Microbial Degradation of Superabsorbent HSPAN Gel by an Indigenously Isolated Bacterial Culture

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ABSTRACT: The *N*,*N*′-dimethyl formamide (DMF) purified, alkali-hydrolyzed starch-*graft*-polyacrylonitrile superabsorbent gel (HSPAN) and its starch-free acid-depolymerized fraction were found to be capable of degradation by an indigenously isolated, naturally occurring, Gram positive cocco-baccillary rod-shaped bacterial culture, having centrally located endospores. This was evidenced by the ability of the culture to grow in a medium containing HSPAN and its fractions as the sole carbon or sole nitrogen source. Side chain polyacrylonitrile obtained by acid depolymerization of the starch-*graft*-polyacrylonitrile followed by alkali hydrolysis (AD−HPAN) was used by the isolated bacterial culture as source of nitrogen only. AD−HPAN was observed to be insoluble in water and DMF. Thus, viscosity changes that would occur on microbial degradation, were determined using alkali-hydrolyzed PAN synthesized without grafting onto starch (SYN−HPAN). Interestingly, SYN−HPAN supported bacterial growth by serving as a sole source of both carbon and nitrogen. The specific viscosity of SYN−HPAN exposed to the bacterial culture was lower than that of SYN−HPAN unexposed to bacterial culture. The degraded fragments of the gel could not be identified due to the complexity of the culture medium at the end of the experiment. Extrapolating the results of SYN−HPAN that was soluble in water, to the cleaved fragments of AD−HPAN, suggested that the side chain could indeed be degraded by selective cleavage. Gravimetric measurements on DMF-purified HSPAN and AD−HPAN showed that the former was degraded by about 94% while the latter was degraded by about 53% after 21 days of continuous culturing.

Introduction

Superabsorbents are capable of absorbing large quantities of water and are increasingly being used as water-retaining aids in products of personal hygiene,[1] seed coatings,[2] root ball tip coating,[3] bandages,[4] etc. It has been well-reported that HSPAN, a gel synthesized by grafting starch with polyacrylonitrile (PAN) followed by alkaline hydrolysis, has the ability to absorb large quantities of water.[5] The side chain of PAN can be alkali hydrolyzed to amide and carboxylate groups, a process that has been established using model compounds.[6−9]

The authors have already shown that HSPAN not purified by DMF extraction can serve as the sole carbon source for growth and proliferation of an indigenously isolated strain of bacteria.[10] The morphological characteristics of the culture were identified as Gram positive cocco-baccilary rods with centrally located endospores, apparently genus *Bacillus*. HSPAN synthesized by keeping a starch−acrylonitrile ratio of 1:2 with subsequent alkaline hydrolysis was characterized and found to have a water absorption capacity of about 230 times its own weight.[10] The homopolymer content was found to be 14.41% while the grafting ratio was found to be 115.90%. The grafting efficiency, percent add on, and monomer conversion were found to be 78.91%, 53.94%, and 85.51% respectively.

Living organisms exist as thermodynamically open, steady-state systems, exchange both energy and matter with their environment, and transform them into usable forms.[11] Matter transported across the cell wall from the surrounding environment is metabolized to obtain chemical energy, to degrade the available nutrients into building blocks of cellular components, to assemble these building blocks into proteins, nucleic acids, etc., and to form and degrade biomolecules essential for specialized functions of the cells.

Cells may be autotropic (use carbon dioxide as the sole source of carbon and construct carbon skeletons of their organic biomolecules) and heterotropic (obtain the carbon source in some more complex form from their environment).[11] Nitrogen, though abundant in nature, is chemically inert and cannot be used directly by most forms of life. The great majority of the organisms use nitrogen in some simple reduced or combined form such as nitrate or ammonium salts by transporting them across the cell wall. The microorganisms then utilize the nitrate or ammonium salts to synthesize amino acids within the confines of the cell that in turn are used to build nitrogenous cell components such as proteins.[11]

In this paper, we study the degradation of the two fractions of copolymer HSPAN, i.e., DMF-purified alkali-hydrolyzed SPAN (HSPAN) and the side chain PAN obtained after acid depolymerization of starch followed by alkali hydrolysis (AD−HPAN). These fractions were tested for bacterial utilization by incorporating them in carbon- and nitrogen-free basal media, as the sole source of carbon or the sole source of nitrogen. Alkali-hydrolyzed PAN synthesized without grafting to starch (SYN H−PAN), was used to study the viscosity changes that result due to bacterial degradation, as side chain H−PAN from HSPAN was insoluble in water or DMF. The weight loss of the two fractions of the copolymer, when maintained as a sole source of nitrogen in a continuous bacterial culture, was also studied.

Materials and Methods

Agar from Sisco Research Laboratories was used. Sucrose, sodium chloride, sodium nitrate, dipotassium phosphate, magnesium sulfate, potassium chloride, and ferrous sulfate

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were obtained from Sd Fine Chem Ltd. Himedia Laboratories Pvt. Ltd. was the source of beef extract, peptone, and yeast extract. Phosphoric acid and *N*,*N*′-dimethyl formamide was obtained from Qualigens Chemicals Ltd. Mycostatin manufactured by Sigma Chemical Co. was used. Acrylonitrile (ACN) was obtained locally from M/s Modern Chemicals. Ethyl alcohol was of commercial grade and was distilled prior to use.

Synthesis and Fractionation of the Gel. (A) Synthesis of the Gel. The starch-*graft*-polyacrylonitrile gel was synthesized using the procedure outlined by Fanta.[5] Gelatinized starch (10.0 g) was grafted with acrylonitrile (20.0 mL) using ceric ammonium nitrate (0.6 g) dissolved in 6.0 mL of 1.0 N nitric acid as an initiator, under an atmosphere of nitrogen gas for $2^1/_2$ h. A free radical was formed on starch, while the bond between carbon atoms 2 and 3 of the glucopyranosyl unit was broken. The free radical so formed on polymeric starch reacted with the acrylonitrile (ACN) monomer initiating graft copolymerization.[5] Thus, polyacrylonitrile (PAN) was grafted on the starch as a pendant side chain.[12] The reaction product, starch-grafted polyacrylonitrile copolymer (SPAN), was precipitated in excess ethanol and dried at 90 °C.

(B) Preparation of DMF-Purified HSPAN. The copolymer SPAN, synthesized as described in the earlier paragraph, was powdered in a blender and purified using the procedure described by M. Vera Pacheco et al.[13] Ungrafted homopolymer PAN was removed from 2.0 g of the powdered SPAN, using 100 mL of *N*,*N*′-dimethyl formamide (DMF), under constant stirring for 24 h at room temperature, in a glass-stoppered Erlenmeyer flask. Thus, only starch-grafted PAN remained behind as an insoluble mass. The suspension was centrifuged and the residue washed with 50 mL of ethanol and dried to a constant weight. The DMF-purified copolymer was alkali-hydrolyzed in a 0.5 N sodium hydroxide solution to convert the refractile nitrile groups of SPAN to acrylamide and to acrylic acid.[9] This is henceforth referred to as DMF-purified HSPAN. It has been established by Castel et al.[14] that, on completion of hydrolysis, the ratio of amide groups to acidic groups in the side chain PAN is approximately 3:1. The residual alkalinity was neutralized with dilute phosphoric acid; the copolymer HSPAN was precipitated in excess ethanol and dried at 90 °C.

(C) Preparation of AD−HPAN. Side chain PAN was recovered by refluxing 2.0 g of DMF-purified SPAN for 2 h in 125 mL of hydrochloric acid (0.5 N).[13] The starch portion of SPAN was hydrolyzed to soluble sugars by hydrochloric acid and the insoluble residue of side chain PAN recovered. This residue of side chain PAN was washed to neutrality with distilled water and dried to constant weight. The side chain PAN was subsequently alkali-hydrolyzed using 0.5 N sodium hydroxide solution. The excess alkalinity was neutralized using dilute phosphoric acid and the hydrolyzed mass precipitated in excess ethanol. This was subsequently dried to a constant weight at 90 °C and shall henceforth be referred to as acid-depolymerized alkali-hydrolyzed PAN (AD−HPAN).

(D) Preparation of SYN−HPAN. PAN was synthesized without grafting onto starch using the procedure described by Sorensen and Campbell.[15] A 22.0 mL aliquot of ACN was added to deareated water maintained at 40 °C under constant stirring for 10 min. Potassium persulfate (0.3 g in 10.0 mL of water) was added followed by sodium bisulfite (0.5 g in 10 mL of water) after 1 min. The reaction was allowed to proceed for 3 h; a white opalescent polymer formed, was filtered, and dried at 60 °C to a constant weight. The synthesized homopolymer was alkali-hydrolyzed in 0.5 N sodium hydroxide solution and precipitated using excess ethanol. This precipitate was dried to a constant weight and shall henceforth be referred to as SYN−HPAN.

Organisms and Growth Conditions.[16,17] The bacterial culture was isolated from the water swollen contaminated HSPAN gel, lying neglected in the laboratory. As has been stated earlier, organisms draw their nutrient requirements from the surrounding environment, which in this case was HSPAN. The culture isolated from the neglected water-swollen gel was obviously drawing its carbon and nitrogen requirements from the gel. The only way the culture could do so was to degrade the gel components into biomass useful for its progenesis. This culture capable of degrading HSPAN was isolated by enrichment procedure as described in our earlier paper[10] and was maintained at 4 °C on a nutrient agar slant[16] (1.0 g of peptone, 0.5 g of sodium chloride, 0.3 g of beef extract, and 3.0 g of agar in 100 mL of distilled water; pH 7.0). A pinch of mycostatin was added to prevent fungal contamination.

(A) Preparation of Bacterial Inoculum.[17] The isolated bacteria were rejuvenated in 5 mL of Luria−Bertani Broth[16] (tryptone 50 mg, yeast extract 20 mg, and sodium chloride 50 mg in 5 mL of distilled water) for 24 h in a shake culture. The growth of bacteria greatly depletes the nutrients in the broth, and this is referred to as spent broth. Five milliliters of the spent broth containing the rejuvenated bacteria was used as inoculum for all experiments. As this inoculum would be diluted with 100 mL of fresh broth, defined as per each experimental condition, any contaminant carryover nutrient from the spent broth would be subsequently diluted 20 times so as to be insufficient to support bacterial growth.

B) Growth on Water Swollen DMF-Purified HSPAN. DMF-purified HSPAN was employed as the sole source of carbon and as the sole source of nitrogen in the medium of two independent sets of Czapek Dox broth[16] (CD Broth). The standard composition of Czapek Dox medium is as follows 30.0 g of sucrose, 3.0 g of sodium nitrate, 0.5 g of magnesium sulfate, 0.5 g of potassium chloride, 1.0 g of dipotassium hydrogen phosphate, and 0.01 g of ferrous sulfate in 1000 mL of distilled water with pH adjusted to 7.0. DMF purified HSPAN was incorporated as a carbon source by eliminating sucrose from the Czapek Dox basal medium and retaining sodium nitrate as the nitrogen source. To study the use of DMF-purified HSPAN as a nitrogen source, sodium nitrate was eliminated and sucrose retained in the basal medium. Needless to say, the entire experimental apparatus, medium, and broth were autoclaved by steam (pressure 10 lb/in.^{2}) for 15 min prior to inoculation by bacteria. As stated earlier, DMF-purified HSPAN consists of only starch-grafted PAN (alkali-hydrolyzed) with absence of any ungrafted PAN. Hence, pure grafted copolymer was available for degradation studies.

Two grams of DMF purified HSPAN gel was equilibrated overnight in carbon-free or nitrogen-free CD broth as per the conditions of the experiment. The pH values of the carbon-free and nitrogen-free broths (100 mL) were adjusted to 7.0 and reconfirmed after 24 h. The broth, containing HSPAN gel (HSPAN−CD broth), was blended in a blender to a fine slurry, steam sterilized at a pressure of 10*l*b/in^2 and inoculated with 5% v/v of spent broth containing the rejuvenated culture. This was maintained as a shake culture at 37 °C for 24 h. It needs to be mentioned that, although the gel was blended, it retained its shape of small water-swollen globules in the water phase. When the shaking was stopped, these globules of the gel separated out by settling down.

Visual confirmation of bacterial growth was ascertained by streak inoculating a streaking loop−full volume of the shake culture on a sterilized, agar-solidified Petri plate of the same composition as the shake culture. The volume of the streaking loop approximates to 7 μL as its diameter is about 3 mm. This streak-inoculated Petri plate was incubated at 37 °C overnight. The dilution of 5 mL of spent Luria−Bertani broth with 100 mL of the corresponding HSPAN CD broth, the growth of bacteria in this diluted broth, and the subsequent streak inoculation of a Petri plate with a miniscule 7 μL of this used broth eliminated the probability of carryover nutrients aiding bacterial growth on the agar-solidified Petri plate. The bacterial culture was also inoculated on a control CD agar, free from sucrose or HSPAN as sole source of carbon, to rule out the possibility of the culture being autotropic or degrading agar for its growth. A similar nitrogen-free agar-solidified Petri plate was inoculated as a control for ascertaining that the bacteria were not fixing nitrogen from the air as a nitrogen source.

For continuous culture, 0.52 g of of DMF-purified HSPAN gel was finely ground and the powder equilibrated in distilled water. CD broth free from any source of nitrogen (150 mL)

was added to the water-swollen HSPAN gel, and this broth was sterilized. Bacteria were inoculated in one flask, and a second was maintained as control. The flasks, control and inoculated, were maintained as shake cultures at 37 °C. Every 3 days 50 mL of the broth in the flask was removed aseptically by a micropipet (capacity 1.0 mL) after allowing the globules of the gel to settle for 1 h. Fifty milliliters of fresh sterilized nitrogen-free broth of the same composition as that in the flask was aseptically replenished in both flasks (inoculated and uninoculated) for supplying fresh nutrients, and a similar experimental protocol was maintained. The pH was adjusted to 7 using sterilized 0.5 N potassium hydroxide solution.

A micropipet of 1 mL capacity was used so as to minimize the possibility of any gel particles being lost due to suction in a pipet of larger capacity, having a larger orifice diameter. When no growth was observed in the inoculated flask (absence of turbidity), the contents of both flasks were centrifuged at 10 000 rpm for 1 h to sediment both the gel particles and the bacteria. The residues of both the flasks were washed by resuspending in water and centrifuged at 2000 rpm for 15 min so as to sediment the gel particles only while the bacteria remained in suspension. The washed gels were then filtered through a weighed Whatman 40 filter paper and dried. The weight difference between the gel in the control and cultured flask was reported as the weight loss of the gel while the loss of weight in the control at the end of the experimental period was accounted for as the process loss.

(C) Growth on Water-Swollen AD−HPAN. AD−HPAN was incorporated in the CD medium as the sole source of carbon and separately as the sole source of nitrogen, replacing sucrose and sodium nitrate, respectively. It was equilibrated in CD broths using the same procedure described for DMF-purified HSPAN. Care was taken to maintain the pH of all media at 7.0. The broth was steam sterilized at 10 lb/in.^{2} pressure, inoculated with bacteria, and maintained as a shake culture for 24 h. The AD−HPAN, too, retained its globular water-swollen shape and tended to settle down on stoppage of shaking.

To visually ascertain the growth of bacteria, the procedure mentioned earlier was followed, i.e., streak inoculation of one loopful shake culture on a sterile agar-solidified Petri plate of the same composition as the shake culture and incubation at 37 °C overnight.

The procedure used for DMF-purified HSPAN continuous culture was repeated for AD−HPAN by replacement of HSPAN with AD−HPAN. This medium containing AD−HPAN as a sole nitrogen source was then inoculated with bacteria.

The FTIR studies for the exposed and unexposed fractions were also conducted to observe any change in their IR signature.

(D) Growth on Water Swollen SYN−HPAN. The use of SYN−HPAN was necessitated as the AD−HPAN was insoluble in water or DMF. Thus any change in viscosity or molecular weight as a result of microbial degradation could not be estimated for the insoluble AD−HPAN.

SYN−HPAN prepared as described earlier was incorporated in CD broth basal medium as the sole source of carbon or nitrogen, replacing sucrose or sodium nitrate. As already stated, sterilization of all apparatus medium and broths was done by the procedure defined earlier.

The SYN−HPAN too was used in the continuous medium over the same period as the sole source of nitrogen. As the SYN−HPAN was soluble in the medium, a periodic change of the medium could not be executed. The control and inoculated flasks were shake cultured at 37 °C over the same period as that for the HSPAN fractions, without change in nutritive medium or periodic adjustments to pH. At the end of the continuous culture period, the control and inoculated broth were centrifuged at 10 000 rpm for 1 h to settle the suspended bacteria.

Viscometric measurements were made using an Ostwald capillary viscometer. Measuring the efflux time that was required for a volume of SYN−HPAN solution to flow through the capillary viscometer was used for determination of solution viscosity. Only the specific viscosity of both control and inoculated supernatant solutions was calculated, since the complexity of the growth medium made it difficult to evaluate the average molecular weights. It has already been reported that the insolubility of side chain AD−PAN in DMF had prevented us in determining the molecular weight of the grafted PAN in SPAN in our earlier publication.[10]

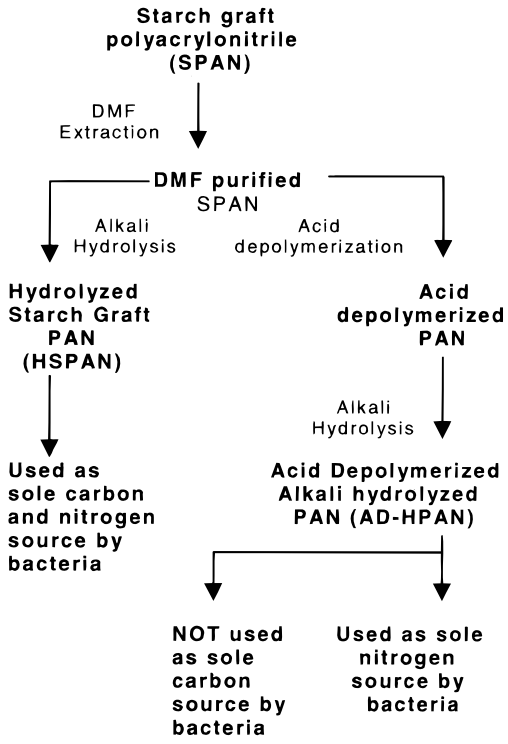

Figure 1. Sequence of fractionation of HSPAN gel and the various fractions used in biodegradation studies. PAN = polyacrylonitrile.

Protein Estimation by Lowry's Method.[18] Since the gel fractions imparted an initial turbidity to the medium, turbidometric measurements were rendered difficult, and the growth of bacterial population was monitored as a rise in protein content of the broth, using Lowry's procedure for protein estimation. As has been clarified earlier, the reduced or combined nitrogen from the surrounding environment only, in this case the surrounding environment being limited to DMF-purified HSPAN or AD−HPAN, would be used by the bacteria for the construction of their nitrogenous cell substances. Thus, there would be a corresponding rise in the protein content of the shake culture under study only if bacterial numbers were rising.

Two milliliters of the experimental shake culture broths were removed under aseptic conditions, and cellular protein from the individual sample was extracted with 2 mL of 2 N NaOH in a boiling water bath for 20 min and estimated by Lowry's procedure.

Estimation of Carbon and Nitrogen. The carbon and nitrogen content of the DMF-purified HSPAN and AD−HPAN before and after bacterial exposure was estimated on a Carbo Erba Instruments, Italy, elemental analyzer (Model CHNS-O EA1108).

Discarding of Used Culture Media and Petri Plates. All the bacterial cultures, used media, etc. were discarded only after autoclaving at 15 lb/in^2 pressure for 20 min as a routine microbiological safety procedure.

Results and Discussion

The purification and fractionation of the gel is summarized in Figure 1. The alkali-hydrolyzed DMF-purified HSPAN was studied for bacterial support as a sole source of carbon by inoculating bacteria rejuvenated in Luria−Bertani broth as a shake culture. The growth

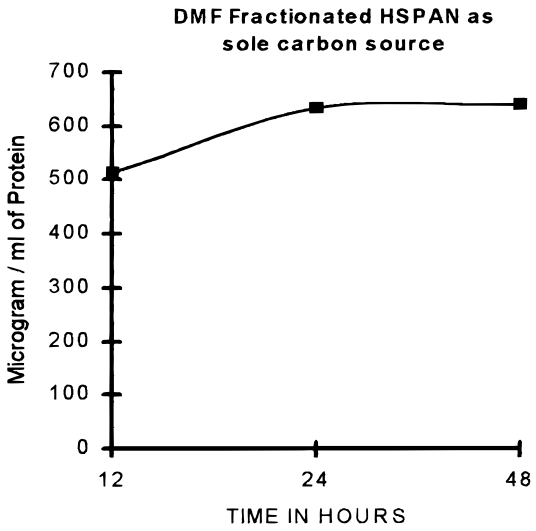

Figure 2. Rise in bacterial protein as monitored by Lowry's procedure wherein the sole source of carbon was DMF-fractionated HSPAN.

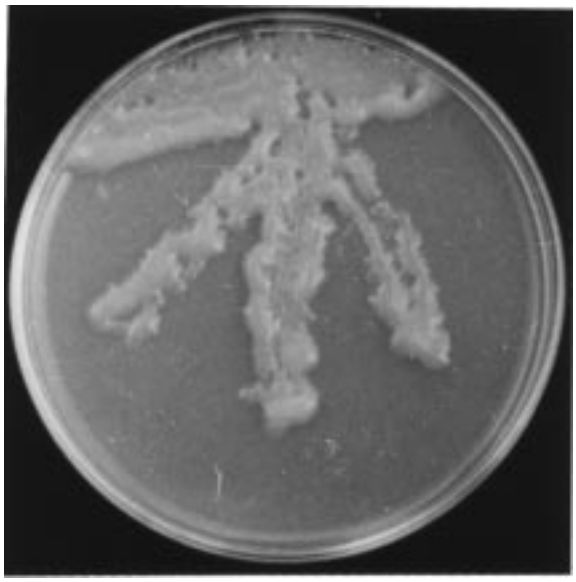

Figure 3. Plate culture photograph of bacterial growth using agar-solidified, DMF-fractionated HSPAN as the sole carbon source.

of bacteria was evidenced in terms of turbidity imparted to the medium. The increase in bacterial population was monitored in terms of rising protein content, and it was observed that the total protein content of the shake culture broth was indeed rising (Figure 2). Inoculation of a sterile agar-solidified Petri plate having the same medium composition as the shake culture, by a flame-sterilized inoculation loop dipped in the turbid shake culture, yielded a lush growth of bacteria on overnight incubation (Figure 3). Microscopic examination too had confirmed the presence of bacteria as reported in our earlier communication.[10] This visually confirmed that the turbidity was indeed due to growth of the bacterial culture. The procedure followed ensured that the carryover nutrients of the rejuvenating Luria−Bertani medium were depleted by bacterial growth, diluted 20 times when inoculated in the shake culture and were further reduced in the small volume of the loop (approximately 7 μL). The probability that any carryover nutrients of the rejuvenating medium supporting bacterial growth was thus eliminated. Even if we assume that some nutrients were carried over, the growth of bacterial population would be infinitesimal and not luxuriant as observed in Figure 3.

It is well accepted biologically that any substance capable of supporting a life form is first degraded by the organism and the degraded components are used as substrates for the enzymatic synthesis of compounds essential to the organism. By the same logic and the fact that the bacteria were not autotropic, i.e., they failed to grow on a control of carbon-free Czapek Dox medium using carbon dioxide from the air, the bacteria were indeed using components from the DMF-purified HSPAN as a source for building their carbon skeleton and increasing their population numbers. This also bolstered the contention that carryover nutrients from the rejuvenating Luria−Bertani broth were insufficient for bacterial growth. It can thus be clearly stated that DMF-purified HSPAN was degraded by the heterotropic bacteria occurring in nature, by using it as a sole source of carbon.

The fate of the starch-free AD−HPAN was studied by incorporating it in a sterile carbon-free CD medium. The bacteria were found to be incapable of using AD−HPAN as a carbon source as was observed by lack of turbidity in the shake culture and absence of growth on the agar solidified Petri plate. Matsumura[19] has reported that the poly(acrylic acid) having molecular weights below about 4000 only appear to be biologically active. That AD−HPAN comprises chains of acrylic acid interspersed with acrylamide units is well-reported. The AD−HPAN fraction was insoluble in DMF, indicating that its molecular weight was very high or the polymer had suffered some cross-links during polymerization. The absence of bacterial growth on the AD−HPAN as the sole source of carbon clearly indicated that the starch component of HSPAN served as a carbon source and that the side chain H−PAN was not usable as a carbon source.

The acrylamide groups interspersed along the side chain H−PAN could be potential sources of nitrogen, and their ability to serve thus required establishment. Experiments were performed wherein CD broth containing sucrose as carbon source and HSPAN as the sole nitrogen source was used as medium. Similarly, AD−HPAN was exposed as the sole nitrogen source in a CD broth of similar composition. It was found that DMF-purified HSPAN and AD−HPAN both supported the growth of bacteria as the sole source of nitrogen. The protein content of the broth too was found to rise in both cases (Figure 4). That the turbidity imparted to the medium and the rise in protein content was indeed due to bacteria was visually reconfirmed in a sterile agar-solidified Petri plates streak inoculated with approximately 7 μL of the respective turbid shake culture broth (Figures 5 and 6).

In other words the bacteria were sustaining themselves on DMF-purified HSPAN and AD−HPAN, using them for the synthesis of nitrogenous compounds required for their growth and proliferation. That the bacteria were capable of sustaining themselves on the various fractions of the gel as source of either carbon or nitrogen was now experimentally established. It remained to be seen whether the bacterial population was being sustained by degradation of the HSPAN gel into smaller particles or by mere modification to the end groups, keeping the polymer chain intact. Starch degradation by bacteria was a very well-established fact,

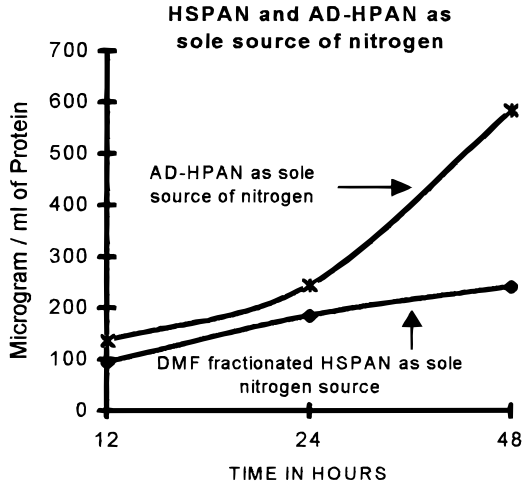

Figure 4. Rise in protein content as monitored by Lowry's procedure wherein the sole source of nitrogen was either DMF-fractionated HSPAN or H−PAN.

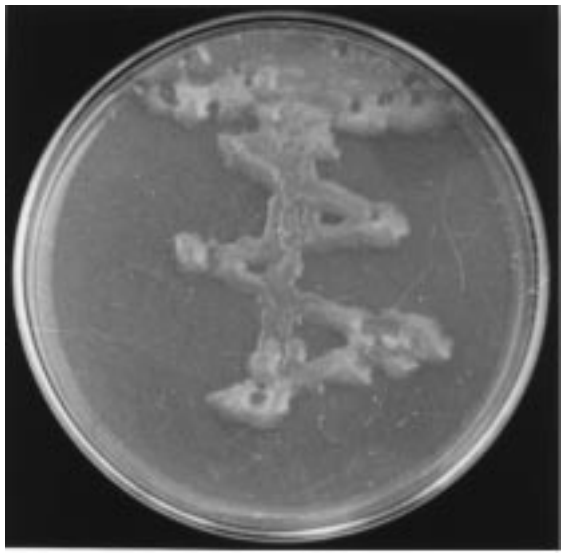

Figure 5. Plate culture photograph of bacterial growth using DMF-fractionated HSPAN as the sole nitrogen source.

and the degradability of side chain H−PAN needed exploration.

AD−HPAN is essentially an acrylic compound made up of acrylamide and the sodium salt of acrylic acid. Its utilization as a nitrogen source (though not as a carbon source) had been established by the above experiments. As has been stated by Lenz,[20] a molecular weight decrease should occur if the polymer is being degraded, and it becomes the single most important parameter of degradation.

The determination of any changes in viscosity or molecular weight on degradation by bacteria was hindered due to the insolubility of side chain AD−HPAN in either water or DMF. This insolubility in the two solvents stated was also the limiting factor for the methods usable for determination of degradation. Thus, SYN−HPAN that was soluble in water was used to study changes in specific viscosity that would occur in side chain H−PAN after bacterial degradation. The bacteria grew in the CD broth containing SYN−HPAN as the sole source of nitrogen (Figure 7).

Determination of the molecular weight of the SYN−HPAN after bacterial degradation was not possible as growth of bacteria caused depletion of the nutrient

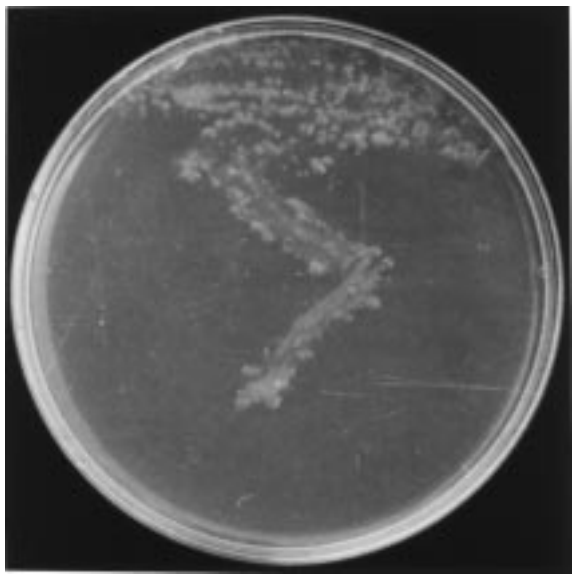

Figure 6. Plate culture photograph of bacterial growth using acid-depolymerized H−PAN as the sole nitrogen source.

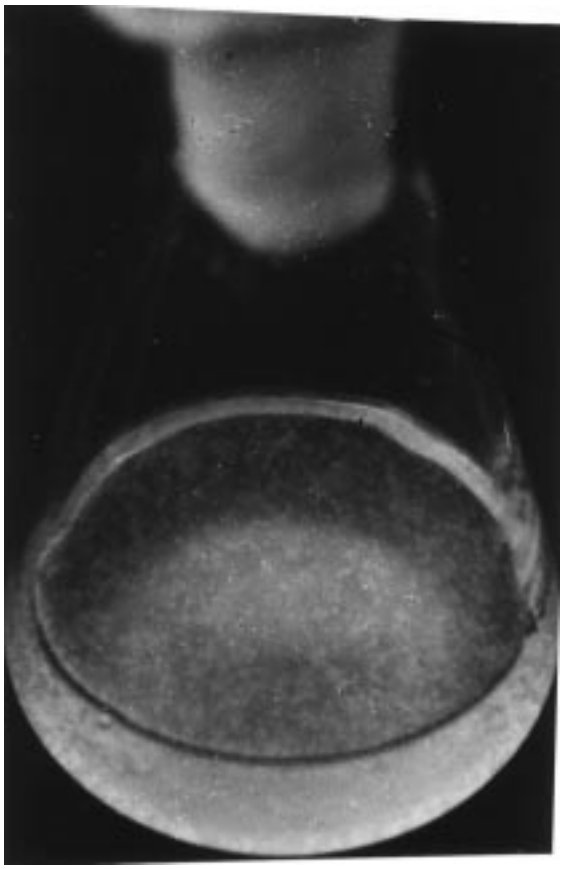

Figure 7. Shake culture photograph of bacterial growth, seen as globules, with synthetic alkali-hydrolyzed PAN as the sole nitrogen source. Synthetic alkali-hydrolyzed PAN also served as the sole carbon source for the bacterial culture.

components and addition of soluble metabolic byproducts in the broth. Thus, the composition of the broth would constantly change in the inoculated flask while remaining constant in the control. The presence of metabolic byproducts probably resulted in the pH of the inoculated broth dropping from 7 to 4. The metabolic byproducts would be absent in the control as no bacteria were inoculated and the pH remained 7. Precipitating of the soluble polymer after bacterial exposure was not

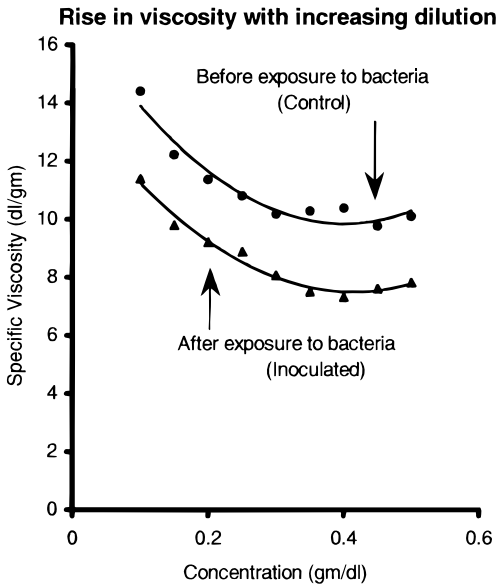

Figure 8. Graph of specific viscosity vs concentration clearly shows that the viscosity of the SYN−HPAN exposed to bacteria was lower than that unexposed to the bacteria at all dilutions. The figure also indicates the rise in viscosity with serially increasing dilution.

possible as metabolic byproducts and the polymer SYN−HPAN were soluble in water and precipitated with the same precipitating solvents. The next closest parameter to molecular weight, that of specific viscosity, was calculated to ascertain the degradation of SYN−HPAN. The centrifugate made free from bacteria was evaluated for viscometric changes. The specific viscosity of the control and inoculated broth was found to be 10.08 and 7.82 dL/g for a concentration of 0.5 g/dL, respectively, while, at 0.1 g/dL concentration, it was found to be 14.4 and 11.4 dL/g respectively (Figure 8). The specific viscosity of the control was found to be higher than that of the sample inoculated by bacteria at all concentrations ranging from 0.5 to 0.1 g/dL indicative of the fact that the bacteria were indeed degrading SYN−HPAN (Figure 8). The decrease in viscosity after bacterial exposure indicated the degradation of SYN−HPAN. Any further degradation would be hampered due to the bacterial growth being inhibited with pH changing from 7 to 4 as confirmed experimentally. This inhibition of bacterial growth would reflect as a stoppage of the degradation of SYN−HPAN. Investigation of SYN−HPAN not being the primary topic of this paper and its use being limited to as a model compound for establishing viscosity changes, no further investigation into its degradation was done.

The specific viscosity of polymeric materials is expected to decrease with increasing dilution. However, it was observed that the viscosity of SYN−HPAN rose with increasing dilution of the broth with distilled water (Figure 8). This phenomenon of rise in viscosity can be explained by the fact that the presence of multivalent ions such as Fe^{2+} and Mg^{2+}, as well as monovalent ions of K^+ in the CD broth, probably led to screening of the COO^- ions present on SYN−HPAN.[14] As the dilution is increased, the concentration of these mono- and divalent ions decreases, probably leading to a reduced screening effect. This would probably lead to a more open structure of the polymer chain that in turn would result in a resistance to flow through the capillary of the viscometer. This increased resistance to flow would translate into higher specific viscosity of both control and inoculated SYN−HPAN solutions. This indicated the presence of undegraded SYN−HPAN that was confirmed by FTIR. The viscosity measurements suggested polymer degradation, and weight loss studies were conducted to ascertain this manifestation.

Table 1: Gravimetric Assessment of HSPAN and AD−HPAN Degradation on Exposure to Bacteria[a]

	DMF-purified HSPAN	side chain AD−HPAN
	Control	
initial weight (g)	0.520	0.520
final weight (g)	0.421	0.467
	Process Loss	
loss (g)	0.099	0.053
	Inoculated	
initial weight (g)	0.520	0.520
final weight (g)	0.032	0.248
	Degradation	
% degraded	∼94	∼53
	Elemental Analysis	
% carbon		
initial	17.04	30.96
final	*b*	42.00
% nitrogen		
initial	0.37	3.31
final	*b*	0.37

[a] The nitrogen content of degraded DMF-purified HSPAN was not estimated as the nitrogen source was the side chain H−PAN, a component of HSPAN. [b] Not estimated.

The two fractions of the gel, viz. DMF-purified HSPAN and AD−HPAN, were exposed to bacteria by maintaining them as a continuous culture in CD medium having these gel fractions as the sole nitrogen source. It may be recalled that starch and not the AD−HPAN served as the carbon source. That starch is assimilated by the biosystem is too well established to bear repetition, and hence studies on the use of the gel as a carbon source were not conducted. Continuous culture experiments using DMF-purified HSPAN and AD−HPAN as the sole nitrogen source were monitored to establish their fate. Control flasks were treated similarly to those exposed to bacteria with the exception that they were not inoculated with bacteria. After a 21 day continuous culture, the 0.52 g of dry weight DMF-purified HSPAN gel was found to decrease to 0.421 and 0.032 g of dry weight for control and inoculated flasks, respectively (Table 1). The continuous culture could not be sustained beyond 21 days. The lower weight of the DMF-purified gel after bacterial exposure indicated that the bacteria were degrading it into smaller chains before assimilating the required compound.

The gravimetric studies with AD−HPAN indicated that the residue of the gel after bacterial exposure was 0.248 g while that for the control was 0.467 g (Table 1). In other words, about 53% is degradable and about 47% is not degradable. The residue of this AD−HPAN after 21 days of bacterial exposure is much higher than that of DMF-purified HSPAN, indicating that probably the low molecular weight segments of AD-HSPAN support bacterial growth whereas the higher molecular weight or cross-linked fractions are incapable of supporting bacterial growth. The polymer remnants were separated by filtration, washed thoroughly with distilled water, and dried to a constant weight. The dried remnants were then checked for their FTIR signature. The AD−HPAN gel fraction after continuous culture was found to have the same IR signature as that of the undegraded

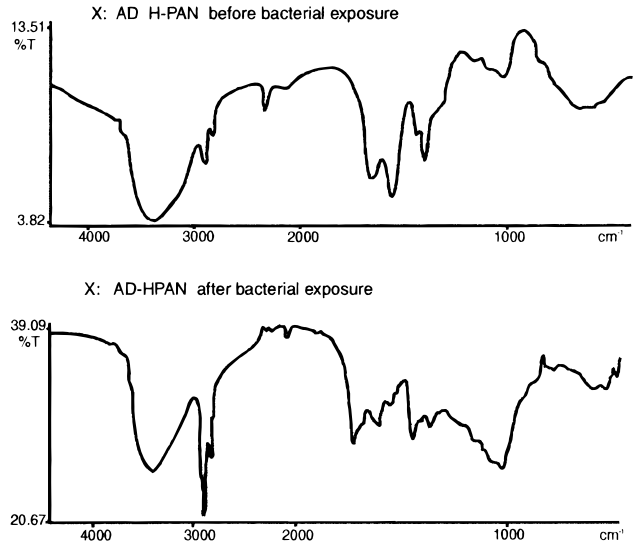

Figure 9. FTIR spectra of HSPAN before (top) and after exposure to bacteria in continuous culture (bottom).

AD−HPAN (control) indicating that the residue was largely that of the undegraded gel (Figure 9).

Table 1 shows the ample presence of carbon in both DMF-purified HSPAN and AD−HPAN. Carbon from AD−HPAN remained unutilized by the bacterial isolate and had been experimentally established earlier on in this paper. The carbon utilized by the bacterial culture in DMF-purified HSPAN was from the starch component, and biodegradation of starch is well established. Hence, estimation of the carbon content after bacterial action was not done. Interestingly, the percent carbon in AD−HPAN was found to rise from about 30.96% to about 42% on degradation. This rise of about 9% could be attributed to the entrapment of bacteria or bacterial metabolites in the nondegradable remnant AD−HPAN.

The nitrogen content of the degraded DMF-purified HSPAN was not estimated as the source of nitrogen was the side chain AD−HPAN and not the former. The nitrogen content of the AD−HPAN decreased dramatically from 3.31% to 0.37%. This dramatic decrease of about 89% clearly indicated the utilization of the nitrogen from AD−HPAN.

That the bacteria were simultaneously using starch as a carbon source is evident from the gravimetric results obtained from DMF-purified HSPAN. The weight fraction of starch in the DMF-purified gel was calculated as the weight difference between the DMF-purified gel before acid hydrolysis with that recovered after acid hydrolysis. When 2.0 g of DMF purified gel was acid hydrolyzed, the recovered product weighed 1.025 g. In other words, about 48% of the gel was composed of starch. Thus, in the sample of 0.52 g used for bacterial degradation studies, 0.25 g is starch and 0.27 g is PAN. It has been established that the bacteria degraded about 53% of AD−HPAN and that about 47% was not degradable. In other words, about 0.14 g is degradable from the 0.27 g of side chain PAN. The loss of HSPAN gel during processing was calculated from the control to be 0.099 g (Table 1). Substituting these values in the formula

$$\text{nondegradable HSPAN} = \text{weight of total gel} - \text{weight fraction of starch} - \text{weight fraction of degradable AD−HPAN} - \text{process loss}$$

the weight fraction of the nondegradable gel was calculated to be 0.031 g, which is close to the experimental value of 0.032 g. Expressing this in a percentage, 94% was degraded by the bacteria over 21 days in continuous culture and 6% remained nondegraded.

It needs to be pointed out that the complexity of the culture medium after bacterial exposure (the medium contained the byproducts of bacterial metabolism along with the soluble degraded components, insoluble gel fragments, and the unconsumed CD broth) severely hindered the attempts to isolate the degradation products. The added complexity was that both the bacteria and the soluble cleaved fragments were being removed during replenishment of the culture medium. As the bacteria, soluble-cleaved fragments, and metabolites existed in a dynamic state, assignment of quantitative figures to the degradation products would be an incorrect representation.

It was observed that the gel remnants in the CD broth after bacterial degradation were neither separable nor precipitated by excess ethyl alcohol, as was the case for nondegraded HSPAN and AD−HPAN. What was separable were the nondegradable globular remnants of the gel components. This probably meant that the gel fractions were degraded by selective cleavage to low molecular weight water-soluble fragments, not precipitable by alcohol. Studies with SYN−HPAN that was water soluble have shown that the specific viscosity of the SYN−HPAN solution decreases on exposure to bacteria. This indicated that the bacteria were capable of degrading water-soluble SYN−HPAN. Lenz[20] had defined that biodegradation of polymers involves random chain cleavage with corresponding change in viscosity and molecular weight. Reading our viscometric and gravimetric results with this definition of degradation, we observed that AD−HPAN appeared to have been selectively cleaved into fragments and would hence be classifiable as degradable. Thus the 94% loss of weight in DMF-purified HSPAN after bacterial action could be attributed to bacterial degradation of the HSPAN.

Conclusion

The grafting of starch with PAN and its subsequent alkaline hydrolysis contributes superabsorbance to the resultant gel without hindering the inherent biodegradability of starch. The gel used in the present study has starch cross-linked with PAN. The −CN groups in PAN are hydrolyzed to −COONa and $-CONH_2$ groups after alkali hydrolysis. Indigenously isolated bacteria that were determined to be Gram positive, cocco-baccillary rod-shaped and to have centrally located endospores utilized the amide nitrogen present on the gel for their metabolic needs. The ability of the bacteria to grow in a culture medium with the gel as the sole carbon source and as the sole nitrogen source underscores the degradability of HSPAN.

SYN−HPAN was used to determine the specific viscosity, and the same was found to decrease after its exposure to bacteria as a source of nitrogen. The increase in specific viscosity on dilution with distilled water indicated the presence of undegraded polymer SYN−HPAN in the continuous culture. Differential behavior of SYN−HPAN and AD−HPAN as a carbon source (only the former acting a carbon source) suggests that a more degradable superabsorbent gel can be synthesized by avoiding the probable cross-linking of the

pendant PAN side chains and by restricting the molecular weight to a more biologically active value.

It was found that the nitrogen content in AD−HPAN decreased after bacterial degradation, indicating its use by the bacteria. The percent carbon in AD−HPAN was found to rise after bacterial degradation and was probably due to the entrapment of bacteria or bacterial metabolites in the nondegraded AD−HPAN.

FTIR of the AD−HPAN sample after degradation had confirmed that it was the polymer remnants that were left behind after degradation. These would probably be the high molecular weight or cross-linked portions of the side chain H−PAN that were resistant to biodegradation. The weight loss observed on the DMF purified HSPAN and that in AD-HSPAN indicated that the gel was certainly being degraded by the bacteria to a smaller size by chain scissions. The results indicated that 94% of the DMF-purified HSPAN was lost after bacterial degradation whereas 6% remained behind as nondegraded remnant.

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