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Exopolysaccharide Production by a Marine Cyanobacterium *Cyanothece* sp.

Application in Dye Removal by Its Gelation Phenomenon

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Abstract

Cyanobacterium *Cyanothece* sp. ATCC 51142 has been shown to produce an exopolysaccharide (EPS) at a high level. EPS production was found to be influenced by the concentration of salt, pH, and type of nitrogen source. Maximum polysaccharide production was found to occur at a 4.5% (w/v) NaCl salt concentration, pH 7.0, and in the presence of $NaNO_3$ as the nitrogen source. The gelation of EPS in alkaline conditions was employed to remove the dyes from the effluents. The effect of organic molecules and metal ions on the efficiency of dye removal capacity was investigated. A laboratory-scale reactor was prepared to treat artificial textile effluent.

Index Entries: *Cyanothece*; cyanobacterium; dyes; exopolysaccharide; gelation; wastewater treatment.

Introduction

Because of the growth of industries in the last few decades, a wide variety of man-made (anthropogenic) compounds have been generated worldwide, and the production of such compounds is associated with the formation of large amounts of wastes. Although the process of industrialization is important for nations' prosperity, the pollution caused by the industries cannot be neglected. With the increasing awareness for the need to protect the environment, the treatment and disposal of industrial wastes have acquired great significance.

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The wastes generated by the food coloring, cosmetics, paper, and textile industries are polluted by dyes, which are relatively recalcitrant. When these colored effluents enter rivers or other bodies of water, they upset the biological activity. They can cause water-borne ailments such as nausea, hemorrhage, ulceration of the skin and mucous membranes, dermatitis, perforation of the nasal septum, severe irritation of the respiratory tract, and cancer, or may also enter the food chain *(1,2)*. Furthermore, such wastes are esthetically objectionable. Therefore, there is a considerable need to treat such effluents prior to discharge.

Although physical and chemical methods are available for the treatment of such wastes, they do not show any significant effectiveness or economic advantage *(3)*. Moreover, they are found to be resistant to biooxidation. Dye color removal by adsorption is a better treatment option *(4,5)*. The use of biological materials as adsorbents in the removal of color from either synthetic solutions or textile effluents has recently attracted much attention *(3,6,7)*. We have found that the marine cyanobacterium *Cyanothece* sp. ATCC 51142 possesses an ability to produce exopolysaccharide (EPS); EPS exhibits an excellent property of gelation that can be exploited for dye removal.

The present study deals with EPS production by *Cyanothece* under various conditions and describes the phenomenon of gel formation and its exploitation for removal of dyes from industrial effluents.

Materials and Methods

Microorganism

Cyanothece ATCC 51142 was obtained as a gift culture from Prof. D. O. Hall, King's College, London. It was maintained at 27°C in ASN III medium with a light/dark cycle of 16/8 h under fluorescent light of 3000 lux light intensity.

Growth and Polysaccharide Production

The EPS production and growth pattern of *Cyanothece* were studied under batch process. Growth of the culture was monitored spectrophotometrically measuring the optical density (OD) at 660 nm. Polysaccharide estimation was carried out by gravimetric analysis, and the results presented are in g% (w/v).

The effect of salt concentration, pH, and type of nitrogen source on EPS production was studied by modifying the ASN III composition as required.

Extraction of EPS

EPS was separated by centrifugation at 10,000*g* for 10 min at 4°C using 30-d-old *Cyanothece* culture grown in ASN III medium.

Table 1
Dyes Used

Dye	λ_{max} (nm)	Color	Type of dye
Porocion golden yellow	406	Golden yellow	Reactive
Porocion brilliant blue	604	Blue	Reactive
Porocion red brown	536	Brownish red	Reactive

Study of Dye Removal

Three textile dyes were used and are listed in Table 1 with their characteristics. Concentrations were determined with the HP-Diode Array UV-visible spectrophotometer (model no. 8452, Hewlett Packard) using their corresponding wavelength showing maximum absorbance (λ_{max}) of the dye. All dyes were used with an initial concentration of 1 mg/mL.

Adsorption experiments were conducted by mixing EPS solution (2 mL, 100 µg of polysaccharide/mL as measured by the method of Dubois et al. *[8]*) with an increasing quantity of dye solution, followed by addition of ammonia to make an alkaline condition (pH of about 11.0). This resulted in the immediate formation of gel, without any incubation. Samples were filtered and filtrates were checked for the presence of dye spectrophotometrically. The control experiment was performed without using ammonia.

Effect of Organic Molecules and Metal Ions

The dye removal capacity of EPS was studied in the presence of organic molecules, namely ethanol, acetic acid, formic acid, glucose, lactose, EDTA, and tartrate, and also in the presence of metal ions, especially sodium, potassium, mercury, cobalt, and zinc. In a typical experiment, 1 mL of any one of these organic compounds or metal salts (solution of 100 m*M*) was mixed with 2 mL (200 µg of polysaccharide) of EPS and 2 mL of Porocion golden yellow dye (1 mg/mL). A few drops of ammonia were then added to make an alkaline condition.

Reactor Study for Removal of Mixture of Dyes

A laboratory-scale glass reactor was prepared as depicted in Fig. 1. The reactor contained a graduated glass vessel of 500-mL capacity connected to a reservoir of EPS, a reservoir of artificial textile effluent, an acid container (50% [v/v] sulfuric acid), and a chamber containing ammonia solution. At the bottom an outlet was provided to remove filtrate or concentrated dye. The junction of the outlet and reactor vessel was packed with glass wool, which served as a filter. This allowed only filtrate to pass, not the gel. The reactor was also equipped with a pH electrode to monitor the pH.

Artificial textile effluent was prepared by mixing 10 textile dyes (Novatic blue BC S/D, Novatic jade green X BN S/D, Porocion golden yellow HR, Porocion blue H5G, Reactive blue 3R, Reactive black RL, Reactive

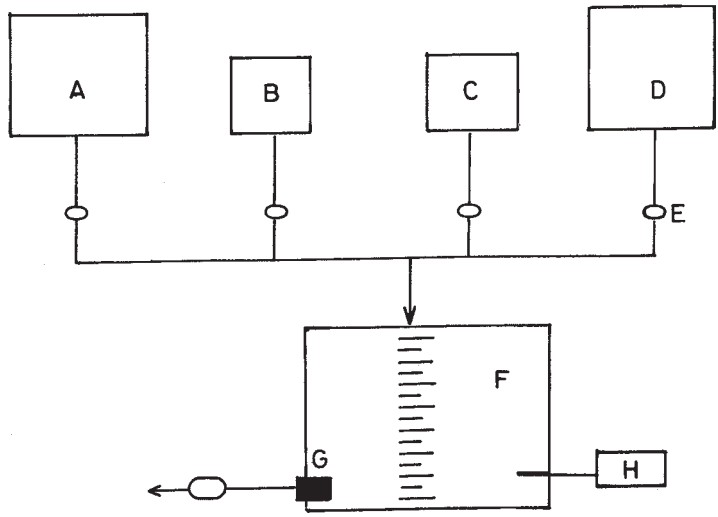

Fig. 1. Laboratory-scale glass reactor used to remove the color from artificial textile effluent using the EPS produced by *Cyanothece* sp. A, EPS reservoir; B, acid container; C, ammonia container; D, effluent reservoir; E, valve; F, graduated treatment reactor; G, glass wool filter; H, pH probe.

golden yellow, Porocion red H7B, Reactive black B, and Porocion red brown H4R) in distilled water to a final concentration of 25 mg/L each.

In a typical experiment, 100 mL of EPS and 100 mL of dye mixture were allowed to flow into the reactor, followed by addition of ammonia solution to obtain a pH of about 11.0. This resulted in gel formation. Treated filtrate from the reactor was allowed to flow out through the filter. After complete removal of filtrate, the gel was then mixed with 5 mL of acid to reduce the pH and solubilize the gel. This was collected as concentrated dye. All experiments were carried out in quadruplicate.

Results and Discussion

A cyanobacterium *Cyanothece* sp. was shown to have an ability to produce EPS that was released into the medium and could be separated easily by centrifugation. This EPS possesses an ability to remove color from textile effluents through the phenomenon of gel formation.

Effect of Physiological Parameters on EPS Production and Growth of Cyanothece

To determine whether production of EPS is growth associated, nongrowth associated, or mixed, we studied the growth and production profile in the batch process. In general, we found that the higher the growth, the better the EPS production, indicating that EPS production was growth linked.

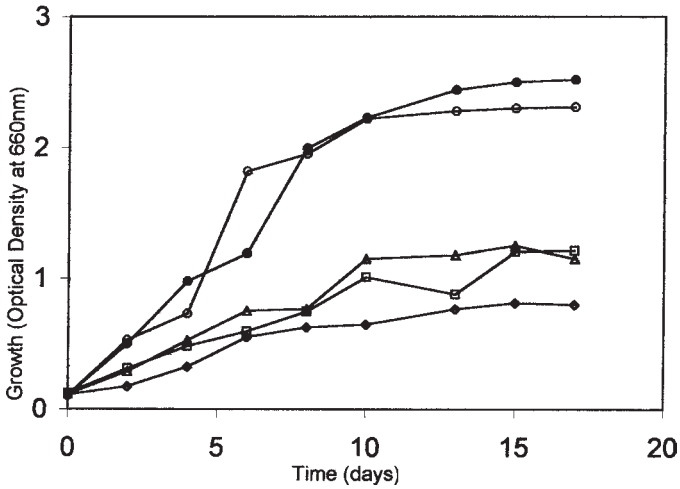

Fig. 2. Effect of different NaCl concentrations on growth of *Cyanothece* sp. ◇, 0%; □, 1.5%; △, 2.5%; ○, 3.5%; ●, 4.5%.

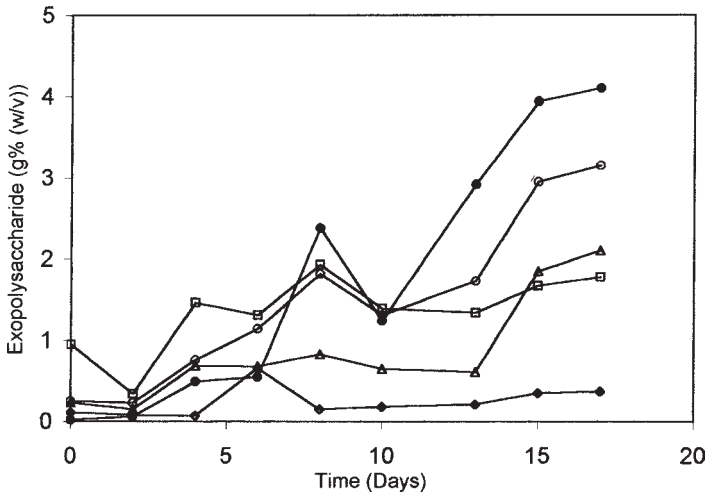

Fig. 3. Effect of different NaCl concentrations on EPS production by *Cyanothece* sp. ◇, 0 %; □, 1.5%; △, 2.5%; ○, 3.5%; ●, 4.5%.

In marine culture, the concentration of NaCl is critical. It was found that with an increase in NaCl concentration, the growth and EPS production increased proportionately (Figs. 2 and 3), reaching maximum at a 4.5% NaCl concentration. Nitrate is the source of nitrogen for marine cyanobacterium. Under nitrogen-limiting conditions, or in the presence of potassium nitrate, the growth and EPS production declined drastically compared with that in the presence of sodium nitrate (Figs. 4 and 5). pH also influences polysaccharide synthesis. Even though the optimum pH for growth is toward the alkaline side, maximum EPS production was seen at

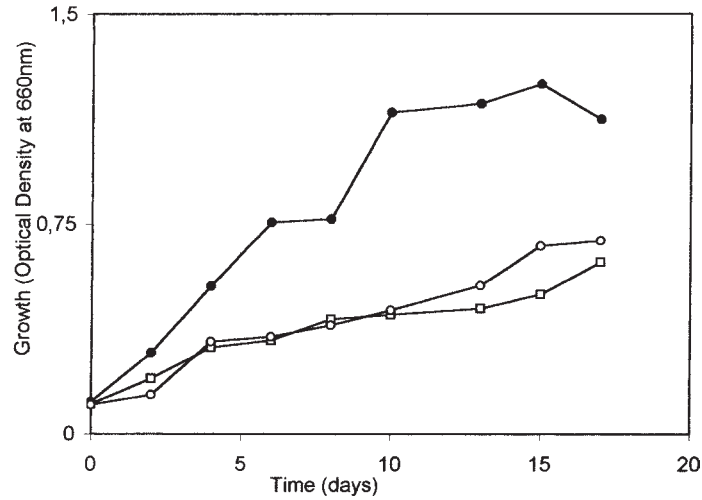

Fig. 4. Effect of nitrogen source on growth of *Cyanothece* sp. ●, $NaNO_3$; ○, KNO_3; □, nitrogen free.

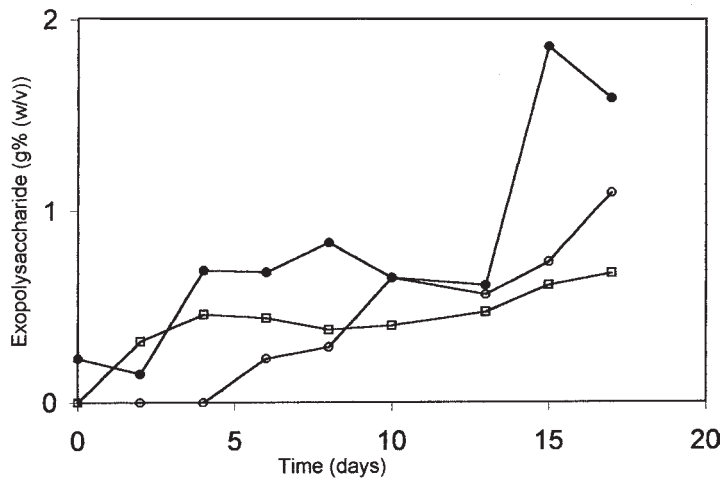

Fig. 5. Effect of nitrogen source on EPS production by *Cyanothece* sp. ●, $NaNO_3$; ○, KNO_3; □, nitrogen free.

neutral pH (Figs. 6 and 7). Our preliminary investigations revealed the presence of anionic groups, mainly OH^- and SO_2^-, in EPS. Thus, the production at a higher rate under alkaline conditions is unlikely.

Gelation of EPS and Dye Removal

The EPS of *Cyanothece* have anionic groups, mainly SO_2^- and OH^- (based on our infrared and nuclear magnetic resonance data and preliminary investigations). It seems that the phenomenon of gel formation is owing to simple ionic bridging of anionic groups by di- or trivalent cations. Similar

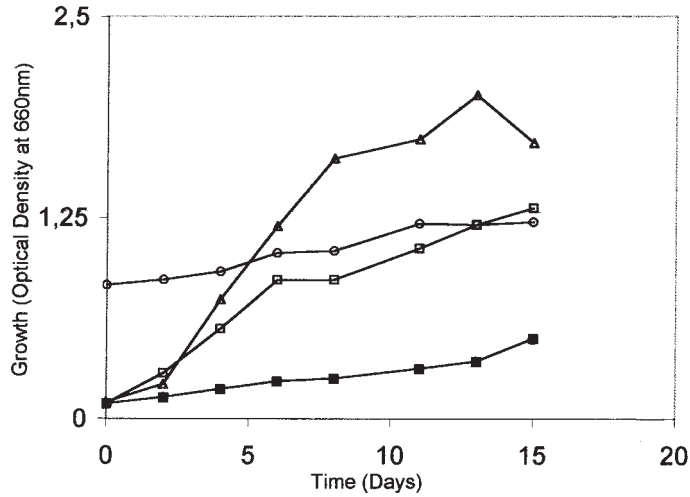

Fig. 6. Effect of pH on growth of *Cyanothece* sp. ■, 5.0; □, 7.0; △, 9.0; ○, 11.0.

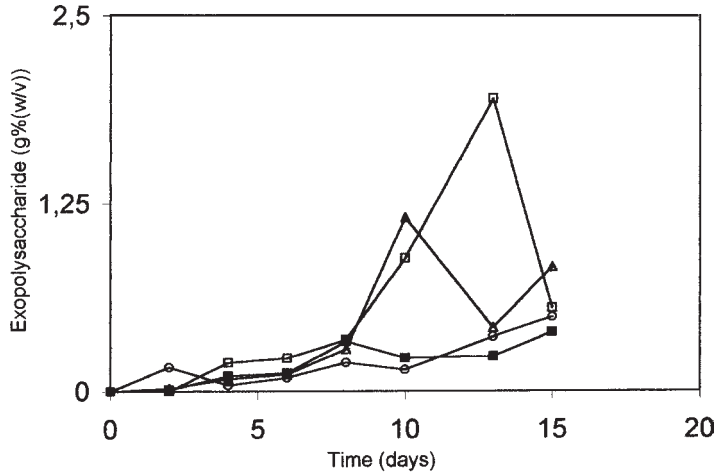

Fig. 7. Effect of pH on EPS production by *Cyanothece* sp. ■, 5.0; □, 7.0; △, 9.0; ○, 11.0.

gelation phenomenon has been reported by Choi *(9)* with polysaccharide produced by *Methylobacterium organophilum*. However, the gelation process by EPS of *Cyanothece* demanded alkaline conditions.

As shown in Fig. 8, maximum color removal was noted with Porocion golden yellow. The EPS can remove >90% of Porocion golden yellow even at a concentration of 5 mg of dye (5 mL of dye solution/2 mL [200 µg] of EPS). However, 200 µg of EPS could remove color of Porocion red brown and Porocion brilliant blue up to 80 and 71%, respectively, at a maximum concentration of 1 mg of dye (1 mL of dye solution).

We presume that dye may be entrapped within the gel during gel formation and also that some amount of dye may then be adsorbed on the gel.

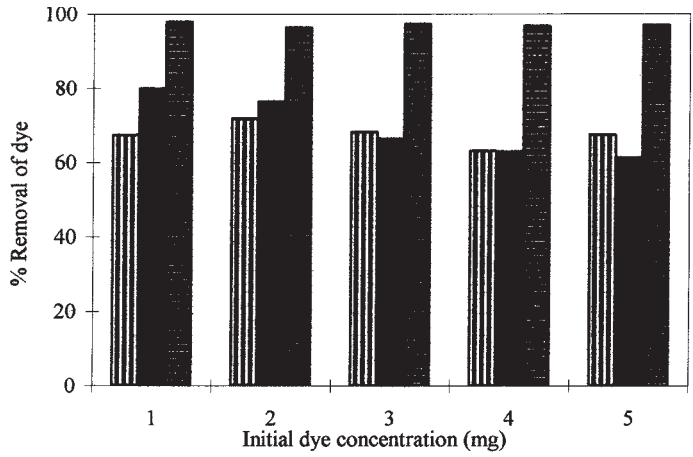

Fig. 8. Percentage of efficiency of EPS produced by *Cyanothece* sp. to remove color from suspensions at various initial dye concentrations. ▤, Porocion golden yellow; ▥, Porocion brilliant blue; ■, Porocion red brown.

The presence of organic compounds and metal ions was shown to influence the dye removal capacity of the EPS (Figs. 9 and 10). The presence of EDTA and tartrate completely prevented any gel formation and thus no dye removal was seen. The presence of organic molecules reduced the efficiency of dye removal in the following manner:

EDTA, tartrate > lactose > acetic acid > glucose > formic acid > ethanol

The presence of metals also reduced the dye removal capacity of EPS as follows:

$$Na^{+} > K^{+} > Hg^{+2} > Mn^{+2} > Ca^{+2} > Zn^{+2}$$

The effect of monovalent ions was drastic because it may bind to the anionic groups of the polysaccharide and prevent the bridge formation required for the gel formation, thus reducing the gel formation capacity.

In the laboratory-scale reactor, >98% of the color was removed from the artificial textile effluent. The economic viability of the process increased when the dye removed from the effluent was recycled. It was observed that on addition of acid, the gel was dissolved and the dye was liberated from the gel. We found that >80% of the dye that was adsorbed within the gel was recovered on addition of acid.

Several processes, such as chemical, biological, and physicochemical, including reverse osmosis, have been employed for color removal. Conventionally, coagulation and flocculation using Fe, Al, and Mg salts have been practiced for decolorization of dye-bearing wastewaters *(10)*. However, limitations of chemical coagulation-flocculation to produce good-quality effluent (in addition to sludge-handling problems, technical and economical constraints of reverse osmosis *[11]* owing to short membrane life, economical nonfeasibility and possible detrimental effects of chemical

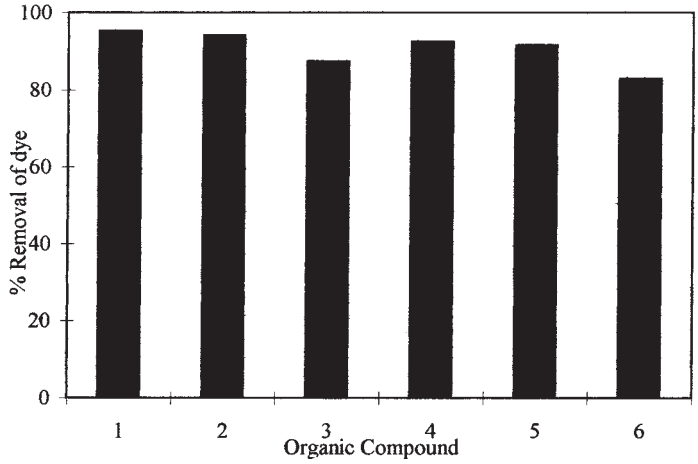

Fig. 9. Effect of different organic molecules (100 μ*M*/mL) on the percentage of efficiency of EPS produced by *Cyanothece* sp. to remove color from suspensions. 1, Control; 2, ethanol; 3, acetic acid; 4, formic acid; 5, glucose; 6, lactose.

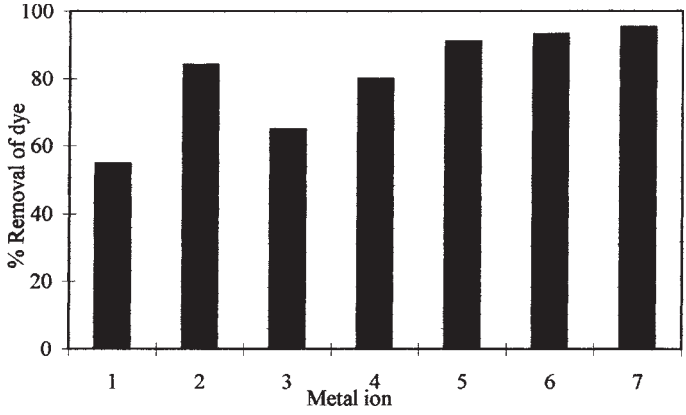

Fig. 10. Effect of different metal ions on the percentage of efficiency of EPS produced by *Cyanothece* sp. to remove color from suspensions. 1, Sodium; 2, manganese; 3, potassium; 4, mercury; 5, calcium; 6, zinc; 7, control.

oxidation, and ambiguity of applicability and shake flask status of biological processes *[12]*) make adsorption a more attractive alternative. The prohibitive cost of activated carbon limits the use of adsorption at least in developing countries. At lower concentrations of dyes, many support materials have been shown to be effective adsorbents *(3–7)*. Our study provides an alternative approach for quick and easier removal of dyes, even with high concentrations from the solutions, including industrial effluents, with an advantage of reusability of dyes.

Acknowledgment

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References

1. Ray, P. K. (1986), *J. Sci. Ind. Res.* **45,** 370, 371.
2. Liversidge, R. M., Lloyd, G. J., Wase, D. A., and Forster, C. F. (1997), *Process Biochem.* **32,** 473–477.
3. Namasivayam, C., Muniasamy, N., Gayatri, K., Rani, M., and Ranganathan, K. (1996), *Bioresour. Technol.* **57,** 37–43.
4. McKay, G. (1982), *J. Chem. Technol. Biotechnol.* **32,** 759–772.
5. Low, K. S., Lee, C. K., and Heng, L. L. (1994), *Environ. Technol.* **15,** 115–124.
6. Nassar, M. M. and El-Geundi, M. S. (1991), *J. Chem. Technol. Biotechnol.* **50,** 257–266.
7. Low, K. S. and Lee, C. K. (1997), *Bioresour. Technol.* **61,** 121–125.
8. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956), *Analyt. Chem.* **28,** 350–356.
9. Choi, J. H. (1998), *Biotechnol. Lett.* **3,** 253–255.
10. Lathia, S. G. and Joyce, T. W. (1978), *TAPPI* **61,** 67–70.
11. Cooper, P. (1993), *J. Soc. Colorists Dyers* **109,** 97–100.
12. Joyce, T. W., Chang, H. M., Campbell, A. G., Jr., Gerrard, F. D., and Kirk, T. K. (1984), *Biotechnol. Adv.* **2,** 301–308.