Bioinduced Changes in the Colored Nitrocellulose-Coated Cellophane Films, Induced by the *Shewanella* J18 143 Strain

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Received 3 September 2009; accepted 30 January 2010
DOI 10.1002/app.32212
Published online 10 June 2010 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Two types of the nitrocellulose (NC)-coated cellophane films, denoted 335 MS films (uncolored) and 335 MSC films (dyed with C.I. Direct Red 81), were incubated with *Shewanella* J18 143 for a period of 1 month at 50°C. The colored films were decolorized by *Shewanella* strain throughout this process. Changes in the NC coating of the films were studied by FTIR analysis, by determination of the surface wettability, and by ESEM evaluations. The colored films that were exposed to the *Shewanella* culture and decolorized, lost a significant amount of nitrate groups and became enriched in the hydroxyl group content. Moreover, the critical surface tension of the colored NC-coated cellophane films increased, from 18.7 mN/m, for the original films, to 33.1 mN/m, for the film that was treated by the *Shewanella* strain. Unlike the colored film, the uncolored NC-coated films did not give any considerable changes in their NC coating when exposed to the *Shewanella* culture, for the same time period. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 118: 2009–2014, 2010

Key words: biodegradable; films; waste; nitrocellulose; coating

INTRODUCTION

Regenerated cellulose packaging films

Regenerated cellulose (cellophane) films have been a huge success in the history of the packaging industry. Cellophane films are thin, flexible (when plasticized), and transparent materials derived from wood pulp, a natural fibrous form of cellulose.[1] Products made of regenerated cellulose are relatively easily degraded by a variety of cellulolytic microorganisms.[2,3] However, the application of surface coatings to the surfaces of the cellophane films impacts negatively on the ease of biodegradability of the coated films.

Cellulose-based films possess good barrier properties against assorted gasses. However, they show poor moisture resistance and heat-sealing ability properties. Therefore, in the majority of applications, the cellulose base film is further processed and coated with a thin layer of a barrier coating of nitrocellulose (NC), of copolymers of vinyl chloride and vinyl acetate, of copolymers of vinylidene chloride and of polyesters.[1] Having a thickness of less than 1 μm, the coating provides most of the properties that the packaging film requires with respect to its intended application. In achieving this, the base film is converted into a multilayer film, a noncompostable product, a cause of concern to those involved in waste disposal issues.

The increasing pressures on the manufacturing of packaging materials to ensure adequate, safe, and cost effective disposal of their packaging products in manufacturing waste-streams have led to concerted efforts being made to create compostable packaging systems that fulfil the global market's demands.[4] In the case of coated regenerated cellulose films, the concept of the compostable coating system leads to the need to create a coated base cellulose film that is digestible by a variety of microorganisms.

Degradation of NC ("cellulose nitrate")

NC is known to be resistant to microbial decomposition and to chemical decomposition. Waste NC is also considered to be a hazardous waste because of its explosive and flammability properties.[5] Depending on the degree of substitution per anhydroglucose unit of the cellulose, the physical and chemical properties of the nitrate ester can be varied.[6] The maximum value of the nitrogen content (14.14%) corresponds to cellulose trinitrate, a highly explosive material when exposed to shock, abrasion, or sparks. NCs having a lower nitrogen content (10.5–11.13%)

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Contract grant sponsors: Innovia films Ltd (Dr. C. Marshall).

have uses in paints, printing inks and varnishes, pharmaceuticals, photographic films, and artificial leather.[7] The lower nitrated cellulose is also used to coat regenerated cellulose base films.

The chemical and the biological degradation of NC wastes has been among the more challenging issues, with limited successes, for more than 2 decades.[8–18] Developing a technique for converting waste NC using microorganisms in the soil and seawater is an objective. Although cellulose and cellulosics are known to be relatively biodegradable,[2,3] any small changes in its molecular structure render it to be more resistant to biodegradation.[19–22] Depending on the degree of substitution and on the size of any substituted groups, enzymatic hydrolysis is retarded or effectively blocked.[23]

Chemical hydrolysis provides another option for the disposal of the cellulose nitrate. The efficiency of chemical digestion in sodium hydroxide solution depends on the temperature of the digestion, on the concentration of the alkaline medium, on the particle size of NC-containing waste, and on the time of the contact.[15] The by-products of alkaline hydrolysis are nitrate ions and nitrite ions with the ratio of 1 : 3. This ratio is independent of the alkali strength, the temperature, and the degree of digestion.[24] Denitrification of cellulose nitrate and the release of inorganic nitrite ions have also been observed by application of organic sulphydryl-containing compounds.[14] The chemical treatment of the NC is neither economical nor a complete process. Therefore, the materials left from the chemical hydrolysis route need further biological treatment for safe disposal to be realized.

Great effort has been put into the bioremediation of cellulose nitrate by various fungi and microorganisms to provide safe and economical processes for its disposal.[8–13,16,18,25] In all of these studies, an additional carbon source (electron donor) was present during the bioremediation of cellulose nitrate, with its inherent variable heterogeneity in the nitrogen content. Brodman and Devine[18] have shown that the *Aspergillus fumigatus* organism is capable of partial denitration of cellulose nitrate. Application of a naturally occurring fungus, Penicillium cory-lophilum Direckx, for transformation of NC (13.17% N) gave 20% utilization over a period of 3 days, providing that no other nitrogen source was available.[8]

Biotransformation of NC, as a contaminant in wastewater, has been studied in a mixed methanogenic enrichment culture.[11] As a consequence of this treatment, the nitrate content and the heat of combustion of the NC, processed in the growth culture, were decreased, leading to a less explosive compound. Apparently, the methanol-grown culture has the ability to transfer externally electrons to the insoluble NC particles. This work was continued into an investigation of the biotransformation of NC under denitrifying and sulphidogenic conditions.[10] Incubation of the NC (13.1–13.2%) in both enrichment cultures reduced the nitrogen content of the polymer to a value of 12.2–12.4%. This transformation was impossible in the absence of primary substrates (methanol for the denitrifying culture and lactate ions for the sulfidogenic culture). FTIR analysis indicated the occurrence of enrichment of the hydroxyl groups in the biologically exposed cellulose nitrate.

Study of the degradation of NC using varieties of lignocellulolytic and cellulolytic fungi gave considerable decreases in the amount of NC when the NC was used as the only source of nitrogen, with starch or carboxymethyl cellulose being the carbon source.[16]

Recently, published work on the transformation of NC concerned the application of a sulphate-reducing bacterium, *Desulphovibrio desulphuricans* 1388, which resulted in a 4.9–9.3% mass loss.[9] Here, nitro groups were cleaved from the β-D-glucopyranose chain and reduced to ammonium ions by the nitrate and nitrite reductases.[13] In other words, substitution of the nitrate groups by the hydroxyl groups took place.[25] Calorimetric results indicate that NC-induced changes in the metabolism of the bacteria were caused by both chemical (nitrate) and physical (biofilm formation) factors.[12]

Breakdown of the recalcitrant compounds by *Shewanella* bacteria

Shewanella strains exhibit remarkable respiratory versatility under anaerobic conditions, which makes them unusual among respiratory organisms.[26] When grown under anaerobic conditions, this bacterium locates the majority of its membrane-bound cytochromes to its outer membrane, a novel finding for bacteria.[27] Formation of the outer membrane cytochromes resulted in reduction of an extensive array of soluble electron acceptors, insoluble electron acceptors, and extracellular substrates by *Shewanella* strains.[28–31]

Certain strains of the *Shewanella* bacterium are known to be denitrifiers and to contain reductase enzyme that can consume nitrate ions and nitrite ions as the terminal electron acceptor.[32]*Shewanella* strain J18 143 has been previously used in reduction of colored compounds such as azo dyes, metal complex dyes, and azo pigments that have large molecular structure and are recalcitrant to bioremediation.

NC-coated, regenerated cellulose film can be considered as a potential source of nitrogen for metabolism by microrganisms. The objective of this work was to determine the effects of the *Shewanella* J18 143 strain on the breakdown of the coating of NC-coated cellophane films. Here, the breakdown of the

nitrocellusic coating was investigated both in the presence and in the absence of a reducing compound such as disazo dye to investigate whether or not their presence could promote reduction of the cellulose nitrate by enhancing the bacterial electron transfer in an anoxic environment.

MATERIALS AND METHODS

The chemicals used in this study were supplied by Sigma–Aldrich, Gillingham, Dorset, UK, unless otherwise mentioned.

NC-coated films

NC-coated regenerated cellulose films were provided by Innovia films, Wigton, Cumbria, UK. The uncolored 335 MS type films and the red-colored 335 MSC type films (dyed with C.I. Direct Red 81) were cut into 5.25 cm × 7.5 cm size strips and used in each experiment. A sample of the C.I. Direct Red 81, liquid dye was also used in a decoloration test using *Shewanella* J18 143 resting cells. The chemical structure of C.I. Direct Red 81 is given as Figure 1.[33]

Shewanella strain J18 143

In 1996, this strain was originally isolated from soil that was contaminated by textile wastewater.[34] The ability of *Shewanella* strain to reduce large molecular structures outside its cells walls has led researchers to use this organism for the decoloration of dyes and colorants in waste water streams. The reduction of reactive azo dyes was one of the studies conducted.[35] Samples from the bank of the *Shewanella* J18 143 strain (accession number of 96081,914)[36] were applied in this study.

Growth medium

Triptone Soy Broth (TSB) was prepared according to the manufacturer's instructions. One loop full of the *Shewanella* Strain J18 143 was transferred into 50 cm^3 of the sterilized TSB medium and left overnight (30°C, shaking at 200 cycles/min). Growth of this aerated culture was stopped after 16 h, when the cells were in their stationary growth position. The anaerobic growth culture was prepared by incubation of 9 cm^3 of the aerated culture in 90 cm^3 of the anoxic TSB for 2 h, to generate cells with physiological characteristics that suited the decoloration procedure.

Resting cells

Anaerobic growth cultures were centrifuged and washed twice with a phosphate buffer-saline (PBS) solution. The concentration of the resting cells was determined by measurement of the optical density of their diluted mixture (in the PBS buffer) at 600 nm using a Specord S100 spectrophotometer.

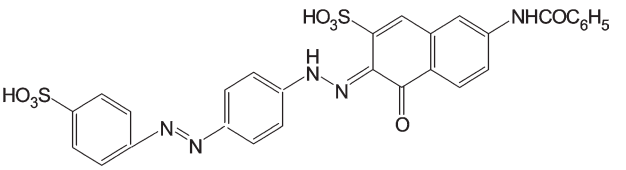

Figure 1 Molecular formula of the C.I. Direct Red 81 dye.

Reduction of C.I. direct red 81 using *Shewanella* strain J18 143 resting cells

Although *Shewanella* J18 143 has been previously used for decoloration of variety of azo dyes and pigmentary compounds, there was no recorded evidence that this bacterium is capable of reducing C.I. Direct Red 81. Therefore, a 100 μ*M* sterilized solution of C.I. Direct Red 81 was prepared and incubated with anaerobic cell suspensions of *Shewanella* J18 143 (5% of assay volume) under anaerobic conditions at 30°C. The absorbance of the dye solution was measured periodically at the λ_{max} for C.I. Direct Red 81 (507 nm) using a Specord S100 spectrophotometer.

Bioremdiation of NC-coated film using *Shewanella* strain J18 143 resting cells

In all of the studies, sodium formate (SF) was used as the electron donor, at a final concentration of 21 m*M*. Anthraquinone-2,6-disulphonate (AQDS), the exogenous extracellular redox mediator, was added to a final concentration of 100 μ*M*. NC-coated film was added to a 100 cm^3 vial containing 95 cm^3 of the medium (PBS, SF, and AQDS). For each study, a cell-free control was allocated to eliminate all the effects that medium could have on the films. Vials were loosely sealed with Teflon-butyl rubber septa. After sterilization, each cap was completely sealed. Nitrogen gas was bubbled into each vial for 10 to 15 min to remove all the oxygen that was present in the head space of the vials or dissolved in the medium. Using a microsyringe, specific amounts of known concentration of the resting cells were added to the vials, as appropriate. Samples were incubated for 1 month at 50°C in an oven. The resultant films after washing and drying (at 40°C) were subjected to FTIR analysis, determination of surface wettability and environmental scanning electron microscopic evaluation.

ANALYTICAL METHODS

Fourier transform infrared spectroscopic analysis

Samples of NC-coated, cellophane film from each study, were analysed using a Perkin-Elmer Fourier

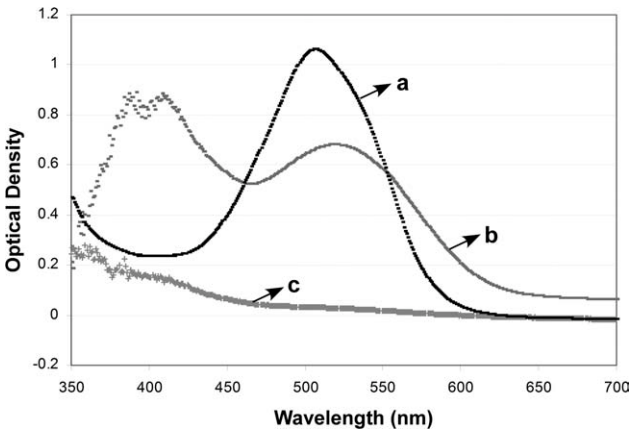

Figure 2 Changes in the optical density of 100 μ*M* dye solution of C.I. Direct Red 81 after incubation with *Shewanella* J18 143 resting cell under anaerobic condition, (a) preincubation, (b) 5 min after incubation, and (c) 16 h after incubation.

Transform Infrared (FTIR) spectrophotometer. Before the analysis, the NC-coated film samples were dried at 40°C overnight, to ensure that any hydroxyl groups from the bound aqueous moisture could not interfere with the result. The characteristic vibrations related to the nitrate groups of the NC coating can be identified at 820 cm^{-1}, 1272 $cm^{-1,}$ and 1631 cm^{-1} of the FTIR spectrum. To eliminate the effects of the amount of samples used in the FTIR analysis, all of the spectra were normalized, based on the intensity of the 2919 cm^{-1} (stretch vibrations of —CH_2 group), allowing comparisons to be made.

Critical solid surface tension evaluation

The Zisman method for ascertaining the surface tension of a solid substrate was used.[37,38] The procedure consists of application of series of liquids with known surface tension to the solid substrate and measurement of the contact angle between the liquid and the solid surface. Plotting cosines of the contact angle (θ) against the surface tension of the respective liquid and extrapolation of the data to cos (θ) = 1 (zero contact angle) gives the basis of the critical surface tension of the substrate. Measurement of the static contact angles was achieved using a Contact θ-meter, (Pearson-Panke, Ltd, UK).

Environmental scanning electron microscopy

Analysis of the films was carried out using a Phillips XL30 ESEM ODP environmental scanning electron microscope (ESEM). One problem in the application of the ESEM technique for the analysis of the NC-coated films is the sensitivity of films to the electron beam, causing degassing of the samples at higher electron beam currents. Therefore, an accelerating voltage of 5 kV and vacuum of 0.4 Torr were chosen for the analysis. Under these conditions, the electron beam of the ESEM did not damage the films.

RESULTS AND DISCUSSION

Figure 2 shows changes in the optical density of the 100 μ*M* solution of C.I. Direct Red 81, before incubation with cell suspension (a), 5 min after incubation (b) and 16 h after incubation with *Shewanella* cell culture (c). According to this figure, the reduction of the dye solution started by discoloration of C.I. Direct Red 81 (shift in the λ_{max} from 507 nm to

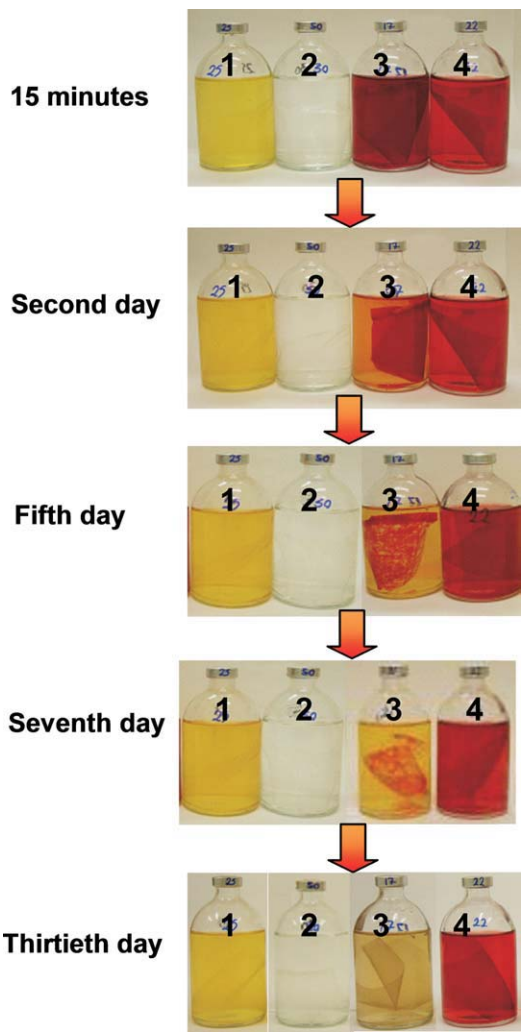

Figure 3 Changes in the NC-coated cellophane films that were incubated with *Shewanella* J18 143 resting cells for the period of 1 month at 50°C. Here, No.1 and No. 3, respectively show vials that contained cells and the 335 MS film (uncolored) or 335 MSC film (colored 335 MS). No.2 and No.4, respectively, relate to the samples from the cell-free control studies. The time period of incubation was as indicated. The resultant films after washing and drying (at 40°C) were subjected to environmental scanning electron microscopic evaluation, to the determination of their surface wettability, and to FTIR analysis. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

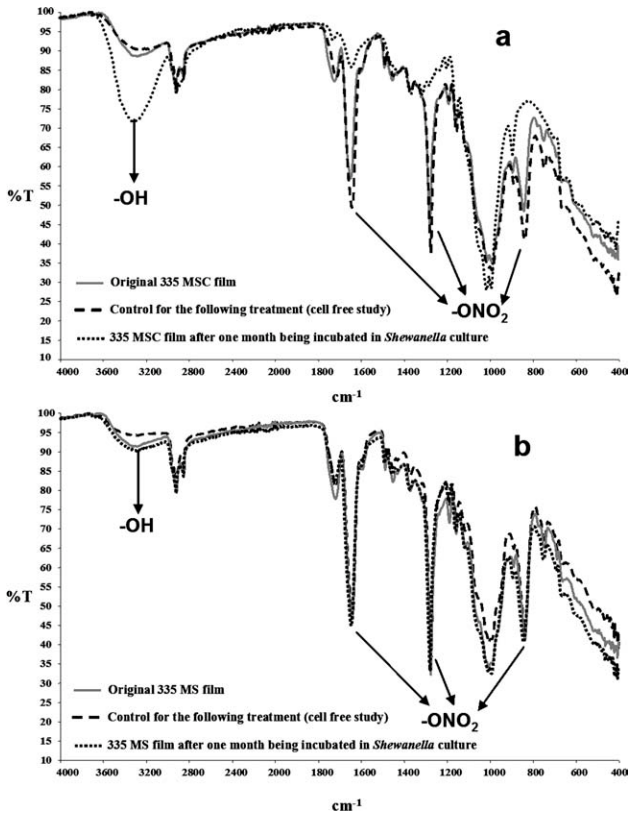

Figure 4 FTIR analysis of the NC-coated cellophane films, (a) shows the changes over the colored 335 MSC film and (b) shows the changes over the 335 MS film. All the spectra are normalized based on the $-CH_2$ group (2918–2919 $cm^{-1)}$ having an absorbance of 0.1.

523 nm) and then continued until the dye solution become completely colorless. This shows that *Shewanella* J18 143 is able to reduce C.I. Direct Red 81 dye solution, under anaerobic condition.

In the next step, changes in the NC-coated cellophane films that were incubated with *Shewanella* J18 143 resting cells were monitored for the period of 1 month at 50°C (Fig. 3). Here, the terms No.1 and No. 3, respectively, relate to vials that contained cells and the 335 MS film (uncolored) or 335 MSC film (colored 335 MS). No.2 and No.4, respectively, are the cell-free control studies. As Figure 3 illustrates, *Shewanella* strain J18 143 resting cells were able to decolorize completely the colored 335 MSC films in a period of 1 month.

Investigations of the chemical changes that occurred to the NC coating of the resultant films of 335 MS and 335 MSC were carried out. Figure 4(a) compares typical FTIR spectra of the original 335 MSC films and that of the 335 MSC films that were treated with *Shewanella* cells. The control is also represented. Figure 4(b) shows a similar comparison for the uncolored NC-coated film (335 MS). Figure 4(b) indicates that there was no significant nitrate reduction occurred in the coating of the uncolored coated

TABLE I
Critical Wetting Tension (γ_C) for the Typical NC-Coated Cellophane Films (Colored and Uncolored) and for Those That Were Treated with *Shewanella* Cells and their Controls. The Zisman Method Was Applied for γ_C Evaluations That Are Presented in this Table: A, Original Film. B, Films That Were Incubated in Cell Free Medium for a Period of 1 Month. C, Films That Were Incubated with Resting Cell Suspension of *Shewanella* J18 143 for a Period of 1 Month

	Critical wetting tension (γ_C) mN/m at different stages		
Film sample	A	B	C
335 MS	22.73 ± 0. 41	28.25 ± 0.91	28.43 ± 0.11
335 MSC	18.65 ± 0.29	28.61 ± 0.49	33.14 ± 0.08

film treated with the *Shewanella* J18 143. The spectrum of the film from the cell-free study (control) was very similar to the spectrum of the original film. Unlike the spectrum of the uncolored film, Figure 4(a) shows a significant nitrate-peak reduction at 1272 cm^{-1} and at 1631 cm^{-1} for the NC coating on the dyed film (335 MSC). Moreover, the characteristic vibration at 820 cm^{-1}, related to the nitrate groups of the treated film, disappears.

The results of the surface characterisation (hydrophilicity related to hydrophobicity) are shown in Table I. According to this table, relative to the original NC-coated films, there is an increase in the critical wetting tension (γ_C) of all of the films that were incubated in the *Shewanella* culture. However, this

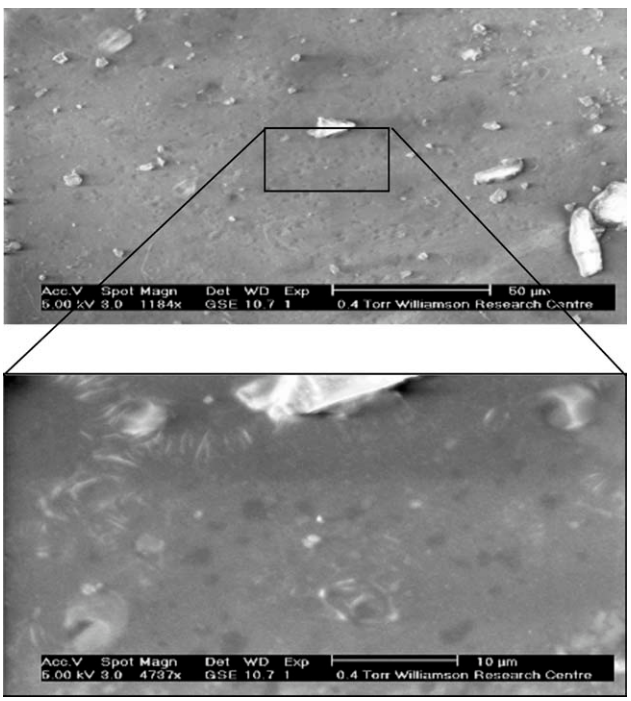

Figure 5 ESEM analysis of the colored NC-coated film that were incubated in the *Shewanella* culture for 1 month at 50°C.

increase in the value of the critical surface tension for the colored NC-coated films that were exposed to the *Shewanella* cell culture, ($\gamma_C = 33.14$ mN/m) is significantly greater than the γ_C value of the 335 MSC films that were used in the cell free study ($\gamma_C = 28.61$ mN/m).

To ensure that this observation for the treated 335 MSC was not due to formation of a biofilm over the NC coatings when they were exposed to the cells, an ESEM study was conducted. *Shewanella* cells are rod-shaped microorganisms, having a length of 2–3 μm and diameter of 0.4–0.7 μm. Thus, they are easily observed by ESEM. However, no biofilm was observed over the colored NC-coated films that were treated by *Shewanella* cells (Fig. 5). This shows that the results from FTIR analysis and surface characteristic study are realistic.

CONCLUSIONS

FTIR analyses and critical surface tension, γ_C, evaluations indicate that the NC coating over the dyed cellophane films underwent changes when the films were treated with the *Shewanella* J18 143 strain for period of 1 month. The colored NC-coated films were decolorized by this treatment. Moreover, during such treatment, the NC lost a significant proportion of its nitrate groups becoming more hydrophilic. The reducing matrix formed between the direct red azo dye and the *Shewanella* cells results in the color removal of the film. An electron reflux could migrate through the NC coating to have access to the dye in the cellophane base film. There might be an effect of this reducing environment that leads to denitrification of the cellulose nitrate as a coating and raises the surface energy of the colored films.

The authors acknowledge Dr. C. Marshall for supplying materials that were used in this project. The authors also would like to thank Prof. J. Lloyd and Dr. C Pearce, University of Manchester, for their input and for providing the ESEM analysis facilities.

References

1. British Standards Institution. In Packaging Code (Regenerated Cellulose Film, Films Made of Plastics, Aluminium Foil, Flexible Multilayer Structures and Metallized Materials; British Standard Institution BS 1133-21, 1991.
2. Malburg, L. M.; Tamblyn, L.; Forsberg, C. W. Microbial Degradation of Natural Products; VCH Publisher: New York, 1992; p127.
3. Ghosh, B. K.; Ghosh, B. Microbial Degradation of Natural Products; VCH Publisher: New York, 1992; p 83.
4. Reske, J. Beauty of bioplastics. Available at: www.waste-management-world.com, Accessed on 2005, 19/07.
5. American Water Work Association Water Quality and Treatment; McGraw-Hill Inc: New York, 1990.
6. Quinchon, J. In Nitrocelluloses, the Materials and Their Application in Propellants, Explosives and Other Industries; Tranchant, J. Q. A. J., Ed.; Ellis Horwood Limited, 1989; p 63.
7. Gaudry, J.; Ronssin, S. Mem Poudres 1962, 44, 284.
8. Sharma, A.; Sundaram, S. T.; Zhang, Y. Z.; Brodman, B. W. J Appl Polym Sci 1995, 55, 1847.
9. Petrova, O. E.; Tarasova, N. B.; Davydova, M. N. Anaerobe 2002, 8, 315.
10. Freedman, D. L.; Chashwell, G. M.; Kim, B. J. Waste Manage 2002, 22, 283.
11. Freedman, D. L.; Caenepeel, B. M.; Kim, B. J. Water Sci Technol 1996, 34, 327.
12. Petrova, O. E.; Tarasova, N. B.; Alyabyev, A. J.; Davydova, M. N.; Loseva, N. L. Thermochim Acta 2006, 445, 67.
13. Tarasova, N. B.; Petrova, O. E.; Davydova, M. N.; Khairutdinov, B. I.; Klochkov, V. V. Biochemistry (Moscow) 2004, 69, 809.
14. Gold, K.; Brodman, B. W. U.S. Pat. 4,814,439 (1989).
15. Wendt, T. M.; Kaplane, A. M. J Water Pollut Control Fed 1976, 48, 660.
16. Auer, N.; Hedger, J. N.; Evans, C. S. Biodegradation 2005, 16, 229.
17. Christodoulatos, C.; Su, T. L.; Koutsospyros, A. Presented at the Proccedings of the 3rd International Conference on Protection and Restoration of the Environment, China, Gr, 1996.
18. Brodman, B. W.; Devine, M. P. J App Polym Sci 1981, 26, 997.
19. Siu, R. G. H. Microbial Decomposition of Cellulose with Special Reference to Cotton Textile; Reinhold publishing corp: New York, 1951.
20. Selby, K. In Biodeterioration of Materials; Walters, A. H., Elphich, J. J., Eds; Elsevier: New York, 1968; p 62.
21. Kaplan, A.; Mandels, M.; Pillion, E.; Greenberger, M. Appl Microbiol 1970, 20, 85.
22. Greathouse, G. A.; Wessel, C. J. Deterioration of materials; Reinhold Publishing Corp: New York, 1954.
23. Pfeil, A. Propell Explos Pyrotech 1999, 24, 156.
24. Christodoulatos, C.; Su, T. L.; Koutsospyros, A. Water Environ Res 2001, 73, 185.
25. Tarasova, N. B.; Petrova, O. E.; Faizullin, D. A.; Davydova, M. N. Anaerobe 2005, 11, 312.
26. Wigginton, N. S.; Rosso, K. M.; Lower, B. H.; Shi, L.; Hochella, M. F. Jr., Geochim Cosmochim Acta 2007, 71, 543.
27. Myers, C. R.; Myers, J. M. FEMS Microbiol Lett 1993, 108, 15.
28. Myers, C. R.; Myers, J. M. J Bacteriol 1997, 179, 1143.
29. Fredrickson, J. K.; Zachara, J. M.; Kennedy, D. W.; Liu, C.; Duff, M. C.; Hunter, D. B.; Dohnalkova, A. Geochim Cosmochim Acta 2002, 66, 3247.
30. Neal, A. L.; Lowe, K.; Daulton, T. L.; Jones-Meehan, J.; Little, B. J. Appl Surf Sci 2002, 202, 150.
31. Konishi, Y.; Tsukiyama, T.; Ohno, K.; Saitoh, N.; Nomura, T.; Nagamine, S. Hydrometallurgy 2006, 81, 24.
32. Dichristina, T. J.; Delong, E. F. J Bacteriol 1992, 176, 1468.
33. Society of Dyers and Colourists. Colour Index International (electronic resources), 4th ed; Society of Dyers and Colourists, 2002.
34. Willmott, N. J. The Use of Bacteria-Polymer Composites for the Removal of Colour from Reactive Dye Effluents; University of Leeds: Leeds, 1997.
35. Pearce, C. I.; Christie, R.; Boothman, C.; Canstein, H. V.; Guthrie, J. T.; Lloyd, J. R. Biotechnol Bioeng 2006, 95, 692.
36. Nelson, G.; Willmott, N. J.; Guthrie, J. T. GB Pat 2316684B (2000).
37. Zisman, W. A. Contact Angle, Wettability and Adhesion; American chemical society: Washington, DC, 1964.
38. Patton, T. C. Paint Flow and Pigment Dispersion: A Rheological Approach to Coating and Ink Technology, 2nd ed.; Wiley: New York, 1979; p 205.