions interconnected by a cheese-like structure where the microorganisms can be supported and/or entrapped.

Introduction

Hydrogels are polymeric materials that have attracted the interest of industrial and academic researchers mainly because they have potential applications (just to mention a few) in bioreactors and biomedical applications.[1,2]

In the bioreactor field a substantial number of hydrogels that may be useful materials for supporting or entrapping the microorganisms required for the biological degradation of organic material have been reported.[3–5] Hydrogels based in poly(vinyl alcohol)-Ca-alg[6] and poly-(vinyl pyrrolidone)-Ca-alg[7] have been proposed; however, none can support the high shear stress found in bioreactors,[8] and very few can support the coarse chemical environment found in the treatment of municipal wastewater treatment plants, i.e., the high concentration of phosphates. The hydrogels mentioned above are chemically degraded because they are prepared with Ca-alginate, a very rigid and ionically cross-linked polymer matrix.[9,10] Therefore, robust hydrogels,

in terms of mechanical, chemical resistance properties and without detriment in the affinity for the microorganisms, are needed in order to see a generalized use of these materials in bioreactor applications.

To deal with the challenge of producing robust hydrogels, research activity has been addressed in two directions: (1) the formation of highly cross-linked gels[9,10] and (2) the possibility of entrapping or immobilizing microorganisms in highly hydrophobic polymers. It is well known that hydrophobic polymers usually possess excellent mechanical properties, and they are used to support biofilms in wastewater treatment plants. In fact, polystyrene[11] and polysulfone[12] have been studied as possible candidates for the entrapment and immobilization of microorganisms.

In this paper research activity has focused on the formation of hydrogels made of a copolymer that bears a hydrophilic polymer, PVOH, grafted to a hydrophobic polymer, polystyrene, PS. In particular, effort is placed on synthesis of the copolymer, formation of a macro- and microporous film of the hydrogel, and evaluation of the affinity of this material for aerobic microorganisms.

Experimental Procedure

Materials. The vinyl acetate and styrene monomers as well as the stable free radical 2,2,6,6-tetramethyl-1-piperidiniloxy, TEMPO, were kindly donated by CID,

---

* To whom correspondence should be addressed. Tel.: (52) 55 5950-4074. Fax: (52) 55 5950-4279. E-mail: alberto.ruiz@uia.mx.
† Universidad Iberoamericana.
‡ Universidad A. del Estado de México.
§ Centro de Investigación y Desarrollo Tecnológico.
‖ Universidad Nacional Autónoma de México.

Centro de Investigación y Desarrollo Tecnológico S. A. de C. V. The aerobic microorganisms were collected from the wastewater treatment plant of Universidad Ibero-americana. The benzoyl peroxide, BPO, initiator, toluene, and dimethyl sulfoxide solvents were reactive-grade compounds.

Syntheses of the PVOH-*g*-PS Hydrogel. A typical synthesis consisted of formation of a poly(vinyl acetate) homopolymer stabilized with TEMPO at the end of the bulk polymerization. The synthesis starts with vinyl acetate and BPO at $T = 60$ °C, and after 6 h of reaction TEMPO is added to the reactor using a molar ratio of 1.3 mol of TEMPO/mol of BPO. After the addition of TEMPO the temperature is increased from 60 to 140 °C, where it remains for two more hours to produce the macroinitiatior of PVAc stabilized with TEMPO, PVAc-T*. The macroinitiator is then precipitated in hexane and then Soxhlet extracted for at least 2 days with hexane to wash out any residual monomer and TEMPO. The macroinitiatior is then used to produce the PVAc-*g*-PS under controlled free-radical polymerization at high temperature using different amounts of styrene as suggested elsewhere.[13,14] The copolymers were synthesized increasing the temperature of the reaction mixture up to 140 °C for 7 h. The copolymers are precipitated in methanol and then Soxhlet extracted with methanol for 2 days to extract the PVAc homopolymer and then Soxhlet extracted with cyclohexane for two more days to eliminate the polystyrene homopolymer and styrene residual monomer.[14] Afterward, the copolymers are carefully dried in a convention oven at 40 °C followed by a vacuum-drying period of 2 days at 80 °C. The PVOH-*g*-PS copolymers were obtained by a methanolysis reaction, which consisted of reacting 2 g of dried PVAc-*g*-PS in 100 mL of methanol with 1 mL of an aqueous 40 wt % NaOH solution at 40 °C for 1.5 h. The copolymers were then dried overnight in a conventional oven at 60 °C.

Film and Hydrogel Formation. The films were formed by solution-casting a 10 wt % PVOH-*g*-PS copolymer solution dissolved in a mixture of solvents composed of toluene and DMSO. A typical solution consisted of 10 wt % polymer and 70 wt % toluene with the rest being DMSO. After slow evaporation of the mixture of solvents, the films were removed from the glass plates dried in a conventional oven at 40 °C for 2 days, and then vacuum-dried at 80 °C for two more days. The film's average thickness was around 1 mm, and they were somewhat translucent, probably due to their thickness or to some indication of microphase separation. To produce the hydrogels the mentioned films were washed in warm water at 90 °C for 1 h in order to remove some PVOH not grafted to PS. Afterward, every film was subjected to three, five, and seven freeze/thaw cycles to crystallize the PVOH present in the PVOH-*g*-PS copolymers. The freeze cycles last 12 h at −20 °C, whereas the thaw cycles lasted 12 h at room temperature. These hydrogel copolymers were dried at 40 °C and then vacuum-dried at 80 °C for 2 days.

Copolymers and Films Characterization. Proton nuclear magnetic resonance, [H]NMR, Bruker Avance 400 Spectrometer, was used to characterize the PVAc-T* macroinitiator and all copolymers, whereas gel permeation chromatography, GPC Waters with a 515 HPLC pump, 717 auto sampler, and RI 410 Differential Refractometer, was used to determine the average molecular weight of the PVAc-*g*-PS copolymers. Differential

calorimetric studies, DSC, STA 650 Instrument Specialists, were also carried out on PVOH-g-PS hydrogel copolymers to follow the melting endothermic transitions typical of the crystallization of PVOH, which is dependent on the number of freeze/thaw cycles. DSC scans were run at a 5 °C/min temperature ramp from 30 to 300 °C. The affinity of these hydrogels to support/entrap microorganisms was followed by contacting the hydrogel copolymer films with wastewater solutions containing the aerobic microorganisms. Low-vacuum scanning electron microscopy, JEOL SEM 5600 LV resolution 3 μm, and atomic force microscopy, AFM, Nanoscope E Digital Instrument, were then used to characterize the surface of the hydrogel films with and without microorganisms.

Determination of the Number of Colony-Forming Units (CFU) Immobilized in Copolymer. Aerobic sludge was taken from the reactor of the wastewater treatment plant of Universidad Iberoamericana, centrifuged, and washed with 0.9% saline solution. A 20 mL sample of the suspension was set in contact with samples of the copolymer with an area of 1 cm^2 in a medium containing $MgSO_4$ (0.078 g/L), NH_4Cl (0.56 g/L), Na_2HPO_4 (0.022 g/L), KH_2PO_4 (0.138 g/L), yeast extract (0.5 g/L), and 2 g/L of glucose as carbon source. The suspension was incubated for 5 days and bubbled with air continuously at room temperature; each day the suspension was centrifuged and a fresh medium added.

After incubation each copolymer sample was washed by immersing and removing the films at least three times in a 50 mL saline solution in order to eliminate the loosely bound microorganisms. Then the films containing the entrapped microorganism were smashed in 10 mL of saline solution; 1 mL was sequentially diluted, and 0.1 mL of each dilution was spread in nutrient agar. After 48 h of incubation at room temperature (20 °C) the number of colony-forming units (CFU) was determined. This procedure was done in triplicate with three different samples.

Results and Discussion

The PVAc-*g*-PS copolymers form a white powder that according to DSC determinations show two glass-transition temperatures corresponding to poly(vinyl acetate), $T_g = 40$ °C, and polystyrene homopolymer, $T_g = 100$ °C. Figure 1a shows a ^{1}H NMR spectrum of such copolymers where the characteristic peaks for both PVAc and PS appear at 4.1 ppm for the methine protons of PVAc and from 6.4 to 7.2 ppm for the aromatic protons of PS. Figure 2 plots the average molecular weight, M_n, for the PVAc-*g*-PS copolymers synthesized with different amounts of styrene and PVAc-T*. The average molecular weight of PVAc-T*, which is initially 40 000 Daltons, increases substantially when the amount of styrene is above 70 wt %. In fact, a copolymer synthesized with 90 wt % styrene and 10 wt % macroinitiator PVAc-T* lead to the formation of very nice films having an average molecular weight close to the 100 000 Daltons.

It was previously mentioned that mechanically robust materials are needed to support the high shear stresses in biological reactors,[8] but they also need to have functional groups to promote the affinity between the material and microorganisms. Thus, the PVAc part of the PVAc-*g*-PS copolymer may be transformed into PVOH to provide the hydrophilic domains. In fact,
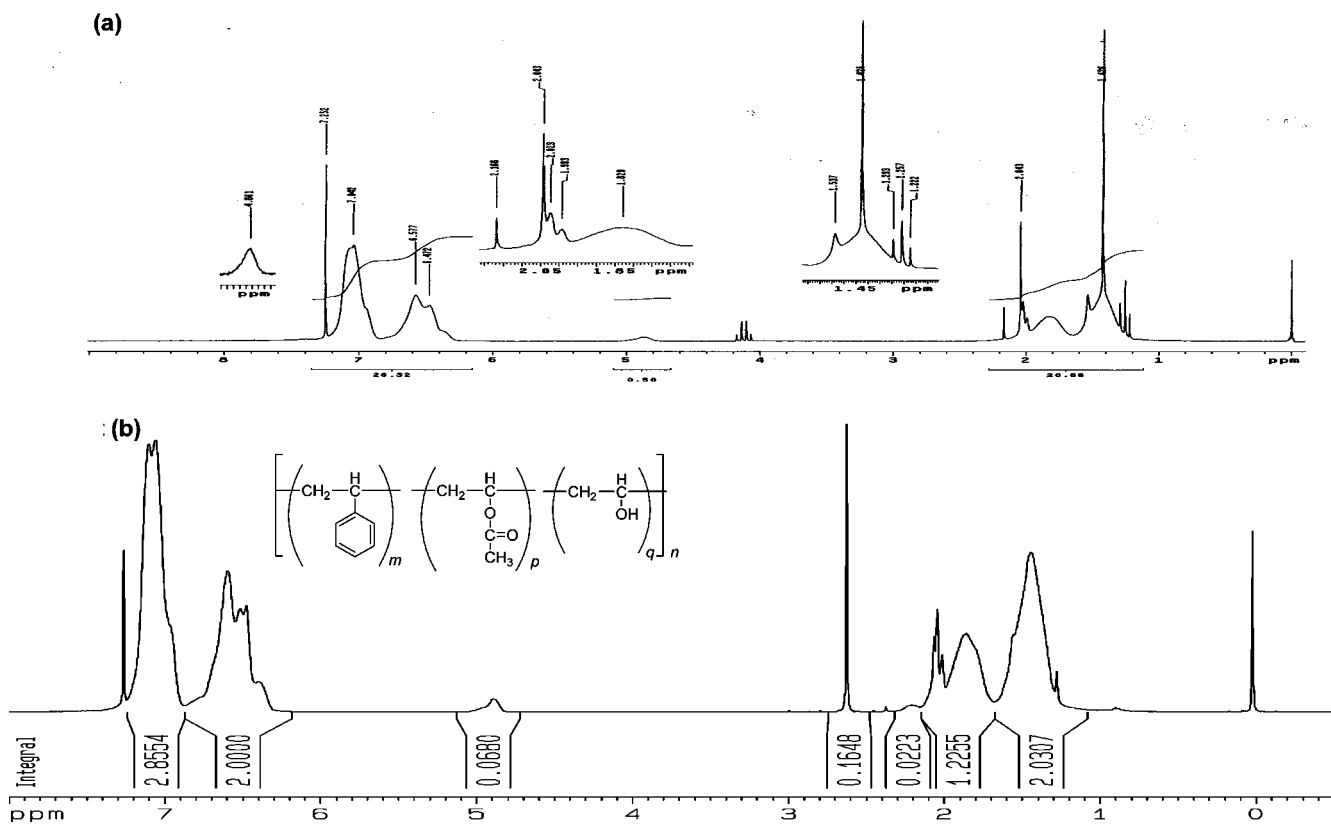
(a)

(b)

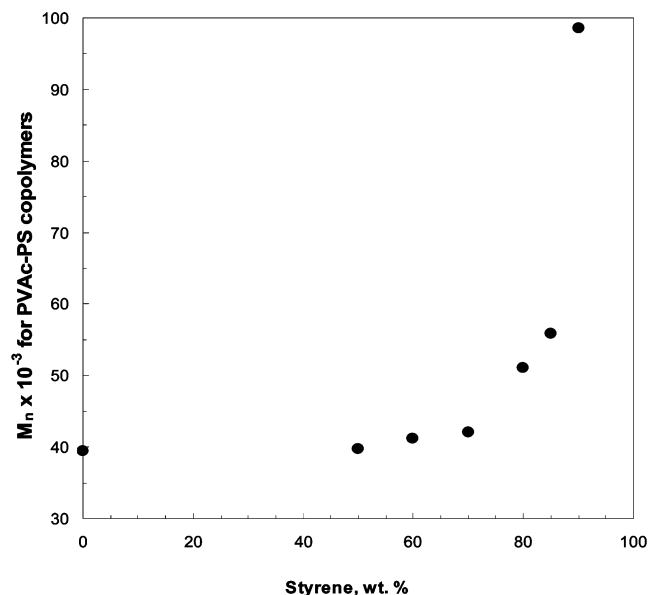**Figure 1.** ¹H NMR for (a) PVAc-*g*-PS and (b) PVAc−PVOH-*g*-PS.

Figure 2. Average molecular weight measured for the PVAc-*g*-PS copolymers as a function of the amount of styrene used in the synthesis of styrene with the PVAc-T* macroinitiator.

Figure 1b shows the ¹H NMR spectrum for a PVAc-*g*-PS copolymer synthesized with 90 wt % styrene and 10 wt % PVAc-T*, which was subjected to a methanolysis reaction for 1.5 h. The signals corresponding to the aromatic polystyrene fragments (6.40−7.10 ppm), methyl group of poly(vinyl acetate) units (singlet at 2.62 ppm), and hydroxyl group of PVOH (broadened singlet at 4.88 ppm) can be easily identified. Simple calculations using integral data show that the copolymer contains 88.76%, 6.21%, and 5.03% of PS, PVOH, and PVAc units, respectively. This result confirms that some of

the PVAc has been transformed into PVOH, and thus, a copolymer based on hydrophobic and hydrophilic domains can be formed. Of course, increasing the methanolysis reaction time and concentration of NaOH in the aqueous solution should lead to an increase in the amount of PVOH; however, this type of study is not the goal of the present work.

Similarly, to support the microorganisms the material must have pores that facilitate entrapment of the microorganisms and wastewater containing the nutrients the microorganism require for living.[15,16] Thus, to produce a porous material a phase inversion process using a mixture of solvents defined by a good (more volatile) and poor (less volatile) solvent system was selected.[17] For this work the PVOH-*g*-PS copolymer was first dissolved in a mixture of toluene/DMSO, and then films were formed by allowing slow evaporation of the mixture of solvents. Complete miscibility of the PVOH-*g*-PS copolymers was observed when a 77 wt % toluene and 23 wt % DMSO mixture of solvents is used for the dissolution process. Figure 3a is a low-vacuum SEM image of such films. A very homogeneous pore size distribution, in terms of both macro- and micropores,[15,16] is observed and they seem to be interconnected by a cheese-like structure. Figure 3b shows the topography of the same copolymers as viewed by atomic force microscopy. The diameter of the micropores is around 5 mm, which is in the same range of the typical size reported by a single living bacterium found in domestic or industrial wastewater supplies.

To produce the hydrogels the mentioned copolymer films were subjected to different freeze/thaw cycles.[18,19] Figure 4 shows DSC scans for the PVOH-*g*-PS hydrogels produced with three, five, and seven freeze/thaw cycles. There are two well-defined endothermic transitions, one located at 110 °C and the other in the range of 230−
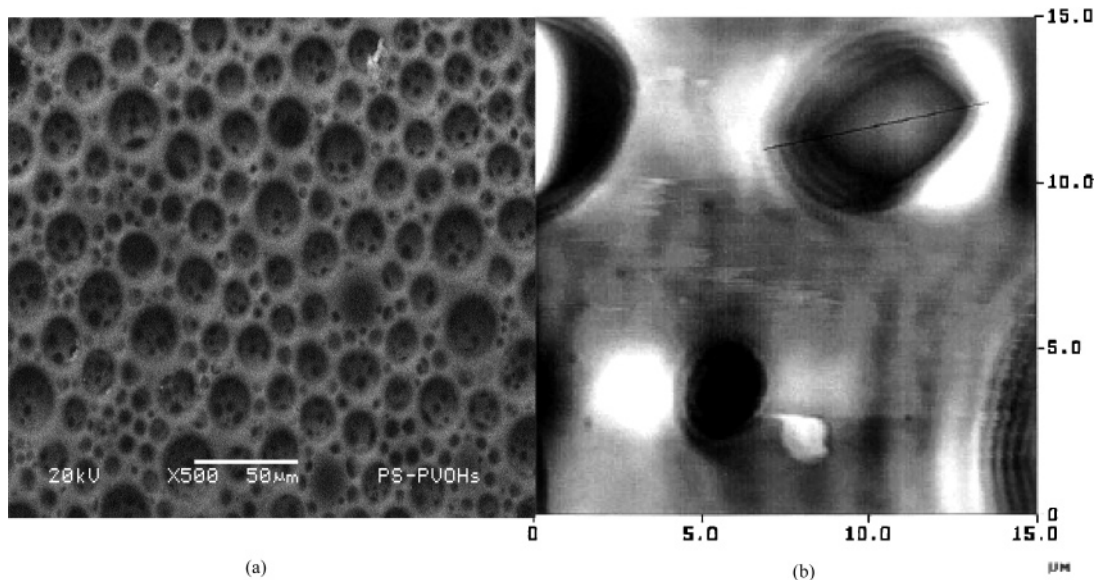
Figure 3. Low-vacuum SEM (a) and AFM (b) images shown for the dried PVOH-*g*-PS copolymer films.

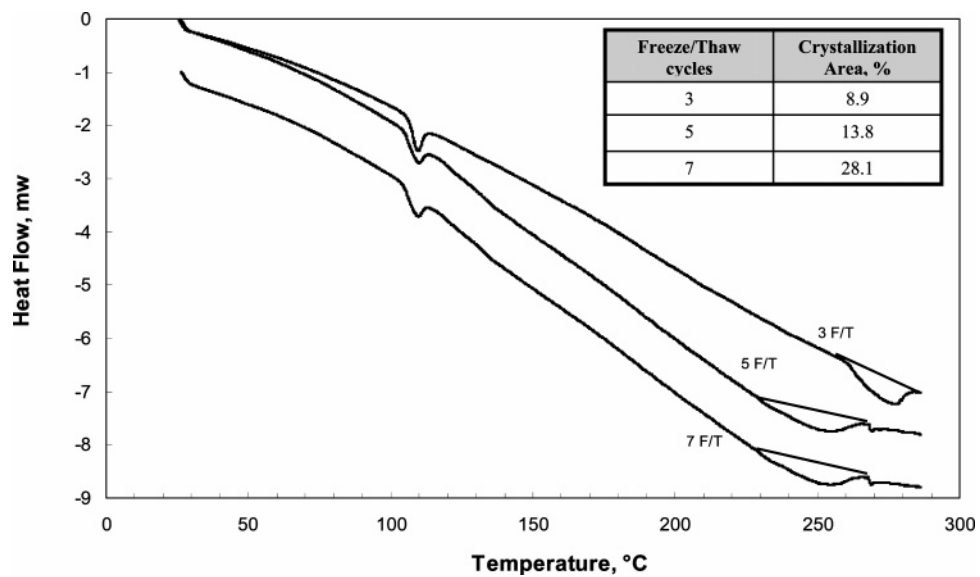| Freeze/Thaw cycles	Crystallization Area, %
3	8.9
5	13.8
7	28.1

Figure 4. Typical DSC scans for PVOH-*g*-PS copolymers that were subjected to three, five, and seven freeze/thaw cycles. The insert in this figure summarizes the increase in the crystallization area.
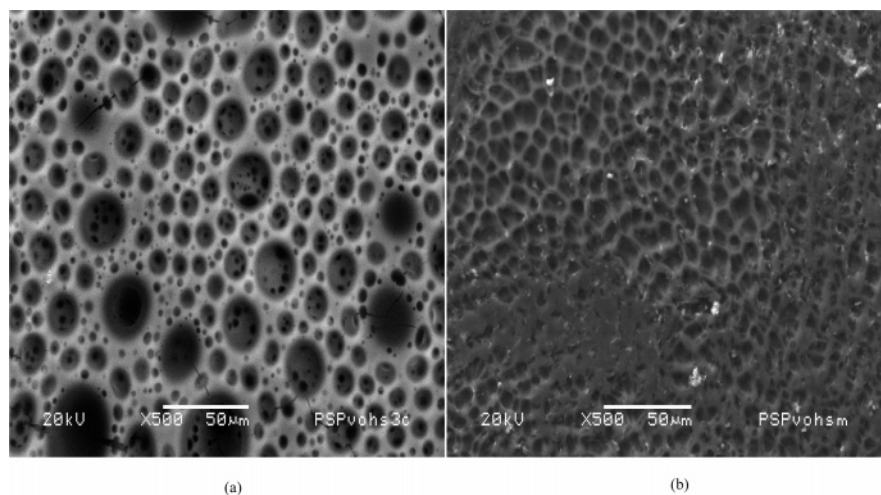

Figure 5. Low-vacuum SEM for PVOH-*g*-PS copolymers subjected to three freeze/thaw cycles: (a) dried material; (b) hydrogel (the PVOH-*g*-PS swollen copolymer containing water at equilibrium).
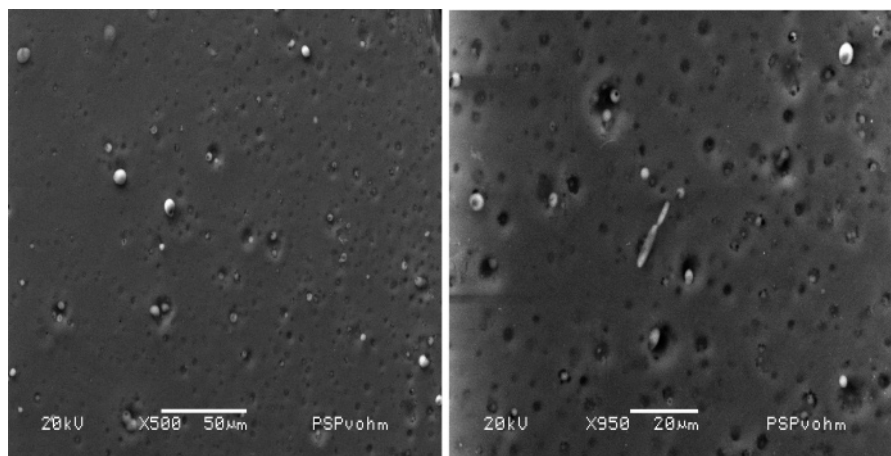
Figure 6. Low-vacuum SEM for PVOH-*g*-PS hydrogels that were subjected to three freeze/thaw cycles and after being in contact with wastewater containing microorganisms (bacteria are the white spots).

270 °C. The first transition corresponds to the glass-transition temperature of the PS domains, whereas the second one corresponds to fusion of the PVOH domains. The melting temperature for pure PVOH is reported as 250 °C, but this value depends on the average molecular weight of the PVOH. For example, Hassan and Peppas[19] reported melting endothermic transitions for pure PVOH hydrogels in the range of 190−230 °C, and the crystallization region for the PVOH-*g*-PS copolymer that was subjected to different freeze/thaw cycles goes, in general, from 230 to 280 °C. Of course, the presence of PS and the relatively high average molecular weight of these copolymers should be playing an important role. Since the interest of this work is focused on formation of a hydrophilic/hydrophobic hydrogel copolymer, the insert in Figure 4 summarizes the crystallization process of the PVOH domains reached in every freeze/thaw cycle studied. As observed in the insert, it is possible to produce hydrogels based on PVOH-*g*-PS copolymers since the crystallization area characteristic of the fusion of PVOH is increasing with the number of freeze/thaw cycles.

Figure 5a shows low-vacuum SEM images of a dried PVOH-*g*-PS copolymer that was subjected to three freeze/thaw cycles. Figure 5b shows the image of the same copolymer after contact with water. The image in part a shows a macro- and microporous material with a cheese-like structure that may be useful to accommodate water on its structure. In fact, the image in part b reveals the surface morphology of a water-swollen copolymer, which is typically termed a hydrogel copolymer.

Finally, Figure 6 shows low-vacuum SEM images of PVOH-*g*-PS hydrogles with three freeze/thaw cycles that were incubated in wastewater containing aerobic microorganisms. The bacteria are the white spots with a ca. 5 μm diameter. It is interesting to note that the microorganisms are residing on top of the material, see part a, and also inside the pores, see part b, of the hydrogel copolymers made up of a polystyrene matrix containing some crystalline domains of poly(vinyl alcohol). Careful examination inside and on top of some of the pores may lead to the conclusion that the microorganisms are also entrapped beneath a tiny film of the copolymer. In fact, from the experiments performed to test the affinity of the microorganisms for the copolymers, the number of microorganisms counted in three samples of copolymer with an area of 1 cm^2 each was 5

$\times 10^6 \pm 2 \times 10^2$ colony-forming units (UFC)/cm^2. This number is in agreement with the CFU reported for other hydrogels.[6]

Conclusions

Poly(vinyl acetate)-*g*-polystyrene copolymers synthesized by stable free-radical polymerization with TEMPO are useful materials to produce poly(vinyl alcohol)-*g*-polystyrene copolymers that lead to formation of hydrogels as determined by the crystallization process that suffers the PVOH domains when the number of freeze/thaw cycles is increased. Hydrogel copolymer films may be easily produced with macro- and micropores interconnected by a cheese-like structure via the slow evaporation of an appropriate toluene/dimethyl sulfoxide mixture. The resulting materials may be used to support and/or entrap aerobic microorganisms as demonstrated by the proliferation of bacteria observed by the low-vacuum SEM technique and counted after incubation with wastewater sludge.

Acknowledgment

Literature Cited

(1) *Polymer Gels Fundamentals and Biomedical Applications*; DeRossi, D., Kajiwara, K., Osada, Y., Yamauch, A., Eds.; Plenum Press: New York, 1991.

(2) Ratner, B. D.; Hoffman, A. S. *Hydrogels for Medical and Related Applications*; Andrade, J. D., Ed.; American Chemical Society: Washington, DC, 1976; Vol. 31.

(3) Okazaki, M.; Hamada, T.; Fujii, H.; Mizobe, A.; Matsuzawa, S. Development of Poly (vinyl alcohol) Hydrogels for Wastewater Cleaning. I. Study of Poly (vinyl alcohol) Gel as the Carrier for Immobilizing Microorganism. *J. Appl. Polym. Sci.* **1995**, *58*, 2235.

(4) Okazaki, M.; Hamada, T.; Fujii, H.; Kusudo, O.; Mizobe, A.; Matsuzawa, S. Development of Poly (vinyl alcohol) Hydrogels for Wastewater Cleaning. II. Treatment of N, N.-Dimethylformamide in Wastewater with Poly (vinyl alcohol) Gel with Immobilized Microorganisms. *J. Appl. Polym. Sci.* **1995**, *58*, 2243.

(5) Chen, K. C.; Lee, S. C.; Chin, S. C.; Houng, J. Y. Simultaneous Carbon−Nitrogen Removal in Wastewater Using Phosphorylated PVA-Immobilized Microorganisms. *Enzyme Microb. Technol.* **1998**, *23* (5), 311.

(6) Doria-Serrano, M. C.; Ruiz-Treviño, F. A.; Rios-Arciga, C.; Hernández-Esparza, M.; Santiago P. Physical Characteristics of Poly (vinyl alcohol) and Calcium Alginate Hydrogels for the Immobilization of Activated Sludge. *Biomacromolecules* **2001**, *2*, 568.

(7) Doria-Serrano, M. C.; Riva-Palacio, G.; Ruiz-Treviño, F. A.; Hernández-Esparza, M. Poly (N-vinyl pyrrolidone)-Calcium Alginate (PVP-Ca-alg) Composite Hydrogels: Physical Properties and Activated Sludge Immobilization for Wastewater Treatment. *Ind. Eng. Chem. Res.* **2002**, *41*, 3163.

(8) Eiselt, P.; Lee, K. Y.; Mooney, D. J. Rigidity of Two-Component Hydrogels Prepared from Alginate and Poly (ethylene glycol)-Diamines. *Macromolecules* **1999**, *32*, 5561.

(9) Vogelsang, C.; Wijffels, R. H.; Ostgaard, K. Rheological Properties and Mechanical Stability of New Gel-Entrapment Systems Applied Bioreactors. *Biotechnol. Bioeng.* **2000**, *70* (3), 247.

(10) Lee, K. Y.; Rowley, J. A.; Eiselt, P.; Moy, E. M.; Bouhadir, K. H.; Mooney, D. J. Controlling Mechanical and Swelling Properties of Alginate Hydrogels Independently by Cross-Linker Type and Cross-Linking Density. *Macromolecules* **2000**, *33*, 4291.

(11) Travieso, L.; Benitez, F.; Weiland, P.; Sanchez, E.; Dupeyron, R.; Dominguez, A. R. Experiments on Immobilization of Microalgae for Nutrient Removal in Wastewater Treatments. *Bioresour. Technol.* **1996**, *55* (3), 181.

(12) Chung, T. S.; Loh, K. C.; Tay, H. L. Development of Polysulfone Membranes for Bacteria Immobilization to Remove Phenol. *J. Appl. Polym. Sci.* **1998**, *70*, 2585.

(13) Park, E.-S.; Yoon, J.-S. Synthesis of Poly (vinyl acetate)-graft-Polystyrene and Application to Preparation of Porous Membranes. *J. Appl. Polym. Sci.* **2001**, *82*, 1658.

(14) Miwa, Y.; Yamamoto, K.; Sakaguchi, M.; Shimada, S. Well-Defined Polystyrene Grafted to Polypropylene Backbone by Living Radical Polymerization with TEMPO. *Macromolecules* **2001**, *34* (7), 2089.

(15) Lozinsky, V. I.; Plieva, F. M. Poly (vinyl alcohol) Cryogels Employed as Matrices for Cell immobilization 3. Overview of Recent Research and Developments. *Enzyme Microb. Technol.* **1998**, *23* (3), 227.

(16) Chen, J.; Park, H.; Park, K. Synthesis of Superporous Hydrogels: Hydrogels with Fast Swelling and Superabsorbent Properties. *J. Biomed. Mater. Res.* **1999**, *44* (1), 53.

(17) Bungay, P. M.; Lonsdale, H. K.; Pinho, M. N. *Synthetic Membranes: Science, Engineering and Applications;* NATO ASI Series C: Mathematical and Physical Sciences; Reidel Publishing Company: Dordrecht, Boston, Lancaster, and Tokyo, 1983; vol. 181, p 40.

(18) Hassan, C. M.; Peppas, N. A. Structure and Morphology of Freeze/Thawed PVA Hydrogels. *Macromolecules* **2000**, *33*, 2472.

(19) Willcox, P. J.; Howie, D. W.; Schmidt-Rohr, K.; Hoagland, D. A.; Gido, S. P.; Pudjijanto, S.; Kleiner, L. W.; Venkatraman, S. Microstructure of Poly (vinyl alcohol) Hydrogels Produced by Freeze/Thaw Cycling. *J. Polym. Sci., Part B: Polym. Phys.* **1999**, *37*, 3438.