

# Microbial Attack of Nitrocellulose

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## Synopsis

This is a study dealing with the use of a microorganism for partial denitration of the surface of nitrocellulose-based small arms propellants, in order to gain burning rate control. An organism, *Aspergillus fumigatus*, was found to grow on pyroxylin suspended in a nitrogen deficient, carbon-containing nutrient medium. No growth was observed under the same conditions when carbon was absent. This would indicate that the organism utilized nitrogen from nitrocellulose without attacking the carbon backbone. Further, mechanism studies indicated that the organism did not utilize the nitrogen directly from nitrocellulose, but rather relied on a hydrolysis reaction for a source of nitrogen.

## INTRODUCTION

Deterrents are materials diffused some distance into nitrocellulose (NC)-based small arms propellant grains in order to slow down their burning rate early in the ballistic cycle when the propellant bed surface area is at maximum. A variety of deterrent materials are used in both ball and extruded military propellants. In the case of ball propellants, the specific deterrent utilized is di-*n*-butyl phthalate (DBP). A series of past studies<sup>1-3</sup> has revealed the concentration profile produced when DBP is diffused into a spherical propellant grain and established the mechanism involved. Propellant grains deterred in this manner have the disadvantage of lot-to-lot ballistic variations along with the possibility that deterrent migration might occur under extreme storage conditions. Further, the deterring manufacturing operation involves raising the temperature to about 70°C. If this high temperature exposure could be avoided, it would result in a safety improvement. For these reasons an alternate method for control of the gas generation schedule would be desirable.

The present study is directed toward the use of microorganisms to remove energetic nitrate ester groups from the surface of propellant grains in order to obtain the requisite control of the propellant burning rate.

Past work regarding the microbial decomposition of NC produced conflicting results. Bokorny<sup>4</sup> found that mold grew on NC suspended in an aqueous medium containing mineral salts. The suggestion was made that the NC provided the mold with essential carbon and perhaps nitrogen. Malenkovic<sup>5</sup> and Jacque<sup>6</sup> in independent investigations came to the conclusion that the organism was utilizing only dissolved mineral salts and not attacking the NC. The authors concluded that the organisms gave rise to organic compounds such as acids, which in turn can degrade the NC. More recently, studies<sup>7</sup> involved with the microbial decomposition of NC particles in waste water concluded that direct denitration of the NC did not occur; however, appropriate chemical pretreatment, such as

with a mineral acid, accomplished hydrolysis of the nitrate ester. The free nitrate ions could then be utilized by the microorganism.

### EXPERIMENTAL

**Nitrocellulose.** In all cases, ground pyroxylin (11.11% nitrogen) was used.

**Culture medium.** The initial culture medium consisted of  $\text{KH}_2\text{PO}_4$ ,  $\text{K}_2\text{HPO}_4$ , and  $\text{MgSO}_4$  each present at 0.7 g/liter. In addition, the solution contained trace amounts of sodium chloride, iron sulfate, zinc sulfate, and magnesium sulfate.

**Inoculation and incubation.** In all cases, the organism used was *Aspergillus fumigatus*. In each case, the culture medium was added to a 250-ml Ehrlenmeyer flask along with 1% by weight of ground pyroxylin. The flask was then incubated at 31°C at 200 rpm on a rotary shaker for the requisite time.

**Mycelial weight.** The mycelial weight was obtained by filtering the flask contents followed by repeated washing with an ether-alcohol mixture. This wash removed the NC and the isolated mycelia were dried and weighed.

### DISCUSSION

No fungus growth was observed in the initial experiments wherein ground pyroxylin was suspended in the nitrogen deficient culture medium. Growth was observed, however, when 3% glucose was added to the shaking culture flask containing the pyroxylin, culture medium, and organism. Experiments were run in order to determine the weight of mycelial tissue produced by *Aspergillus fumigatus* in the glucose and pyroxylin containing medium. Table I contains the growth data. Examination of Table I indicates that essentially no growth occurred when the pyroxylin was absent. The fact that glucose is needed for growth would tend to indicate that the organism could not utilize the carbon from the NC. Further, the need for pyroxylin would indicate that the organism was utilizing the nitrogen from the NC.

An experiment was run to determine if the microbial attack of NC involved direct utilization of nitrogen or if the nitrate ester group had undergone hydrolysis prior to utilization by the organism. This type of hydrolysis has been previously reported in the literature.<sup>8</sup> Pyroxylin (1 g) was shaken in both a 100-ml flask of distilled water and in a 3% glucose-inorganic salt solution for seven days. The pyroxylin was filtered off, and to the flask containing only distilled water was added a 3% glucose-concentrated salt solution in order to adjust the salt concentration to that used in the previous experiment. All filtrates were

TABLE I  
Mycelial Dry Weights of *Aspergillus fumigatus* Grown on Glucose-Salts Medium Containing Ground Pyroxylin as Sole Source of Nitrogen

Incubation time, days	Mycelial dry weights, mg, nitrogen source	
	None	pyroxylin, 1%
5	0.7, 1.7	39.7, 34.9, 37.6
11	4.6	92.5, 49.8
21	0.6, 1.4	59.0, 63.8, 60.4
40	2.3, 2.3	77.2, 73.4, 74.3

cold sterilized by membrane filtration and then inoculated with a spore suspension of *Aspergillus fumigatus*. In addition, a flask containing a 3% glucose solution and an inorganic nitrogen source (0.67 g of  $\text{NaNO}_3$ ) at a level equivalent to 1 g of pyroxylin (111 mg of N) was inoculated with *Aspergillus fumigatus*.

The flasks were incubated at 31°C for 21 days on a rotary shaker. Weights of mycelia produced with the various conditions are listed in Table II. It should be noted that the mycelia were not washed with ether-alcohol prior to weighing, and thus were higher than washed weights. Examination of Table II indicates that both the distilled-water extract and salt extract contained sufficient nitrogen to support growth. Interestingly enough, the extract made with the glucose-salt solution appeared to extract slightly more nitrogen than did the distilled-water extract. This may have been due to the lower pH of the salt extract which enhanced the hydrolysis rate of the nitrate ester group. As would be expected, the cultures containing the inorganic nitrogen source showed significantly more growth. Also, a culture containing no nitrogen or carbon sources showed essentially no growth.

The next phase of the study involved an effort to establish the rate of hydrolysis of the NC nitrate ester group when suspended in an aqueous medium. Milled pyroxylin (1 g) was suspended in 100 ml of distilled water and shaken on a rotary shaker. At the appropriate intervals, the pyroxylin was separated from the water by centrifugation and the pyroxylin resuspended in fresh distilled water. The nitrate ion concentration of the supernatant liquid was determined by means of an Orion specific ion electrode and an Orion 801 ion analyzer. Table III summarizes the results of the experiment.

Chemical analysis of the dried mycelia of *Aspergillus fumigatus* has shown a nitrogen content of 2%. The values listed in Table III for nitrate ion show that about 0.44 mg of nitrogen can be removed from 1 g of nitrocellulose as nitrate ion, and that 95% of this is removed in the first four days. Based on the 2% N content of *Aspergillus fumigatus* and an available nitrogen content of 0.44 mg, the mycelia mass one would expect would be about 22.0 mg. This value of 22.0 mg for the mycelia mass is lower than the experimental value obtained for five days of growth (Table I).

Table I gives data indicating greater growth than could be accounted for by the experimental hydrolysis data. This fact can be accounted for by enhanced nitrocellulose nitrate ester hydrolysis caused by the organism.

TABLE II  
Mycelial Dry Weights Produced during Growth of *Aspergillus fumigatus* for 21 Days on Solution of Glucose and Mineral Salts with  $\text{NaNO}_3$  or on Extracts of Ground Pyroxylin as Nitrogen Source

Carbon source	Nitrogen source	Mycelial dry weight, mg	
		replicates	average
None	None	5.3, 5.7, 5.5	5.5
3% glucose	0.67 g $\text{NaNO}_3$	634.6, 652.3, 656.6	647.8
3% glucose	pyroxylin extract <sup>a</sup>	22.8, 49.4, 48.1	40.1
3% glucose	pyroxylin extract <sup>b</sup>	33.4, 30.6, 34.3	32.8

<sup>a</sup> Shaken for seven days at 200 rpm at 31°C in solution containing glucose, 30.0 g/liter;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.7 g/liter;  $\text{K}_2\text{HPO}_4$ , 0.7 g/liter;  $\text{KH}_2\text{PO}_4$ , 0.7 g/liter;  $\text{NaCl}$ , 0.005 g/liter;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.002 g/liter;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.002 g/liter;  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 0.001 g/liter.

<sup>b</sup> Shaken for seven days as above except with distilled water alone. Salts and glucose were added to distilled-water extract before cold sterilization of complete medium.

TABLE III  
Nitrate Levels in 100 ml Distilled-Water Extracts of 1 g Pyroxylin

Extraction time, days	Average, <sup>a</sup> NO <sub>3</sub> <sup>-</sup>	Average <sup>b</sup> mg N/flask	Replicates
4, initial extraction	$2.90 \times 10^{-4}M$	0.406	4
6, second extraction	$1.49 \times 10^{-5}M$	0.021	6
4, third extraction	$0.74 \times 10^{-5}M$	0.011	6

<sup>a</sup> Distilled water alone gave (NO<sub>3</sub><sup>-</sup>) readings of less than  $1 \times 10^{-5}M$ .

<sup>b</sup> 1 g pyroxylin contains 111.1 mg nitrogen.

In summary, it has been shown that *Aspergillus fumigatus* can utilize nitrogen from pyroxylin suspended in a nitrogen deficient medium if a supplementary carbon source is provided. It appears that the organism does not directly attack the nitrocellulose but rather utilizes the nitrogen resulting from the hydrolysis of the nitrocellulose nitrate ester group. Further, it appears that the organism caused enhanced hydrolysis of the nitrocellulose.

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