Biodegradation of Nitrate Esters. II. Degradation of Nitrocellulose by a Fungus Isolated from a Double-Base Propellant

A. SHARMA,[1] S. T. SUNDARAM,[1] Y.-Z. ZHANG,[1] and B. W. BRODMAN[2,*]

[1]Geo Centers Inc., Lake Hopatcong, New Jersey 07849, and [2]U.S. Army, Armaments Research, Development and Engineering Center, Picatinny Arsenal, New Jersey 07806

SYNOPSIS

An organism found to be growing on moist double-base propellant, containing nitrocellulose (NC) and nitroglycerine aerobically degraded NC in submerged cultivation. This organism, which was subsequently identified as *Penicillium corylophilum* Dierckx, was able to degrade the NC (13.17% N) when it was present as the sole nitrogen source, in conjunction with either starch or xylan as a carbon source. It was found that 20% of the NC was utilized in a 3-day period. Also, NC degradation was studied utilizing *Fusarium solani* IFO 31093, a denitrifying fungus, in combination with *P. corylophilum;* however, no increased utilization was observed. Evidence for the degradation includes a decrease in the NC weight, an increase in the biomass weight, the presence of cellulolytic and denitrifying enzymes, and other appropriate growth parameters. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

Nitrocellulose (NC) is the major ingredient in most gun propellants. When it is plasticized with nitroglycerin, the resulting mixture, in combination with other nonenergetic materials is referred to as a double-base propellant. One of the nonenergetic ingredients added to propellant compositions is a stabilizer.[1] Typically, this material is diphenylamine, which is present to terminate an autocatalytic decomposition reaction. When the stabilizer is depleted, the propellant becomes unstable and must be destroyed, either by open-air burning or detonation (OB/OD). This scrap propellant coupled with waste from manufacture represents a significant amount of NC-based material that must be disposed of each year. Biodegradation is potentially a more environmentally friendly disposal method than OB/OD.

A number of degradation processes based on the chemical and biological treatment of NC have been investigated.[2-5] Previous work carried out by several researchers on microbial degradation of NC produced conflicting results.[6-10] Brodman and Devine[11] reported that *Aspergillus fumigatus* can utilize the nitrogen resulting from the hydrolysis of the nitrate ester group of NC without attacking the carbon backbone. Kaplan and his co-workers indicated that NC was not subject to direct microbial attack.[3,9,10] Their studies showed that chemical pretreatment of NC by alkaline hydrolysis is necessary to generate a modified polymer which can be attacked by microorganisms. However, IL'Iinskaya and Leshchinskaya[12] reported that *A. fumigatus* was capable of directly attacking NC.

In our earlier work, we have demonstrated aerobic NC degradation in liquid culture by a combination of cellulolytic (*Sclerotium rolfsii* ATCC 24459) and denitrifying (*Fusarium solani* IFO 31093) fungi.[13] In the present work, we demonstrated NC degradation by a naturally occurring fungus, *Penicillium corylophilum* Dierckx, which was isolated from a double-base propellant. To the best of our knowledge, never before has *P. corylophilum* been isolated from a double-base propellant nor has it been reported to degrade NC.

* To whom correspondence should be addressed.

Journal of Applied Polymer Science, Vol. 55, 1847–1854 (1995)
© 1995 John Wiley & Sons, Inc. CCC 0021-8995/95/131847-08

MATERIALS AND METHODS

Organisms

F. solani IFO 31093, a denitrifying fungus[14] was purchased from the Institute of Fermentation, Osaka, Japan.

Isolation and Purification of BB-1

A fungus (BB-1) was isolated from a stored water wet propellant sample (WC 844), SM Lot 609, manufactured by Olin Corp. (St. Marks, FL). The composition of this propellant is given in Table I. The isolated fungal culture was purified by repeated streaking on a malt-extract agar plate and tested on a number of agar media (potato dextrose, sabouraud maltose, and czapek-dox agar) for growth.

Identification of BB-1

A pure culture of BB-1 was sent to Prof. J. Wang, College of Environmental Science and Forestry, State University of New York, Syracuse, New York, and to Dr. P. Hoffmann, Deutsche Sammlung Von Mikroorganismen und Zellkulturen GmbH (the German Culture Collection), Braunschweig, Germany, for identification. The results obtained indicated a close resemblance of this fungus to genus *Penicillium* sp. Dr. Hoffmann, at the German Culture Collection Laboratory, has identified it as *P. corylophilum* Dierckx.

Maintenance of Cultures

F. solani IFO 31093 and *P. corylophilum* were maintained at 4°C on sabouraud maltose agar with monthly transfers.

Chemicals

NC (smokeless grade) was received as a gift from Hercules Inc. (Kenvil, NJ), and it contained 13.17% nitrogen (~ 2.33 nitrate ester groups per repeat unit) and 25.07% moisture. Carboxymethyl cellulose, glucose estimation kit, starch, β-1,4-xylan, *p*-nitrophenyl β-glucopyranoside, protein estimation kit, bovine serum albumin, methyl viologen, sulfanilamide, and dithioerythritol were purchased from Sigma Chemical (St. Louis, MO).

Brucine, *N*-(1-naphthyl) ethylenediamine dihydrochloride, sodium dithionite, sulfanilic acid, 3,4-dimethoxybenzyl alcohol (veratryl alcohol), and 3,4-dimethoxy bezaldehyde (veratraldehyde) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Enzymatic kit for nitrate analysis was purchased from Boehringer Mannheim (Indianapolis, IN). Unless otherwise specified, all other chemicals used were of analytical grade.

Determination of Moisture Content in Nitrocellulose

Moisture content of NC was estimated using DuPont Thermal Analysis System 1090 B equipped with Thermogravimetric Analyzer 951.

Growth Medium

Mineral salts medium used in this study contained (g/L): KH_2PO_4, 1.0; $MgSO_4$, 0.5; NaCl, 0.1; $CaCl_2$, 0.1 and trace metal solution, 100 μl. Trace metal salts solution contained (w/v %): $C_6H_8O_7$ (citric acid), 5.0; $ZnSO_4$, 5.0; $CuSO_4$, 0.25; $MnSO_4$, 0.25; H_3PO_4, 0.05; $Na_2MoO_4 \cdot 2H_2O$, 0.05 and $CoCl_2$, 2.0.

Nitrocellulose Sterilization

Steam sterilization (15 psi, 121°C, 30 min) of NC released a significant quantity of free nitrite ions indicating decomposition of NC (data not shown). Hence, Ultra Violet (254 nm) treatment was tested as an alternative for NC sterilization. UV irradiation of NC for 45 min did not release free nitrite ions. Thus, in all experiments NC was sterilized for 45 min using UV light and then added to the cooled steam sterilized medium.

Inoculum Preparation

Mycelial mats were pregrown in mineral salts medium containing ammonium nitrate (1.0%) as a nitrogen source and xylan (0.1%) as a carbon source. Fungi were grown at 28°C in a gyrotary shaker (~ 150 rpm) for 4 days and harvested by centrifugation (5000 rpm, 4°C, 10 min). The harvested fungal mycelia were washed thoroughly under aseptic conditions with the cooled basal salts medium lacking carbon and nitrogen sources. The washed fungal mycelia were homogenized using a Virtishear explosion proof pilot homogenizer operated at half of the maximum output for 2 min at 4°C with 15 s interruptions.

Biomass Dry Weight Determination

A known volume of homogenized mycelia, used for inoculation, was filtered through a preweighed filter paper (VWR Scientific), washed with distilled water,

and dried at 105°C until a constant weight was reached.

Cultural Conditions

A known volume of homogenized mycelia (~ 20 mg dry wt.) was transferred to the experimental medium (50 mL in a 250-mL Erlenmeyer flask) containing NC (0.3%) as a nitrogen source and starch/xylan (0.1%) as a co-substrate. Inoculated flasks were incubated for various time intervals (0, 3, and 7 days) in a gyrotary shaker (~ 150 rpm, 28°C). Equal volumes of each homogenized fungal culture (1 : 1) were used whenever a combination of fungi was used as a source of inoculum. Three types of controls were run in parallel to the treatment, one of them lacking nitrocellulose but containing the cultures, the other containing NaN_3 (1m*M*), $HgCl_2$ (1m*M*) along with the cultures, while the third control lacked the cultures and fungicidal agents. The fungal biomass was harvested at the desired time intervals by centrifugation (8000 rpm, 4°C, 15 min). The biomass and the supernatant were saved for further analyses.

Enzymes

Preparation of Crude Fungal Enzyme

The fungal mycelia, which were cultivated and harvested as described above, were washed with an excess of potassium phosphate buffer (100 m*M*, pH 7.0) and then resuspended in 5 mL of the same buffer containing phenylmethylsulfonyl fluoride (~ 2 μL, 0.1 m*M*) and ground in a homogenizer, operated at half of the maximum speed for 3 min with 30-s interruptions. The homogenate was then centrifuged (10,000 rpm, 4°C, 20 min) and the supernatant analyzed for intracellular (β-glucosidase, nitrate, and nitrite reductase) enzymes.

Carboxymethyl cellulase (CMCase) (E.C. 3.2.1.4) was assayed as described by Mandels and Weber.[15] Reducing groups generated were estimated by Somogyi reagent. One unit of CMCase was defined as the amount of enzyme which generates 1 μmol of glucose equivalent per minute.

Filter paper cellulase (FPCase) (E.C. 3.2.1.9) was assayed as described by Mandels and Weber.[15] Reducing groups generated were estimated by Somogyi reagent. One unit of FPCase was defined as the amount of enzyme which generates 1 μmol of glucose equivalent per min.

β-Glucosidase (E.C. 3.2.1.21) was assayed as described by Kubackova et al.,[16] using *p*-nitrophenyl β-D-glucopyranoside. One unit of β-glucosidase was defined as the amount of enzyme releasing 1 μmol of *p*-nitrophenol from the substrate per minute.

Xylanase (E.C. 3.2.1.8) was assayed as described by Sharma et al.[17] Reducing groups generated were estimated by Somogyi reagent. One unit of xylanase was defined as the amount of enzyme which generates 1 μmol of xylose equivalent per minute.

Lignin peroxidase (E.C. 1.11.1.7) was assayed spectrophotometrically at 30°C by the method of Tien and Kirk.[18] Activities were expressed as μmol of the substrate oxidized per minute.

Nitrate reductase (E.C. 1.6.6.2) was assayed by measuring the reduction of nitrate to nitrite ions colorimetrically[19] using potassium nitrate as a substrate and methyl viologen as an artificial electron donor. One unit of nitrate reductase was defined as the amount of enzyme producing 1 μmol of nitrite per minute at 25°C.

Nitrite reductase (E.C. 1.6.6.4) was assayed by the procedure of Kakutani et al.,[20] using potassium nitrite as a substrate and methyl viologen as a hydrogen donor. One unit of enzyme was defined as the amount of enzyme required to reduce 1 nmol of nitrite per minute at 25°C.

Determination of the Weight of Biomass and Unutilized Nitrocellulose

Medium containing NC and grown fungi was filtered through a preweighed filter paper (VWR Scientific, 5.5 cm) (W_1) and the filter cake was washed thoroughly with distilled water, dried at 50°C for 3 h and then cooled to room temperature (22°C) in a desiccator having Drierite. The filter paper with filter cake was dried and weighed until a constant weight was attained (W_2). To a preweighed centrifuge tube (W_3) was added the dried filter cake, and the tube with the filter cake was then weighed (W_4). Acetone was added gradually with vigorous stirring until almost all NC in the filter cake dissolved. This acetone NC solution, with suspended biomass, was centrifuged (8000 rpm, 15°C, 1 h). The supernatant transferred to another preweighed centrifuge tube (W_5) and the collected biomass remained in the centrifuge tube. Water was added to the supernatant to precipitate the dissolved NC and the suspension was centrifuged (8000 rpm, 15°C, 1.5 h). The supernatant was transferred to a flask, and the collected NC remained in the centrifuge tube. Acetone was added to the collected biomass with vigorous stirring to let all unutilized NC dissolve, and the suspension was centrifuged (8000 rpm, 15°C, 1 h). The supernatant was then transferred to the centrifuge tube containing the collected NC and water

was added to further precipitate NC. The suspension was centrifuged (8000 rpm, 15°C, 1.5 h). A few drops of water were added to the supernatant to make sure that no more NC precipitated, and the supernatant was then combined with the other supernatant solution mentioned above. If there was some precipitate, more water was added before the suspension was centrifuged once again. Finally, the collected NC with the centrifuge tube was dried at 50°C for 3 h and cooled to room temperature (22°C) in a desiccator having Drierite. It was weighed until a constant weight (W_6) was attained. The collected biomass with the centrifuge tube was dried in the same way and was weighed until a constant weight was attained (W_7). The weight of collected NC and biomass was determined as follows:

$$\text{Weight of biomass} = \frac{(W_2 - W_1)(W_7 - W_3)}{W_4 - W_3}$$

$$\text{Weight of NC} = \frac{(W_2 - W_1)(W_6 - W_5)}{W_4 - W_3}$$

Correction Factors for Determination of the Weight of Biomass and Nitrocellulose

In order to establish a correction factor for determination of the weight of biomass, certain amount of biomass (20, 50, 100, 200, and 300 mg) was extracted with acetone (10 mL) and the suspension was centrifuged (8000 rpm, 15°C, 1 h). The supernatant was decanted and saved for determination of a correction factor for NC. Residual biomass remaining in the centrifuge tube was again extracted with acetone (5 mL). The suspension was centrifuged (8000 rpm, 15°C, 1 h) and the supernatant was combined with the corresponding first extract (total 15 mL). The collected biomass was dried at 50°C for 3 h and cooled at room temperature (22°C) in a desiccator with Drierite until a constant weight was attained. Based on the results, a standard curve was plotted from which a correction factor could be determined.

Acetone extracts described above (15 mL) were mixed with 7.0 mL of an NC acetone solution (1.15 g of NC in 50 mL of acetone). Water (5 mL) was added to each of the above mixtures to precipitate NC. The suspension was centrifuged (8000 rpm, 15°C, 1.5 h) and the supernatant was discarded. Collected NC was dried at 50°C for 3 h and cooled at room temperature (22°C) in a desiccator having Drierite until a constant weight was attained. These results were used to determine the amount of biomass co-precipitated with NC and the correction factor could further be determined.

Analytical Methods

Reducing sugars were estimated by the method of Somogyi[21] with glucose as the standard. Glucose was estimated as described in the Sigma glucose estimation kit. Soluble protein was estimated by the method of Bradford.[22] Nitrate and nitrite ions were measured by the procedure of Nicholas and Nason.[23]

RESULTS AND DISCUSSION

An organism, found to be growing on water wet double-base small arms propellant, was isolated and characterized. This leafy green organism was identified as *P. corylophilum* Dierckx. Examination of Table I indicates that there are four compounds capable of providing nitrogen for the growth of this organism. Of these four compounds, only nitroglycerine (NG) has some water solubility (1 g/800 mL); therefore it is likely that the organism utilized NG from the aqueous phase as a nitrogen source. If the organism utilized water-soluble NG, then it is possible that significant depletion occurred and may have effected the propellant's performance characteristics. This will be a subject of a later study.

Previously, *P. corylophilum* was isolated from several sources including soil, cattle feed, microscope slide mounting medium, pasture grass, diesel storage tanks, and several contaminated cultures.[24] However, thus far, the fungus has not been isolated from NC-based propellant. Among the several agar media (czapec dox, potato dextrose, malt extract, and sabouraud maltose) screened for the growth of this

Table I Propellant Composition from which *P. corylophilum* Was Isolated

Constituent	Percent
Nitrocellulose (13.15% N)	83.45
Nitroglycerine	10.78
Dinitrotoluene	0.13
Diphenylamine	1.11
Dibutylphthalate	4.37
Total volatiles	0.92
Moisture and volatiles	0.93
Residual solvent	0.27
Calcium carbonate	0.07
Sodium sulfate	0.03

fungus, sabouraud maltose produced the greatest amount of growth. The ability of the culture to grow in a liquid medium was tested using starch or xylan as a carbon source and ammonium nitrate as a source of nitrogen. *P. corylophilum* was found to grow well on xylan at 28°C as determined by the increase in biomass weight after 7 days of growth.

Since the *P. corylophilum* was isolated from a propellant formulation, we utilized the culture to degrade NC. NC at 0.3% level was tested as the sole nitrogen source in liquid medium, and starch or xylan was provided as a co-substrate. Pregrown and washed mycelia of *P. corylophilum* were homogenized and used as a source of inoculum. The fungal cultures were grown for 3 and 7 days on NC and appropriate growth parameters measured. *P. corylophilum* utilized the reducing sugars, including glucose, and soluble nitrate as evidenced by the reduction of their levels on the seventh day of growth (Table II). The soluble nitrite was completely utilized by the organism. A measured drop in pH of the media containing the culture indicated fungal growth. The results also revealed that the glucose concentration ranged from 10–20% of the total reducing sugars detected on both the third and seventh day of cultivation. The detection of glucose, nitrate, and nitrite ions in the control medium, lacking the culture, may be due to the fact that NC is capable of undergoing some aqueous hydrolysis. Hence, at this point, it was not clear whether *P. corylophilum* was capable of degrading NC or not. If *P. corylophilum* is able to degrade NC, it is reasonable to expect that the organism should produce the necessary enzymes. For this reason, we have studied the cellulose degrading and denitrifying enzymes of this organism. *P. corylophilum* produced significant levels of soluble protein when grown on NC and starch or xylan (Table III). One of the cellulose degrading enzymes, β-1,4-endoglucanase was detected on the third day in the medium containing starch as a co-substrate; the level of this enzyme declined on the seventh day. However, β-1,4-endoglucanase could not be detected in the medium containing xylan as a co-substrate. It appears that starch is necessary for the production of β-1,4-endoglucanase by *P. corylophilum*. Another cellulose degrading enzyme, β-glucosidase was also detected in the growing cultures in extra- as well as intracellular fractions. Significant levels of intracellular denitrifying enzymes such as nitrate and nitrite reductases were also detected in the growing cultures. The production of both enzymes increased up to the third day and decreased around the seventh day (Table III). In *P. corylophilum*, we have also detected significant amount of lignin peroxidase (Table III), an enzyme which has been shown to participate in the free radical formation in fungal systems.[25,26]

Since the culture produced cellulose degrading and denitrifying enzymes, it was decided to determine the amounts of NC and biomass on the third and seventh day of fungal growth. Biomass produced during the fungal growth on NC was found to be trapped in the NC fibers. Hence it was necessary to separate the biomass and NC to determine the actual amounts of these components. In our previous investigation,[13] we reported a method to determine the NC and biomass weights based on the solubility of NC in acetone. Using that method, in the present investigation, we have determined that the weight of the fungal biomass increased by 1.7-fold on starch and 1.8-fold on xylan (Table IV). Twenty percent of the NC was microbially utilized in a 3-day period

Table II Growth Parameters of *P. corylophilum* on Nitrocellulose in Liquid Medium

Culture	Reducing Sugars (μg/mL)		Glucose (μg/mL)		Nitrate (μg/mL)		Nitrite (μg/mL)	
	3d	7d	3d	7d	3d	7d	3d	7d
None[a]	ND	ND	11.0	0.3	12.2	11.1	9.5	9.4
None[b]	ND	ND	3.4	1.9	11.4	11.4	8.6	8.2
P. corylophilum[a]	104.8	58.4	21.7	11.3	6.0	8.4	0	0
P. corylophilum[b]	39.3	23.2	4.0	2.8	9.9	8.7	0	0

[a] Starch was used as a co-substrate at 0.1% level; ND, not determined.
[b] Xylan was used as a co-substrate at 0.1% level. Starting pH of the medium, 6.0; after 7 days culture growth pH dropped to 5.5.

Table III Enzyme Production by *P. corylophilum* on Nitrocellulose in Liquid Medium

Cultivation Time (d)	Soluble Protein (μg/mL)	Enzyme (U/mL)							
		β-glucanase		β-glucosidase					
		Endo	Exo	Extra	Intra	Xylanase	Nitrate Reductase	Nitrite Reductase	Lignin Peroxidase
3[a]	5.1	4.4	0	0.5	0.8	0	5.7	1.5	0.01
7[a]	16.7	0.1	0	0.8	1.0	0	1.5	0.7	0.02
3[b]	18.4	0	0	0.4	1.2	10.1	11.3	5.9	0.01
7[b]	18.0	0	0	8.2	0.5	0	5.9	0.8	0.01

[a] Starch was used as a co-substrate at 0.1% concentration.
[b] Xylan was used as a co-substrate at 0.1% concentration.

as determined by the decrease in NC weight, and it appears that no further utilization occurred on the seventh day. NC was better utilized in the presence of starch while xylan enhanced the fungal growth on NC. The fungal culture produced the xylan degrading enzyme, xylanase (Table III), and the resulting end product xylose appears to be equally good carbon source for the culture growth. The increased utilization of NC (Table IV) in the presence of starch may be due to the fact that other operative enzyme systems could have facilitated the NC degradation. Since *P. corylophilum* degraded only 20% of the NC, attempts were made to improve the NC degradation by combining the culture with *F. solani* IFO 31093, which was used in our earlier investigation.[13] However, the presence of *F. solani* IFO 31093 along with *P. corylophilum* did not enhance NC degradation.

Table IV Nitrocellulose Utilization of *P. corylophilum* Alone or in Combination with *F. solani* IFO 31093 in Liquid Medium Containing Co-substrate

Culture	Residual Nitrocellulose (mg)[a]		Biomass Dry Weight (mg)[b]		
	0 d	3 d	0 d	3 d	7 d
P. corylophilum[c]	150	120.7	28.2	28.6	46.9
P. corylophilum[d] *F. solani*[c]	150	125.9	18.7	27.3	31.9
P. corylophilum[e]	150	124.5	28.2	37.4	49.6
P. corylophilum[d] *F. solani*[e]	150	132.2	18.7	29.8	33.5

[a] Data corrected for the biomass co-precipitated with NC.
[b] Data corrected for the amount of biomass extracted in acetone.
The biomass dry weight in the control lacking nitrocellulose or any other nitrogen source but having basal salt medium did not increase.
The control containing the fungal cultures but killed at 0 h by the addition of $HgCl_2$ and NaN_3 did not reveal any increase in biomass.
Abiotic control on 3 and 7 days incubation under identical conditions resulted in 1.4% nitrocellulose loss.
[c] Starch was supplemented at 0.1% level.
[d] *P. corylophilum* in combination with *F. solani* was tested.
[e] Xylan was supplemented at 0.1% level.

The mechanism of NC biodegradation remains to be identified. One of the current working hypothesis is that highly reactive oxygen derived radicals are involved in this process. The use of free hydroxyl radical (·OH) as an oxidizing agent for the treatment of hazardous wastes has received much attention in recent years.[25,26] Methods of ·OH production include direct addition of ferrous salt to H_2O_2,[27] and photolysis of H_2O_2 as well as TiO_2.[28,29] Barbeni et al.[30] reported that polychlorinated biphenyls are hydroxylated by H_2O_2 (Fenton's reagent). Sedlak and Andren[31] had shown that chlorinated phenols react with the Fenton's reagent releasing the chloride ions. It is likely that ·OH could be involved in the fungal degradation of a range of compounds, possibly including NC as it is known that ·OH reacts with most organic pollutants and wood.[32–35]

The fungi appear to have a method of generating H_2O_2 from glucose through a glucose oxidase reaction.[33] Lignin peroxidase uses H_2O_2 to produce free hydroxyl radicals.[25] Glucose oxidase was reported to be present in fungal systems including *Penicillium* sp.[36] Also, the *Penicillium* sp. has been reported to produce cellulases, peroxidase, and denitrifying enzymes.[37–39] In the present investigation, we have detected cellulases, certain denitrifying enzymes such as nitrate and nitrite reductases, and lignin peroxidase activity in the *P. corylophilum* culture. Based on the results presented here and those published

earlier, we hypothesize that in the presence of free hydroxyl radicals generated enzymatically by the fungus, NC 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.

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 NC.[40] The nitrate ions released due to the action of nitro esterase on NC 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 NC by aqueous hydrolysis and starch or xylan, which is provided as a co-substrate, initialize growth of the *P. corylophilum* culture. Later, the culture starts denitrifying NC by the use of denitrifying enzymes. The denitrated cellulose is further attacked by the cellulase complex of the studied organism.

In our previous investigation,[13] it was reported that a cellulolytic (*S. rolfsii* ATCC 24459) and a denitrifying fungus (*F. solani* IFO 31093) were required to accomplish aerobic degradation of NC in liquid medium. In the present study, a 20% degradation of NC has been demonstrated by *P. corylophilum* and to the best of our knowledge, never before has this organism been reported to degrade NC aerobically. Studies are currently underway in our laboratory to optimize the conditions to achieve maximal degradation of NC by *P. corylophilum* and also to elucidate the NC biodegradation pathway(s).

We are thankful to Prof. June Wang, mycologist, College of Environmental Science and Forestry, State University of New York, Syracuse, New York, for identifying the fungus.

REFERENCES

1. R. Meyer, in *Explosives,* VCH Verlagsgesellschaft mbH, Weinheim, Germany, 1987, p. 113.
2. J. Quinchon and J. Tranchant, in *Propellants, Explosives and Other Industries,* Wiley, West Sussex, United Kingdom, 1989.
3. T. M. Wendt and A. M. Kaplan, *J. Water Poll Control Fed.,* **48,** 660 (1976).
4. K. Gold and B. W. Brodman, U.S. Pat. 4,814,439 (1989).
5. H. N. Hsieh and F. J. Tai, in *Proc. Nitrocellulose—Fines, Separation and Treatment,* U.S. Army–Purdue University, West Lafayette, IN, 1993, pp. 110–121.
6. T. Bokorny (as quoted by T. Urbanski), in *Chemistry and Technology,* Pergamon Press, London, 1965, p. 313.
7. B. Malenkovic (as quoted by T. Urbanski), in *Chemistry and Technology,* Pergamon Press, London, 1965, p. 313.
8. M. Jacque (as quoted by T. Urbanski), in *Chemistry and Technology,* Pergamon Press, London, 1965, p. 313.
9. D. L. Kaplan, P. A. Railey, J. Pierce, and A. M. Kaplan, *Internatl. Biodeterioration,* **23,** 233 (1987).
10. P. A. Railey, D. L. Kaplan, and A. M. Kaplan, Technical Report 85-004, U.S. Army Natic Research, Development and Engineering Center, Natick, MA, 1984.
11. B. W. Brodman and M. P. Devine, *J. Appl. Polym. Sci.,* **26,** 997 (1981).
12. O. N. IL'Inskaya and I. B. Leshchinskaya, *Biotekhnologiya,* **4,** 495 (1988).
13. A. Sharma, Shanmuga T. Sundram, Y. Z. Zhang and B. W. Brodman, *J. Indust. Microbiol.,* to appear.
14. H. Shoun, D-H. Kim, H. Uchiyama, and J. Sugiyama, *FEMS Microbiol. Lett.,* **94,** 277 (1992).
15. M. Mandels and J. Weber, in *Cellulases and Their Applications,* Vol. 95, R. F. Gould, Ed., Adv. Chem. Ser., American Chemical Society, Washington, D.C., 1969, pp. 391–414.
16. M. S. Kubackova, S. Karacsonyi, and R. Toman, *Folia Microbiol.,* **21,** 28 (1976).
17. A. Sharma, O. Milstein, Y. Vered, H. M. Flowers, and J. Gressel, *Biotechnol. Bioeng.,* **27,** 1095 (1985).
18. M. Tien and T. K. Kirk, *Proc. Natl. Acad. Sci. USA,* **81,** 2280 (1984).
19. C. H. MacGregor, in *Methods in Enzymology,* Vol. LIII, S. P. Colowick and N. O. Kaplan, Eds., Academic Press, New York, 1978, pp. 347–355.
20. T. H. Kakutani, H. Watanabe, K. Arima, and T. Beppu, *J. Biochem.,* **89,** 453 (1981).
21. M. Somogyi, *J. Biol. Chem.,* **195,** 19 (1952).
22. M. M. Bradford, *Anal. Biochem.,* **72,** 248 (1976).
23. D. Nicholas and A. Nason, in *Methods in Enzymology,* Vol. 3, S. P. Colowick and N. O. Kaplan, Eds., Academic Press, New York, 1957, pp. 981–984.
24. *ATCC Catalogue of Filamentous Fungi,* S. C. Jong and M. J. Edwards, Eds., American Type Culture Collection, 18th ed., Rockville, MD, 1991, pp. 280–281.
25. D. P. Barr, M. M. Shah, T. A. Grover, and S. D. Aust, *Arch. Biochem. Biophys.,* **298,** 480 (1992).
26. L. J. Forney, C. A. Reddy, M. Tien, and S. D. Aust, *J. Biol. Chem.,* **257,** 11455 (1982).
27. J. T. Spadaro, L. Isabelle, and V. Renganathan, *Environ. Sci. Technol.,* **28,** 1389 (1994).
28. G. R. Peyton and H. Glaze, *Environ. Sci. Technol.,* **22,** 761 (1988).
29. T. Jesseming and C. P. Haung, in *Proc. 1st Intl. Symp.*

Chem. Oxid., W. W. Eckenfelder, A. R. Browns, and J. R. Roth, Eds., Nashville, TN, 1982, p. 262.
30. M. Barbeni, C. Minero, and E. Pelizzetti, *Chemosphere,* **16,** 2225 (1987).
31. L. Sedlak and A. W. Andren, *Environ. Sci. Technol.,* **25,** 1419 (1991).
32. J. Kochany and J. R. Bolton, *Environ. Sci. Technol.,* **26,** 262 (1992).
33. R. L. Kelly and C. A. Reddy, *Arch. Microbiol.,* **144,** 248 (1986).
34. L. Plant and M. Jeff, *Chem. Eng.,* **101,** EE16 (1994).
35. J. W. Koenigs, *Wood Fiber,* **6,** 66 (1974).
36. K. Welfle, W. Buttner, and J. Behlke, *Studia Biophy.,* **138,** 245 (1990).
37. R. J. Gouka, W. van Hartingsveldt, and R. A. L. Bovenberg, *J. Biotechnol.,* **20,** 189 (1991).
38. M. Claeyssens, H. van Tilbeurgh, and P. Tomme, *Biochem. J.,* **261,** 819 (1989).
39. A. Rodriguez, A. Carnicero, F. Perestelo, G. de la Fuente, O. Milstein, and M. A. Falcon, *Appl. Environ. Microbiol.,* **60,** 2971 (1994).
40. O. N. IL'Inskaya and I. B. Leshchinskaya, *Microbiologia,* **56,** 1033 (1987).

Received August 22, 1994
Accepted October 19, 1994