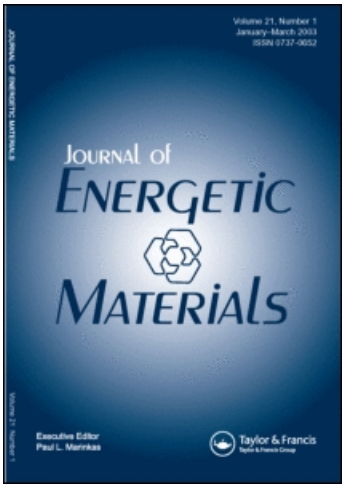

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BIODEGRADATION OF NITROCELLULOSE

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ABSTRACT

The research carried out in the past on the microbial degradation of nitrocellulose produced conflicting results primarily due to the lack of a proper analytical method for nitrocellulose estimation. Using an acetone extraction method, with appropriate corrections, Brodman and his coworkers concluded that certain fungal cultures are capable of degrading nitrocellulose. Results obtained by these investigators indicated that the combined culture containing *Fusarium solani* IFO 31093 and *Sclerotium rolfsii* ATCC 24459 appears to be the best as it degraded 40% of nitrocellulose. In this review, the recent studies on the fungal degradation of nitrocellulose and the possible mechanism (s) of nitrocellulose biodegradation are discussed.

INTRODUCTION

The nitrate ester of cellulose, which is known as nitrocellulose or cellulose nitrate, is the most commonly used energetic ingredient in gun propellant compositions. Nitrocellulose in the dry state is an explosive, and very sensitive to physical treatments such as shock, abrasion, or sparks. Upon storage over long time periods, nitrocellulose is capable of undergoing

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autocatalytic decomposition. A stabilizer, such as diphenylamine, is added to the propellant composition to terminate the autocatalytic decomposition reaction. However, when the stabilizer is depleted, the propellant becomes unstable and dangerous. This scrap propellant, along with the waste generated during the manufacture, are the major sources of nitrocellulose waste generation. Although toxicity studies indicated that nitrocellulose has very low toxicity in the mammalian system [1], it may produce significant abiotic environmental effects [2]. Currently, the disposal of gun propellants is carried out by open air burning or detonation (OB/OD). Recent studies have indicated that OB/OD of certain propellants produces air borne particulates and pan residues containing materials, such as 2,4-dinitrotoluene and 2,6-dinitrotoluene that are toxic [3]. For this reason, OB/OD is becoming prohibited in a number of states. An alternative to OB/OD is incineration, which is costly and time consuming. Hence, it would be appropriate to develop an environmentally safer and economical method for the disposal of gun propellant formulations.

BACKGROUND

A number of degradation processes based on the chemical and biological treatment of nitrocellulose have been investigated in the past [4-9]. The chemical treatment of nitrocellulose produces toxic residues and thus subsequent treatments are necessary to convert these residues into environmentally innocuous products. Bokorny [7] reported that molds can grow on nitrocellulose suspended in aqueous medium containing mineral salts. Studies by Malkenkovic [8] and Jacque [9] indicated that the molds are capable of utilizing only the dissolved mineral salts but incapable of attacking

nitrocellulose. Greathouse and Wessel [10] showed that nitrocellulose is susceptible to fungus growth. Brodman and Devine [11] reported that *Aspergillus fumigatus* can utilize the nitrogen resulting from the hydrolysis of the nitrate ester group of nitrocellulose without attacking the carbon back bone. This was substantiated by Gallo *et al* [12]. Kaplan and his coworkers [5] indicated that nitrocellulose was not subject to direct microbial attack. Their studies showed that chemical pretreatment of nitrocellulose by alkaline hydrolysis is necessary to generate a modified polymer that can be attacked by microorganisms. However, the studies by IL'inskaya and Leshchinskaya [6] showed that *A. fumigatus* is capable of directly attacking nitrocellulose. These investigators further suggested the possibility of nitrate esters removal by immobilized *A. fumigatus* and *Pseudomonas fluorescens* [13]. Duran *et al.*,[14] observed nitrocellulose degradation under anaerobic conditions in a two stage feed reactor. Studies carried out by Hsieh and Tai [15] indicated that only small amount of nitrocellulose could be degraded in a conventional anaerobic digestor. Thus, the earlier investigations on the biodegradation of nitrocellulose did not produce concrete results.

ANALYTICAL METHODS FOR NITROCELLULOSE ESTIMATION

Studies carried out in the past have utilized indirect methods for nitrocellulose estimation. Such a method involes the digestion of nitrocellulose with alkali followed by nitrate and nitrite estimations by high performance liquid chromatography [16]. Duran *et al.*, [14] and Heish Tai [15] measured the quantity of gas produced from nitrocellulose degradation. Based on the detection of reducing sugars during culture growth, IL'inskaya and Leshchinskaya [6] concluded that nitrocellulose was degraded under their

experimental conditions. Griest [17] proposed a method for nitrocellulose estimation based on the size exclusion chromatography. One of the serious drawbacks in his methodology is that nitrocellulose standards, having well defined molecular weights, are not available. Gallo *et al.*, [12] employed another method that involved the separation of nitrocellulose from the fungal biomass by dissolving the former in acetone. The acetone extract was allowed to evaporate and the residual weight was determined. By using this method, the authors came to the conclusion that nitrocellulose can not be directly biodegraded. However, the authors did not apply correction for the amount of biomass solubilized in acetone and thus added weight to the nitrocellulose fraction. Hence, it appears that the inconsistencies in the results obtained in the past with microbial degradation of nitrocellulose may be due to the lack of a proper analytical method for nitrocellulose estimation. During the recent fungal degradation studies of nitrocellulose, the researchers observed that the biomass produced during the growth was trapped in the nitrocellulose fibers[18-21]. Hence, it is necessary to separate the biomass from nitrocellulose to determine the individual amounts. A method was developed to determine the nitrocellullose and biomass weights based on the solubility of nitrocellulose in acetone [18]. The reported method involves the extraction of nitrocellulose from the biomass-nitrocellulose mixture with acetone followed by the precipitation of nitrocellulose with water. The recovered nitrocellulose was dried and the weight determined. The investigators observed that significant amount of solids from the biomass was extracted in acetone with nitrocellulose and that certain amounts of the extracted materials co-precipitated with nitrocellulose and added to the nitrocellulose weight. The amount of extracted material varied significantly depending on the specific fungal culture tested. Therefore, the authors applied correction

factors which were determined for each organism to the biomass and nitrocellulose determinations [18]. This method utilizing the precipitation of nitrocellulose with appropriate corrections appears to be more accurate than the one used by Gallo *et al* [12].

FUNGAL DEGRADATION OF NITROCELLULOSE

Brodman and his coworkers [18] isolated an organism, that was growing on stored wet small arms double-base propellant containing essentially nitrocellulose, nitroglycerine, 2,4-dinitrotoluene, diphenylamine and dibutylphthalate . The isolated leafy green organism was identified as *Penicillium corylophilum* Dierckx. Since the *P.corylophilum* was isolated from a propellant formulation, they utilized the culture to degrade nitrocellulose in liquid medium with starch or xylan as a co-substrate. The amount of residual nitrocellulose was determined using the actone extraction method [18] after three and seven days of fungal growth. The authors observed that 20% of the nitrocellulose was microbially utilized in a three day period as determined by the decrease in nitrocellulose weight and it appears that no further utilization occurred after seven days (Table 1).

Extensive studies on nitrocellulose degradation using several mycelial fungi have been carried out recently [19]. More than 20% nitrocellulose degradation was observed with *Bjerkandera adusta*, *Acremonium persicinum*, *Sclerotium rolfsii* and *Fusarium solani* in three days (Table 1). No further increase in nitrocellulose degradation was measured after the three day growth period. At least seven days of incubation was required with Basidiomycetes sp. *Irpex lacteus*, *Trichoderma pseudokoningii* and *Trametes*

versicolor to achieve ~20% nitrocellulose degradation. *Cyathus stercoreus*

TABLE 1
Fungal degradation of Nitrocellulose in Liquid Medium Containing a Cosubstrate#

Fungus	Nitrocellulose (mg)	
	3 d	7 d
Control*	144.6 (3.6)	143.4 (4.4)
Penicillium corylophilum Dierckx**	120.7 (19.5)	120.7 (19.5)
Penicillium corylophilum Dierckx***	124.5 (17.0)	124.5 (17.0)
Acremonium persicinum ATCC 60921**	118.4 (21.1)	134.8 (10.1)
Basidiomycetes sp. NRRL 6464**	140.5 (6.3)	125.9 (16.1)
Bjerkandera adusta ESF 620**	112.8 (24.8)	125.3 (17.7)
Cyathus stercoreus NRRL 6473**	142.5 (5.0)	138.2 (7.9)
Irpex lacteus Z 212**	136.4 (9.1)	113.6 (24.3)
Phanerochaete chrysosporium ATCC 32629**	132.1 (11.9)	126.8 (15.5)
Trametes versicolor ESF 491**	128.2 (14.5)	118.2 (21.2)
Trichoderma pseudokoningii FTK 228**	124.3 (17.1)	120.8 (19.5)
Sclerotium rolfsii ATCC 24459**	118.0 (21.3)	120.8 (19.4)
Fusarium solani IFO 31093**	114.6 (23.6)	114.6 (23.6)
Sclerotium rolfsii ATCC 24459+ *Fusarium solani* IFO 31093**	103.7 (30.8)	106.9 (28.7)
Sclerotium rolfsii ATCC 24459+ *Fusarium solani* IFO 31093**@	91.2 (39.2)	91.2 (39.2)

*Values given are average of four replicates
**Starch (0.1%) was used as a co substrate
***Xylan (0.1%) was used as a co substrate
Values given in parentheses are percent nitrocellulose utilized
Starting concentration of nitrocellulose, 150 mg
Starting pH of the growth medium 6.0; Final pH 5.5 except for the growth medium containing *S. rolfsii* (pH 2.0)
@pH was maintained at 6.0 using morphilino ethane sulfonic acid buffer (100 mM)
#Data from references 18,19,20 and 21

degraded only 8% of nitrocellulose in a seven day growth period. The authors observed an increase in the nitrocellulose contents after seven days of growth of *A. persicinum* and *B. adusta* as compared to the three day growth

period. This variation may be due to the nitrocellulose correction factors which were determined with the biomass harvested after five days of growth. It appears that these cultures produced/released significant amount of acetone soluble but water insoluble materials between the five and seven day growth period. Hence, it is likely that the nitrocellulose correction factors which were determined with the five day old biomass is not applicable to the seven day data. However, since the cultures degraded only 20% of nitrocellulose, and was not of significant interest, no attempts were made to repeat the correction factors with the seven day old cultures. Gallo *et al.*,[12] were not able to observe the degradation of nitrocellulose by *Phanerochate chrysosporium*, Actinomycetes sp. and *A. fumigatus*. It appears that the method used by these investigators as previously discussed was not accurate enough to detect the nitrocellulose degradation. The difference in nitrocellulose utilization may also be due to the fact that they used different fungal strains.

Although certain fungal cultures were identified that degraded nitrocellulose to a limited extent, none of them totally degraded nitrocellulose. In order to utilize this method for nitrocellulose disposal, complete degradation should occur. For this reason, optimization studies must be conducted. Brodman and his coworkers [20], as part of their on going effort, tested the ability of the combination culture containing the nitrate reducing fungus, *F.solani*, and the cellulolytic fungus, *S.rolfsii*, to degrade nitrocellulose . Their results indicated that more than 28% of the nitrocellulose was utilized by the growing fungi in a three day period (Table 1). No further increase in nitrocellulose utilization was observed up to a period of 28 days. Also, a significant drop in pH (from 6.0 to 2.0) was

measured in the fungal grown medium. At the acidic pH, certain enzymes involved in the degradation of nitrocellulose might have been inactivated. Hence, they tested the effect of maintaining a constant pH on the nitrocellulose degradation. The fungal cultures were cultivated on nitrocellulose and starch dispensed in basal salts medium prepared using 100 mM morphilino ethane sulfonic acid (MES) buffer (pH 6.0). Under these conditions, no drop in pH was measured and the results indicated at least an 11% increase [21] in the nitrocellulose use (Table 1).

The possible routes of the microbial degradation of organic hazardous wastes in liquid culture include (i) mineralization (ii) assimilation into microbial biomass/utilization for microbial growth (iii) volatilization as organic gaseous compounds and (iv) biotransformation to intermediate/end products. During their studies [18-21], the investigators observed several fold increase in the fungal biomass weights of *P. corylophilum*, *B. adusta*, *C. stercoreus*, *A. persicinum*, *T. versicolor*, *S. rolfsii* and *F. solani* and the combined culture containing *S. rolfsii* and *F. solani* (Table 2) when nitrocellulose was used as a sole nitrogen source for growth. However, they observed a decrease in the biomass weight after seven days of growth with some of the cultures, apparently due to the autolysis of the fungal biomass. Autolysis of the biomass has been reported to occur in fungi due to the accumulation of toxic products and/or due to the expression of the enzymatic machinery that cause autolysis [22-25]. No direct correlation between the fungal biomass weight and the amount of nitrocellulose degradation was observed with Basidiomycetes sp, *I. lacteus*, *T. pseudokoningii* and *P. chrysosporium*. They concluded that, in these cultures, the autolysis of the fungal biomass started to occur during the three day growth period. The

TABLE 2
Fungal growth on Nitrocellulose in Liquid Medium Containing a Cosubstrate#

Fungus	Biomass (mg dry weight)		
	0 d	3 d	7 d
Control*	0	0	0
Penicillium corylophilum Dierckx**	28.2	28.6	46.9
Penicillium corylophilum Dierckx***	28.2	37.4	49.6
Acremonium persicinum ATCC 60921**	26.0	58.4	57.4
Basidiomycetes sp. NRRL 6464**	49.8	40.1	33.9
Bjerkandera adusta ESF 620**	27.3	64.9	53.2
Cyathus stercoreus NRRL 6473**	40.6	57.8	58.5
Irpex lacteus Z 212**	23.1	22.5	14.3
Phanerochaete chrysosporium ATCC 32629**	48.7	43.0	48.2
Trametes versicolor ESF 491**	41.7	46.0	47.9
Trichoderma pseudokoningii FTK 228**	33.6	33.2	38.0
Sclerotium rolfsii ATCC 24459**	30.8	83.5	88.4
Fusarium solani IFO 31093**	26.4	77.5	55.0
Sclerotium rolfsii ATCC 24459+ *Fusarium solani* IFO 31093**	6.9	21.1	23.0
Sclerotium rolfsii ATCC 24459**+ *Fusarium solani* IFO 31093***@	9.8	25.6	21.9

*Values given are average of four replicates
**Starch (0.1%) was used as a co substrate
***Xylan (0.1%) was used as a co substrate
Values given in parentheses are percent nitrocellulose utilized
Starting concentration of nitrocellulose, 150 mg
Starting pH of the growth medium 6.0; Final pH 5.5 except for the growth medium containing *S. rolfsii* (pH 2.0)
@pH was maintained at 6.0 using morphilino ethane sulfonic acid buffer (100 mM)

#Data from references 18, 19, 20 and 21

authors predicted that the enzymes, secreted by these fungi at the early stages of growth, might have been released from the fungal hypha. When present in the growth medium, they may have contributed to the degradation of

nitrocellulose. Apparently, the degradation products of nitrocellulose inhibited the growth of Basidiomycetes sp. and *I. lacteus* and marginally supported the growth of *P. chrysosporium* and *T. pseudokoningii* in a seven day growth period (Table 2). Thus, the fungal growth pattern on nitrocellulose varies from culture to culture. Although the investigators reported the nitrocellulose degradation[18-21], they did not make any attempts to identify the products formed during the degradation. It is necessary to determine the biochemical fate of nitrocellulose in the system before suggesting this technology as an alternative treatment method.

MECHANISM OF NITROCELLULOSE BIODEGRADATION

The mechanism of the microbial degradation of nitrocellulose remains to be identified. The use of free hydroxyl radical (.OH) as an oxidizing agent for the treatment of hazardous wastes has received much attention in recent years [26,27]. Barbeni *et al.*, [28] reported that polychlorinated biphenyls are hydroxylated by H_2O_2 (Fenton's reagent). Sedlak and Andren [29] had shown that chlorinated phenols react with the Fenton's reagent releasing the chloride ions. The hydroxyl radicals could be involved in the fungal degradation of a range of compounds, possibly including nitrocellulose as it is known that .OH reacts with most organic pollutants and wood [27,30]. It is likely that in the presence of free hydroxyl radicals which were generated enzymatically by the fungus, nitrocellulose releases the nitrate ions which are further converted to nitrite ions by nitrate reductase. The nitrite ions are possibly metabolized to ammonia by nitrite reductase. The denitrated cellulose polymer is then hydrolyzed to glucose and oligosaccharides by the cellulose degrading enzymes. In this context, it is interesting to note the reports by Brodman and

his colleagues indicating the presence of some cellulose degrading and nitrate reducing enzymes, as well as lignin peroxidase, an enzyme that generates free hydroxyl radicals from hydrogen peroxide [18-21], in some of the fungal cultures used for nitrocellulose degradation.

Another possible mechanism can be the involvement of nitro esterase, an enzyme produced by *Pseudomonas fluorescens*, which has been reported to cleave the $-ONO_2$ group of nitrocellulose [31]. The nitrate ions released due to the action of nitro esterase on nitrocellulose may be converted to nitrite ions by nitrate reductase and possibly to ammonia by nitrite reductase. One can also presume that soluble nitrate and nitrite ions made available from nitrocellulose by aqueous hydrolysis and starch or xylan, which is provided as a cosubstrate, initiates growth of the fungal cultures. Later, the cultures start reducing the nitrate ester group of nitrocellulose. The denitrated cellulose is further hydrolyzed by the cellulase complex to oligosaccharides and glucose.

CONCLUSIONS

The combined culture containing *F. solani* and *S. rolfsii* appears to be better than the other studied fungal cultures in utilizing nitrocellulose. However, thus far, only 40% of nitrocellulose degradation was achieved [21]. The reason for the limited nitrocellulose utilization may be due to the inhibition of certain key degrading enzymes (viz. cellulolytic and nitrate reducing enzymes) by some of the products formed during the degradation of nitrocellulose. Also, at this point, it is not clear whether the degree of nitration has any effect on the biodegradation of nitrocellulose. The nitrocellulose used in the investigations had a 13.17% nitrogen content with

~2.33 nitrate ester groups per repeat unit [18-21]. It appears that more mechanistic information is required in order to optimize the nitrocellulose degradation. For example, the possibility of the presence of nitrocellulose nitrate ester groups in an inaccessible steric environment for the fungal enzymes can not be ruled out at this time. Nitrocellulose is a complex organic material that is insoluble in the aqueous medium. The use of presolubilized nitrocellulose with subsequent addition to the aqueous medium which might increase the bioavailability may be considered for the optimization studies. The utilization of the genetic engineering technology to develop or modify the microbial strains with novel nitrocellulose degradative capabilities and the ability to survive in hostile environments may also be considered. In conclusion, the information on the products formed during the degradative process and the optimization of the degradation may be necessary for the disposal of nitrocellulose.

REFERENCES

1. D. O. Helton, AD-A 036-151, U.S. Army Medical Research and Development Command, Washington, DC. (1976).

2. M. G. Ryan, AD-ORNR-6179, U.S. Army Medical Research and Development Command, Washington, DC. (1986).

3. MacDonald Johnson, Technical report DAAAD09-87-D-0008, U.S. Army Armament, Munitions and Chemical Command, Bangbox Test Series, Volume 2, Rock Island, IL. (1992).

4. K. Gold and B. W. Brodman, US Patent 4814439 (1989).

5. P. A. Riley, D. L. Kaplan and A. M. Kaplan, Technical Report 85-004, US Army Natic Research, Development and Engineering Center, Natick, MA (1984).

6. A. N. IL' inskaya and I. B. Leshchinskaya, Biotekhnologiya. 4, 495 (1988).

7. T. Brodney, (as quoted by T. Urbanski) in "Chemistry and Technology", Pergamon Press, London, England, 1965, p. 313.

8. B. Malenkovic, (as quoted by T. Urbanski) in "Chemistry and Technology", Pergamon Press, London, England, 1965, p. 313.

9. M. Jacque, (as quoted by T. Urbanski) in "Chemistry and Technology", Pergamon Press, London, England, 1965, p 313.

10. G. A. Greathouse and C. J. Wessel, "Deterioration of materials", Reinhold Publishing Corp. New York, NY, 1965.

11. B. W. Brodman and M. P. Devine, J. Applied Polymer Sci. 26, 997 (1981).

12. B. Gallo, A. Allen, R. L. Bagalawis, C. Woodbury, A. Yang, P. Austin and D. Kaplan, in "Proceedings on nitrocellulose-fines,

separation and treatment", U.S. Army-Purdue University, West Lafayette, IN, 1993, p. 78.

13. O. N. IL' inskaya, P. P. Chernov and I. B. Leshchinskaya, Biol Nauki. 6, 87 (1988).

14. M. Duran, B. .J. Kim and R. E. Speece, in "Proceedings on nitrocellulose-fines, separation and treatment",U.S. Army - Purdue University, West Lafayette, IN, 1993, p. 92.

15. H. N. Hsieh and F. J. Tai, in "Proceedings on nitrocellulose-fines, separation and treatment", U.S. Army- Purdue University,West Lafayette, IN, 1993, p. 110.

16. USATHAMA method LY 02, United States Army Toxic and Hazardous Material Agency, Aberdeen Proving Ground, Maryland, USA.

17. W. H. Griest, in "Proceedings on nitrocellulose, fines, separation and treatment", U.S. Army-Purdue University, West Lafayette, IN, 1993, p. 150.

18. A. Sharma, S. T. Sundaram , Y. Z. Zhang and B. W. Brodman, J. Applied Polymer Sci. 55, 1847 (1995).

19. S. T. Sundaram, Y. Z. Zhang, A. Sharma, K. Ng and B. W. Brodman, J. Applied Polymer Sci. (Submitted) (1995).

20. A. Sharma, S. T. Sundaram, Y. Z. Zhang and B. W. Brodman, J. Industrial Microbiol. (In Press) (1995).

21. A. Sharma, S. T. Sundaram, Y. Z. Zhang and B. W. Brodman, Proc. 3rd Intl. Symposium on *In Situ* and On site Bioreclamation (In Press) (1995).

22. D.H. Griffin, "Fungal Physiology", John Wiley & Sons, New York, 1981.

23. F. Reyes, P. Villanueva and C. Alfonso, FEMS Microbiol. Letters. 69, 67 (1990).

24. F. Reyes, , P. Villanueva and C. Alfonso, Letters Appl. Microbiol. 10, 175 (1990).

25. R. Lackner, E. Srebotnik and K. Meessner, Can. J. Micobiol. 37, 665 (1991).

26. D. P. Barr, M. M. Shah, T. A. Grover and S. D. Aust, Arch. Biochem. Biophys. 298, 480 (1992).

27. L. J. Forney, C. A. Reddy, M. Tien and S. D. Aust, J. Biol. Chem. 257, 11455 (1982).

28. M. Barbeni, C. Minero and E. Pelizzetti, Chemosphere. 16, 2225 (1987).

29. L. Sedlak and A. W. Andren, Environ. Sci. Technol. 25, 1419 (1991).

30. J. Kochany and J. R. Bolton, Environ. Sci. Technol. 26, 262 (1992).

31. O. N. IL' inskaya and I. B. Leshchinskaya, Microbiologia. 56, 1033 (1987).