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Saponified starch-*g*-polyacrylonitrile gels as carbon source in bacterial culturing

U. R. Gosavi[a,*], R.L. Deopurkar[b] and V.S. Ghole[a]
[a]*University of Pune, Department of Chemistry, Ganeshkhind, 411 007 Pune, India*
[b]*University of Pune, Department of Microbiology, Ganeshkhind, 411 007 Pune, India*
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Water-absorbing gels are being increasingly used in our daily lives. Some of these gels are synthesized by modification of starch. They are of the superabsorbing type. The chemical modifications to starch, involved in the synthesis of gels, are likely to present an environmental threat on account of the loss of its inherent biodegradability. Alkali hydrolysed starch-graft-polyacrylonitrile (HSPAN), a starch-based gel, was studied for biodegradability using indigenously isolated bacterial culture. Interestingly, the bacterial culture isolated in the laboratory used HSPAN as the sole source of carbon for its growth and progenesis. The microbial characteristics of the culture were Gram-positive rods with centrally located spores. © 1998 Elsevier Science Ltd. All rights reserved.

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INTRODUCTION

Superabsorbent gels are materials capable of absorbing large quantities of their own weight of water. They can be classified into two broad categories, namely those obtained from purely synthetic substrates and those obtained by chemical modification of biopolymers. The synthetic gels use acrylic acid, acrylamide, etc., as their substrates[1]. The modified biopolymer gels are prepared by chemical modification of starch[2] or cellulose[3]. Such gels are partially biodegradable and absorb comparatively lesser quantities of water than their synthetic counterparts.

Today, the biodegradability of materials in everyday use has achieved great prominence as a result of increased awareness of environmental pollution. Superabsorbents, capable of absorbing large quantities of water, present a great potential as water-retaining aids. HSPAN gels[4,5] are one such in the modified biopolymer category. Chemically, the starch backbone is modified with pendant side chains of polyacrylonitrile (PAN) by a process known as grafting. A need was felt to investigate whether such chemical modifications render the final produce biorecalcitrant and hence a consequent environmental threat. An ecofriendly way for management of superabsorbent gels could be achieved if, after use for their primary purpose (namely water absorption), the gels could be degraded either by bacteria, fungi, animal mastication and digestion or ultraviolet radiation-induced degradation.

Superabsorbing gels synthesized by modifying starch present a potentially nutritive media for growth of certain types of microorganisms. Thus starch chemically modified with some monomeric substrate, such as acrylonitrile, might create some difficulties *vis-à-vis* biodegradation of the modified starch. In this paper we report the utilization of HSPAN, a starch-based gel, as a sole source of carbon by the indigenously isolated bacterial culture.

* To whom correspondence should be addressed

EXPERIMENTAL

Materials

Potato starch and agar from Sisco Research Laboratories, India was used. Glucose, sodium chloride, sucrose, sodium nitrate, dipotassium phosphate, magnesium sulphate, potassium chloride and ferrous sulphate were obtained from Sd Fine Chem Ltd, India. Himedia Laboratories Pvt. Ltd was the source of beef extract, peptone and yeast extract. Phosphoric acid, ceric ammonium nitrate, AR grade nitric acid was from Qualigens Chemicals, India. Mycostain manufactured by Sigma Chemicals Company, USA was used. Acrylonitrile was obtained from M/s Modern Chemicals. Ethyl alcohol was of commercial grade and was distilled prior to use.

Synthesis of the gel

The starch-graft-polyacrylonitrile was prepared following the procedure reported by Fanta *et al.*[6]. Ten grams of gelatinized starch was reacted with 20 ml of acrylonitrile (ACN) using 0.6 g of ceric ammonium nitrate initiator dissolved in 1.0 N nitric acid. The reaction product was precipitated in ethanol and dried at 90°C. The dry mass was pulverized in a blender and saponified with 0.5 N sodium hydroxide and the saponified product precipitated with ethanol.

Characterization of the gel

The parameters of the synthesized copolymer were expressed on a weight basis using the procedure described by Vera-Pacheco *et al.*[7]. The amount of homopolymer formed was determined by extracting 2.0 g of the product within 100 ml of dimethyl formamide in a glass stoppered Erlenmeyer flask at room temperature with stirring for 24 h. The suspension was centrifuged, filtered through Whatman 40 paper, washed with 50 ml of ethanol and dried to a constant weight. The extracted homopolymer was calculated by the weight difference after drying the recovered polymer. The content of PAN in the copolymer was

determined by acid depolymerization of 2.0 g of the polymer with 125 ml hydrochloric acid (0.5 N) by refluxing for 2 h. The recovered precipitate was washed to neutrality with distilled water and dried to constant weight. The parameters established were conversion of monomer (%), grafting ratio (%), grafting efficiency (%), add on (%) and homopolymer content (%).

Isolation[8], purification[9] and maintenance of the culture

The bacterial culture was isolated from the water-swollen gel found contaminated, lying neglected in the laboratory. The culture was purified by repeated subculturing on nutrient agar of the following composition[8]: 1.0 g peptone, 0.5 g sodium chloride, 0.3 g beef extract solidified with 3.0 g agar in 100 ml distilled water. The pH was adjusted to 7.0. A pinch of mycostain was added to prevent fungal contamination. The culture was maintained on a nutrient agar slant[9] with frequent subculturing at 4°C for further experiments.

Culturing bacteria in HSPAN

The bacterial isolate was grown in Czapek Dox broth (CD broth) as the basal medium, wherein HSPAN replaced sucrose as a sole source of carbon. HSPAN (2.0 g) was allowed to equilibrate overnight in 200 ml sucrose-free CD broth (3.0 g sodium nitrate, 0.5 g magnesium sulphate, 0.5 g potassium chloride, 1.0 g dipotassium hydrogen phosphate and 0.01 g ferrous sulphate solubilized in 1000 ml distilled water) and then finally blended in a blender to a fine slurry and then autoclaved. Since the gel contributed an initial turbidity to this medium, growth was monitored by protein estimation.

Protein estimation by Lowry method[10]

The growth of the bacteria was monitored by Lowry's method for protein estimation as described by Lowry *et al.* Two millilitres of the bacterial broth was removed under aseptic conditions and the cellular protein from this sample was extracted with 2 ml 2N NaOH in a boiling water bath for 20 min before estimation by Lowry's method.

Microscopic studies[9]

A small quantity of the sample was drawn from the HSPAN–CD broth and was smeared on the slides. The smear was heat-fixed and was then stained using the standard Gram staining procedure.

RESULTS AND DISCUSSION

An HSPAN gel was synthesized by the procedure mentioned above and involves covalent crosslinking of acrylonitrile to random saccharide units in the starch. The ring structure of the starch is opened by the ceric ion, generating a free radical, and leads to covalent linking of polyacrylonitrile to the starch backbone. This polyacrylonitrile appears as a pendant side chain attached to the starch backbone. The ratio of starch to side chain PAN is important as regards water absorbing capacity of the synthesized copolymer. As the length of the side chain increases, there is a corresponding increase in the water-absorbing capacity. The copolymer that was synthesized using the starch:ACN ratio of 1:2 was found to have the maximum water absorbency of about 230 g per gram of dry gel and was thus selected for degradation studies. The properties of this gel are reported in *Table 1*.

In our study, the molecular weight of PAN could not be determined, for it was not completely soluble in DMF. On the contrary, we observed swelling of this isolated PAN sample in DMF. This indicates that some sort of cross-linking must have occurred within the linear PAN chains and is an area for further investigation.

The microorganisms isolated from the contaminated gel flask were cultured in a nutrient broth of the composition stated earlier. These microorganisms were purified on nutrient agar medium, isolated and transferred onto slants for preservation.

Biodegradability was ascertained by inoculating the CD broth containing HSPAN as the sole carbon source. As seen in *Figure 1*, the bacteria grew in the above medium as evidenced by rising protein content of the culture medium. As previously mentioned, direct turbidometric monitoring of growth was not resorted to as HSPAN gave a slight initial turbidity. Since the broth contained no carbon source other than HSPAN, the culture had apparently used HSPAN as the source of carbon for its growth.

The growth in liquid media (HSPAN–CD broth), assessed in terms of rising protein content, was confirmed by visualization of bacterial growth on solid medium such as CD agar. The utilization of HSPAN as carbon source by the bacteria grown in the HSPAN–CD broth was also confirmed using solid media. The culture was inoculated on CD agar containing HSPAN as the sole source of carbon; CD agar with sucrose as carbon source and CD agar without any carbon source (neither HSPAN nor sucrose) were simultaneously inoculated as controls. After incubating at 37°C for 24 h, the colonial growth was observed on CD containing HSPAN as the sole source of carbon and no growth was seen on CD agar free from carbon source (*Figure 2*). The culture grew normally on CD agar with sucrose as the carbon source. This clearly indicated the

Table 1 Parameters of the synthesised gel. Grafting of 20 ml acrylonitrile onto 10 g gelatinized starch[a]

Parameter	Value
(1) Homopolymer content (%) $= \dfrac{\text{Weight of non-grafted PAN}}{\text{Weight of PAN formed}} \times 100$	14.41
(2) Grafting ratio (%) $= \dfrac{\text{Weight of grafted PAN}}{\text{Weight of starch}} \times 100$	115.90
(3) Grafting efficiency (%) $= \dfrac{\text{Weight of grafted PAN}}{\text{Weight of the grafted PAN} + \text{weight of homopolymer PAN}} \times 100$	78.91
(4) Add on (%) $= \dfrac{\text{Weight of PAN in graft}}{\text{Total weight of copolymer}} \times 100$	53.94
(5) Monomer conversion (%) $= \dfrac{\text{Weight of PAN formed}}{\text{Weight of acrylonitrile charged}} \times 100$	85.51

[a] Conditions: 0.6 g ceric ammonium nitrate in 1 N nitric acid, time 2.5 h, temperature 27°C

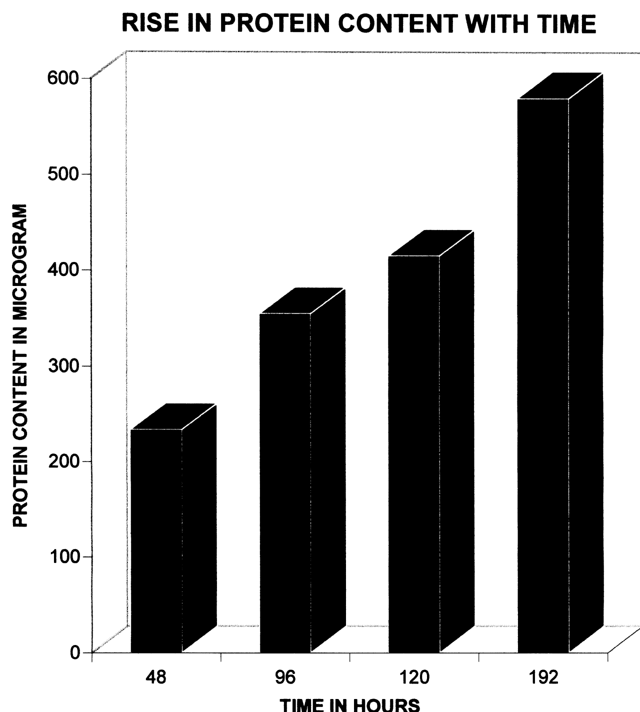

Figure 1 Graphical representation of rise in bacterial population as estimated by increasing protein content determined by Lowry's method. It is evident that the protein content increased from the first estimation after 48 h

Figure 2 Photograph showing growth of culture on HSPAN–CD agar containing Petri plates on the left. The Petri plate on the right shows no growth in the absence of a carbon source in the form of sucrose or HSPAN

ability of the culture to utilize HSPAN as the sole carbon source.

It was observed that the bacterial growth was initially slow on gel containing nutrient media and took about 72 h. When the cultures were repeatedly passaged through this gel, their growth hastened. After three to four passages, bacterial growth was observed within 24 h. This indicated that the bacteria adapted to the use of the gel for their metabolic needs.

This bacterial growth when observed under the microscope revealed that the culture was Gram-positive, of cocco-baccilary rods and with centrally located endospores (*Figure 3*).

Goheen and Wool[11] blended starch with polyethylene and showed using FTIR that the starch portion of the blend is degraded by aerobic microbes present in the soil. However, in their blend no chemical modification of the starch had been resorted to. HSPAN gels, as stated earlier, are chemically crosslinked and do not represent a sheer blend of starch and acrylonitrile. Dennenberg *et al.*[12] have shown that starch-graft-poly(methyl acrylate), in which covalent crosslinking had occurred, could be extruded into films and displayed excellent susceptibility to fungal growth.

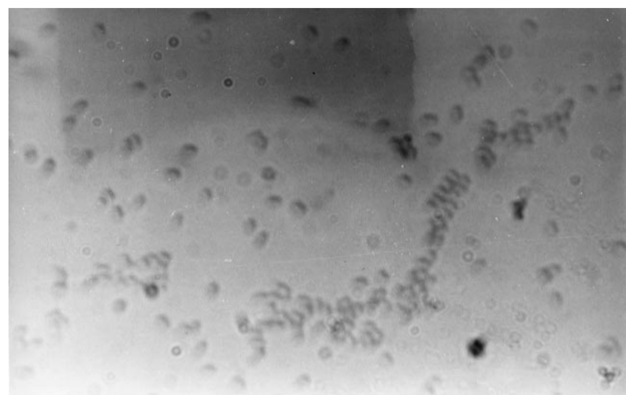

Figure 3 Photograph of the cocco-baccillary rod-shaped bacteria with centrally located spores. Gram staining has shown the bacteria to be Gram-positive

CONCLUSION

The growth of bacteria on synthesized HSPAN indicates that this starch-based gel is susceptible to bacterial attack in spite of chemical modifications to the saccharide parts. They are thus partially biofriendly. Since starch is crosslinked with polyacrylonitrile, it is highly improbable that the bacteria utilized the polyacrylonitrile portion of the gel. The use of HSPAN–CD broth and medium has proved conclusively that the carbon present in the gel is utilized by the bacteria for their metabolic needs.

It has been similarly reported by Dennenberg *et al.* that the starch-*g*-poly(methyl acrylate) copolymer can be degraded by fungus[12]. We feel that the starch backbone is being degraded rather than poly(methyl acrylate). In HSPAN too, the starch portion would be susceptible to microbial attack. This study, along with Dennenberg's, indicates a possibility of synthesizing starch-based gels with good water-absorbing capacity and biodegradability. It may be interesting to further investigate the effects of starch to ACN ratio on the biodegradability of the resulting HSPAN gel. Such gels will not be a severe environmental threat, which otherwise arises from the recalcitrant polymers.

It is possible that these gels may be partially biodegradable and shall only serve to delay the damage inflicted on the ecosystem. This presents an opportunity for the development of fully biodegradable superabsorbents. This group is at an advanced stage in perfecting the synthesis of a different gel that would be more fully biodegradable.

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