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Developing a Slow-release Nitrogen Fertilizer from Organic Sources: II. Using Poultry Feathers

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Abstract. **The structure of feather keratin protein was modified in attempts to develop a slow-release N fertilizer of 12 weeks duration or longer by steam hydrolysis to break disulfide bonds, enzymatic hydrolysis with *Bacillus licheniformis* (Weigmann) to break polypeptide bonds, and steam hydrolysis (autoclaving) to hasten mineralization followed by cross-linking of the protein by a formaldehyde reaction to control the increased rate of mineralization. Release of N in potting substrate within elution columns from ground, but otherwise untreated, raw feathers occurred mainly during the first 5 weeks with a much smaller release occurring from weeks 8 to 12. Steam hydrolysis resulted in an increase of N during the first 5 weeks and a decrease during weeks 8 to 11. Cumulative N release over 11 weeks increased from 12% in raw feathers to 52% for feathers steam hydrolyzed for 90 minutes. This favored an immediately available fertilizer but not a slow-release fertilizer. Microbial hydrolysis with *B. licheniformis* resulted in a modest reduction of N release during the first 5 weeks and a small increase during weeks 8 to 11. Both shifts, while not desirable for an immediately available fertilizer, enhanced the slow-release fertilizer potential of feathers but not sufficiently to result in a useful product. Steam hydrolyzed feathers cross-linked with quantities of formaldehyde equal to 5% and 10% of the feather weight released less N during the first 5 weeks, more during weeks 6 and 7, and less during weeks 9 to 12 compared to raw feathers. The first two shifts were favorable for a slow-release fertilizer while the third was not.**

Many organic materials from different animal sources such as manure (Post, 1956), hoof and horn, blood meal, and blood and bone (Handrek and Black, 1984) have been evaluated as N fertilizers for greenhouse applications. However, Bunt (1988) indicated that 70% of the N was released within 30 days under greenhouse conditions. Williams and Nelson (1992) reported similar results. They evaluated eight organic N sources for mineralization (release of nutrients in plant available forms) in greenhouse chrysanthemum [*Dendranthema ×grandiflorum* (Ramat.) Kitamura] and found durations of N release to be 6 to 7 weeks in all organic materials except poultry feathers. Release of N from feathers was too slow to be of use as a fertilizer.

Feathers are produced in huge quantities as a waste material of the poultry processing industry. Since 90% of feather dry weight consists of crude keratin protein, and feathers as a whole contain about 15% N (Papadopoulos et al., 1985, 1986), they have a strong potential to be used as a slow-release N fertilizer in the greenhouse and nursery industries. However, practical use of feather keratin is currently limited to the animal feed industry where structural modifications are made in polypeptide bonds and disulfide bonds to increase digestibility.

Mineralization of organic N under soil conditions refers to release of NH_4 from proteins, amino acids, and nucleic acids via degradative reaction initiated by soil microorganisms (Paul and Clark, 1989). Slow release of N from feather keratin indicates that soil microorganisms can not readily cleave the keratin structure. Therefore, if keratin structure was modified by the cleavage of specific bonds, the mineralization rate would increase. The cloven feather structure would reduce chemical barriers by providing more reactive sites for microbial enzymes and would reduce physical barriers by providing more surface area for microbial breakdown.

Modification of feather keratin structure in the animal feed industry follows two main approaches: A) steam hydrolysis, which breaks disulfide bridges (McCasland and Richardson, 1967; Morris and Balloun, 1973), and B) enzymatic action of *Bacillus licheniformis* (Williams et al., 1990) and *Streptomyces fradiae* (Waksman and Curtis) Waksman and Henrico (Elmayergi and Smith, 1971) that cleaves polypeptide bonds. An additional possibility is to use carbonyl compounds such as aldehydes that react very readily and reversely with amino groups of proteins to form Schiff's bases (Wong, 1991). After steam hydrolyzing feathers to break disulfide bonds and thereby hasten mineralization, new cross-links could be formed in keratin by reaction with formaldehyde. Many of the new aminobonds would be unnatural and therefore resistant to the proteolytic enzymes of mineralizing microbes (Milligan and Holt, 1977; Seltzer, 1973).

Therefore, the objectives of this research were to modify disulfide bonds and polypeptide bonds in feather keratin by heat hydrolysis, microbial enzyme attack, and heat hydrolysis coupled with formaldehyde cross-linking of keratin and to determine the effects of these modifications on mineralization of N from feather keratin in a potting substrate. It was hoped that these modifications would lead to a slow-release fertilizer with a continually increasing rate of N release over a period of 12 weeks or longer to match the N requirement of many container grown crops.

Materials and Methods

Preparation of raw feathers. White feathers of broiler chickens were obtained from a local poultry processing plant soon after plucking. They were freed of foreign matter, cleaned with tap water, dried 3 d in a forced draft oven at 60 °C, and stored for subsequent use. In each of the following experiments a raw feather

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Table 1. Description of treatments and the final total N concentrations in resulting feather products in Expt. 3.

Formaldehyde[z] (%)	Feathers[y] (%)	N content
0	Raw	14.7
0	Hydrolyzed	15.3
0.005	Hydrolyzed	12.3
0.01	Hydrolyzed	12.3
0.05	Hydrolyzed	12.0
0.1	Hydrolyzed	12.2
0.5	Hydrolyzed	11.5
1.0	Hydrolyzed	11.8
5.0	Hydrolyzed	11.4
10.0	Hydrolyzed	11.5
$LSD_{0.05}$		0.55

control treatment was included. Raw feathers consisted of washed and dried feathers ground in a Wiley mill to a particle size ≤1 mm.

Preparation of steam hydrolysed feathers. Distilled water was added at the rate of 5 mL per 10 g whole, dry feathers. These feathers were then steam hydrolysed in Expt. 1 for 15, 45, or 90 min at a temperature of 135 °C and a pressure of 207 kPa to produce easily degradable N products. Timing of steam hydrolysis began when the specified temperature and pressure levels were reached. Then, feathers were dried overnight at 70 °C and ground to a particle size ≤1 mm. These final products were designated as hydrolyzed feathers.

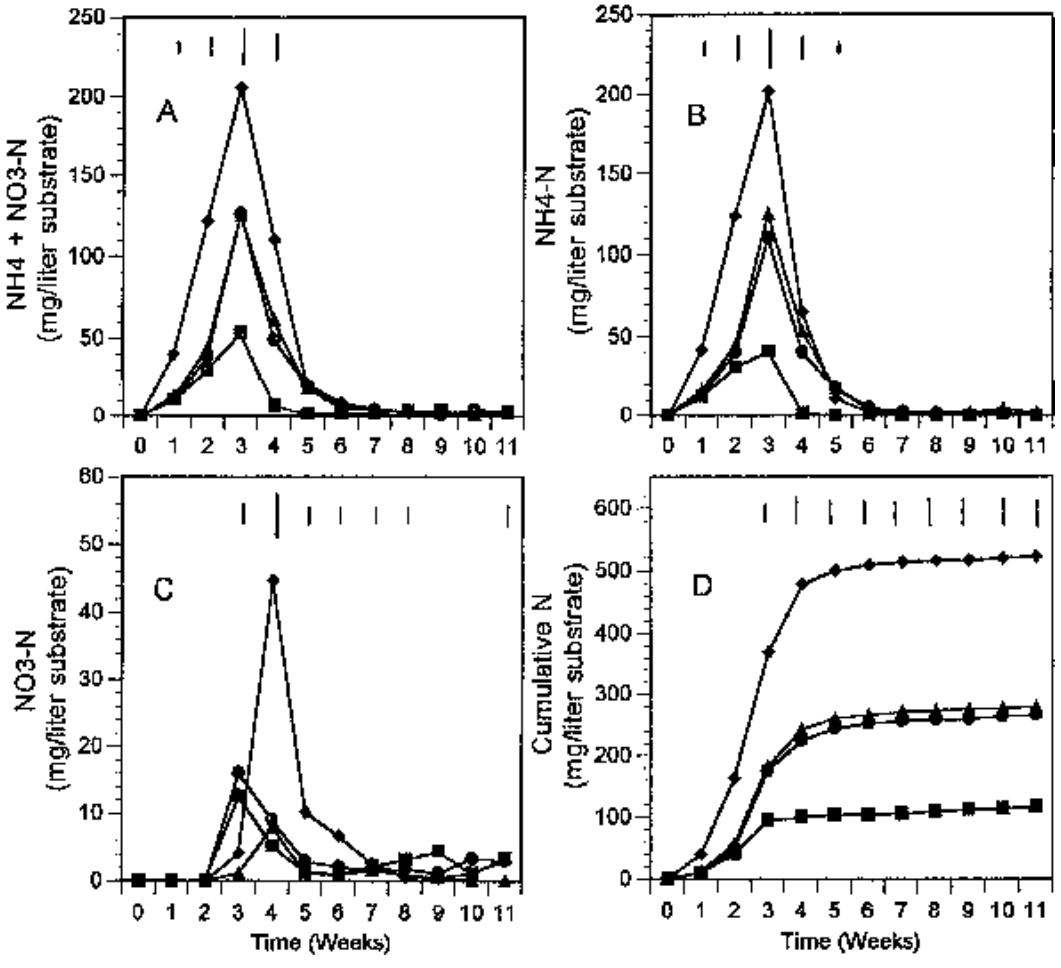
Preparation of microbial hydrolysed feathers. Whole feathers were microbially hydrolysed with a culture of *B. licheniformis* (10^8 cells/mL of culture solution) for 0, 24, 48, 84, or 120 h at 50 °C in Expt. 2. The ratio of feathers to *B. licheniformis* culture solution was 1:1 (w/w). After hydrolysis, feathers were dried for 2 d at 70 °C and ground to a particle size ≤1 mm. These products were designated as microbial hydrolysed feathers.

Preparation of formaldehyde treated feathers. Feathers were steam hydrolysed in Expt. 3, as previously described, for 150 min and were then reacted with weights of formaldehyde (a.i.) equal to 0.1%, 0.5%, 1.0%, 5.0%, or 10.0% of the dry weight of the steam hydrolyzed feathers. The formaldehyde reaction entailed addition of 60 mL distilled water to 30 g steam hydrolysed feathers resulting in a thick slurry. Formaldehyde was added to these slurries and then 3.75 g H_3PO_4 was added to lower the pH level to about 1.5. The slurries were cooled to ambient temperature. The reaction mixture was titrated with solid CaO to a pH of 6.0. The feathers were then dried overnight at 70 °C and ground to a particle size of ≤1 mm. These products were designated formaldehyde-treated feathers.

Column elution procedures. The patterns of N mineralization from the feather materials developed in each of the three experiments in this study were determined by the same column elution procedures described in the previous paper (Choi and Nelson, 1996) with only two exceptions. In the background treatment columns, containing only substrate, a higher limestone rate of 6.0 $g \cdot L^{-1}$ was applied. A lower rate of 2.0 $g \cdot L^{-1}$ was used in the columns with the feather materials because these materials resulted in an increase in substrate pH as NH_4 was released from them. In the first two experiments every three consecutive elutions (Friday, Monday, and Wednesday) were combined and in the third experiment, where the columns were eluted two times per week (Friday and Monday) these two elutions were combined.

Chemical analysis procedures. Total N in feather products was determined by a semimicro Kjeldahl procedure (Eastin, 1978). All other mineral nutrient analyses procedures were as described in the

Fig. 1. Weekly quantities of NH_4-N + NO_3-N (**A**), NH_4-N (**B**), and NO_3-N (**C**) released into the eluent and cumulative release of NH_4-N + NO_3-N (**D**) from columns containing substrate with feathers steam hydrolyzed for various lengths of time in Expt. 1. Each feather product was incorporated at a rate sufficient to supply 1 g N/L of substrate. Points represent mean of three replications. Vertical bars represent 1 SE among weekly treatments (■ = substrate with raw feathers, ● = substrate with 15 min steam hydrolyzed feathers, ▲ = substrate with 45 min steam hydrolyzed feathers, ◆ = substrate with 90 min steam hydrolyzed feathers).



earlier paper in this series (Choi and Nelson, 1996). In addition to the treatment columns in each experiment, background columns were set up with substrate but no feather products. These columns were eluted to determine forms and quantities of N released and these values were subtracted from all treatment values.

Experimental design and data analysis. The elution columns in each experiment were arranged in a randomized complete block design with three replications. One column was used in each plot. The standard error for all treatments was determined within each week for data from column leachate analysis using the CoStat program (CoHort Software, Berkeley, Calif.). Total N concentration data from analysis of the feather products in Expt. 3 were subjected to analysis of variance procedures and means were separated by the LSD test.

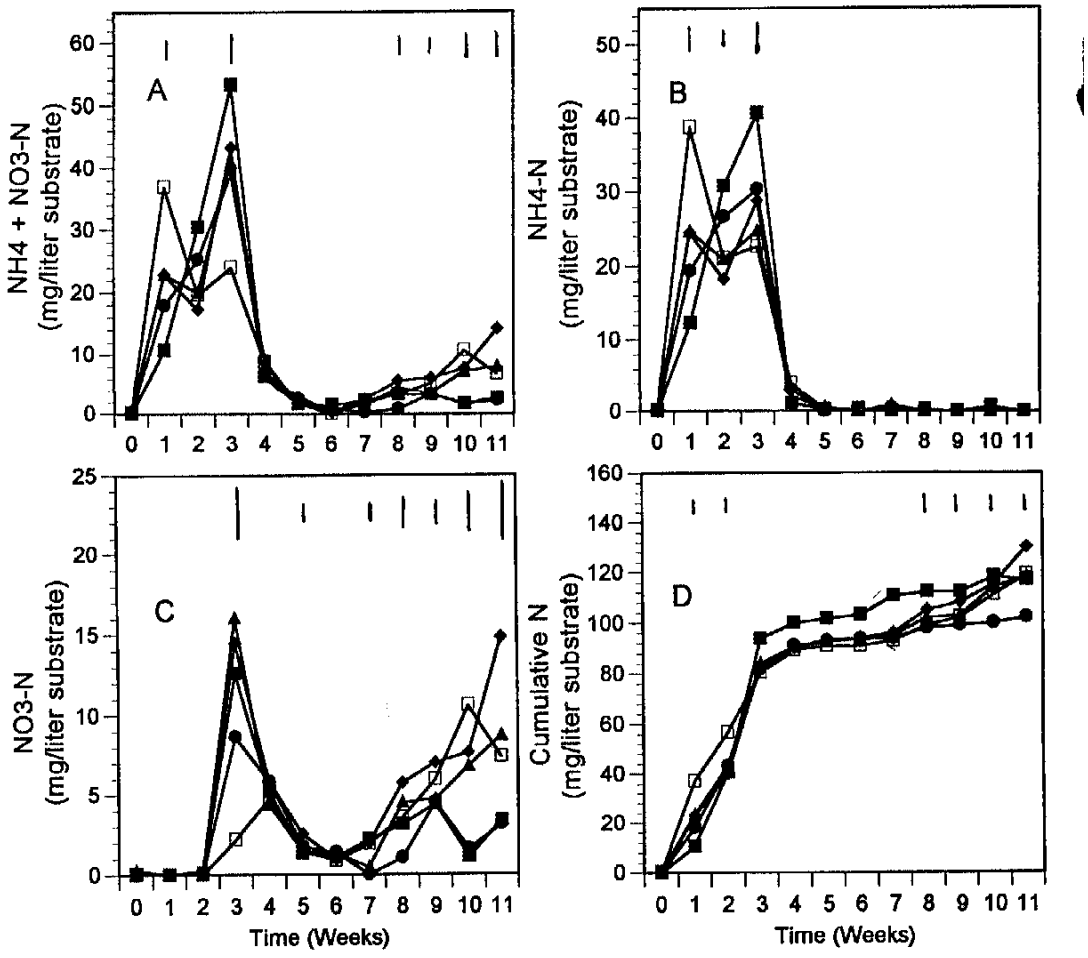
Results and Discussion

Average total N concentration in steam hydrolyzed (Expt. 1) and microbially hydrolyzed (Expt. 2) feathers (data not presented) was 14.7% and did not differ among treatments. The N concentrations of formaldehyde-treated feather products in Expt. 3 (Table 1) were lower in the formaldehyde treatments than in the raw feathers and hydrolyzed feathers. This was due to dilution effects of reactants and titrant used in the formaldehyde reaction.

Experiment 1. Steam hydrolyzed feathers. Most N released from raw feathers occurred during weeks 1 through 3 (Fig. 1A). Steam hydrolysis of feathers for 15 and 45 min resulted in similar increases in the release of N during weeks 1 through 5 compared to raw feathers. Steam hydrolysis for 90 min resulted in a further increase during the same time period. The increased N release was probably due to cleavage of disulfide bonds which rendered the feathers more susceptible to microbial degradation in the substrate (Aderibigbe and Church, 1983; Papadopoulos et al., 1986). The cumulative percentages of the total amount of N applied to the column substrates that were released over 11 weeks from raw feathers, and 15 min, and 90 min hydrolyzed feathers were 12%, 27%, and 52%, respectively (Fig. 1D). The increases in N release caused by steam hydrolysis were not favorable for purposes of a slow-release fertilizer. All of the increased release occurred during the first 5 weeks when an increase was not desired and none during the last 6 weeks where the release should have been highest. These changes were highly desirable, however, for the purposes of an immediately available fertilizer. Longer steam hydrolysis times might serve well for this latter objective to increase N mineralization above the 52% level achieved.

Experiment 2. Microbially hydrolysed feathers. The effect of microbial hydrolysis with *B. licheniformis* during the first 5 weeks was a shift toward earlier release of N. As the time of microbial hydrolysis increased the quantity of N released during the first week increased proportionately while the amount released during weeks 2 and 3 decreased (Fig. 2 A and B). The total cumulative amounts of N released by the end of week 5 (Fig. 2D) from the various microbial hydrolysis treatments did not differ from each other and were only slightly lower than in the raw feather treatment. Overall, there was no advantage gained during the first 5 weeks toward the requirements for a slow-release fertilizer. Increased release during week 1 constituted a disadvantage while reduced cumulative release over the first 5 weeks provided a small advantage. The main advantage of microbial hydrolysis occurred in the release pattern of total N during weeks 8 through 11 (Fig. 2A). Feathers microbial hydrolysed for 48, 84, and 120 h released more N than raw feathers in that period. The largest release of N at

Fig. 2. Weekly quantities of NH_4-N + NO_3-N (**A**), NH_4-N (**B**), and NO_3-N (**C**) released into the eluent and cumulative release of NH_4-N + NO_3-N (**D**) from columns containing substrate with feathers hydrolyzed for various lengths of time with *B. licheniformis* in Expt. 2. Each feather product was incorporated at a rate sufficient to supply 1 g N/L of substrate. Points represent mean of three replications. Vertical bars represent 1 SE among weekly treatments (■ = substrate with raw feathers, ● = substrate with 24 h hydrolyzed feathers, ▲ = substrate with 48 h hydrolyzed feathers, ◆ = substrate with 84 h hydrolyzed feathers, ❑ = substrate with 120 h hydrolyzed feathers).



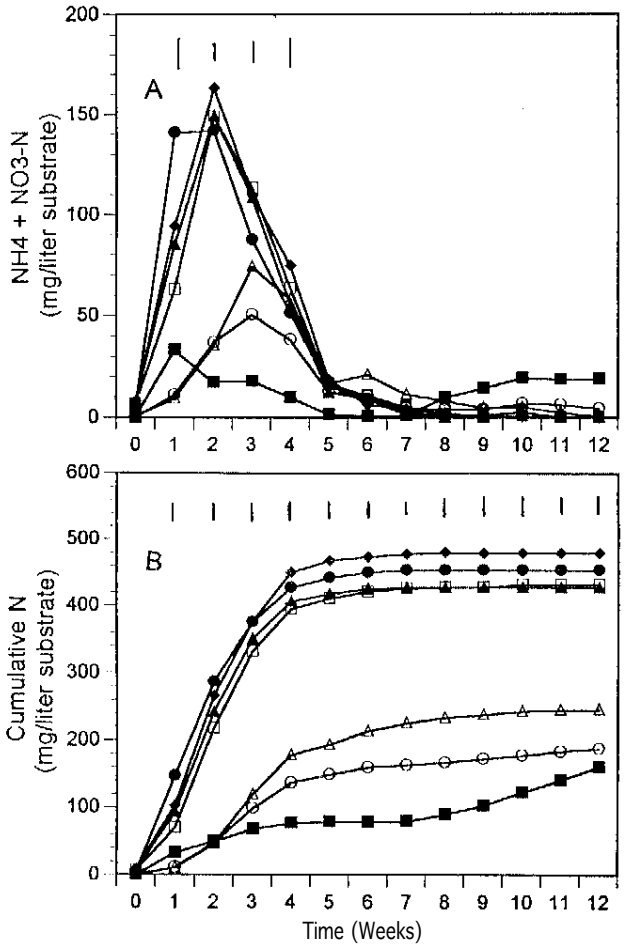


Fig. 3. Weekly quantities of NH_4-N + NO_3-N (**A**) released into the eluent and cumulative release of NH_4-N + NO_3-N (**B**) from columns containing substrate with steam hydrolyzed feathers subsequently reacted with various amounts of formaldehyde, expressed as a weight percentage of feathers, in Expt. 3. Each feather product was incorporated at a rate sufficient to supply 1 g N/L of substrate. Points represent mean of three replications. Vertical bars represent 1 SE among weekly treatments (■ = raw feathers, ● = 2.5 h steam hydrolyzed feathers, ▲ = hydrolyzed feathers reacted with 0.1% formaldehyde, ◆ = hydrolyzed feathers reacted with 0.5% formaldehyde, ❑ = hydrolyzed feathers reacted with 1.0% formaldehyde, ❍ = hydrolyzed feathers reacted with 5.0% formaldehyde, Δ = hydrolyzed feathers reacted with 10.0% formaldehyde).

week 11 occurred from 84 h hydrolysed feathers. Feathers microbially hydrolysed for 84 h released 4.4% of their N during weeks 8 through 11 while raw feathers released 2.6% of their N in the same period. The gain from 84 h of microbial hydrolysis over raw feathers was only 1.8% of the 1000 mg of feather N applied to each column (Fig. 2D).

Comparison of the NH_4 release curves in Fig. 2B with the NO_3 release curves in Fig. 2C reveals a delay in the peak of NO_3 release. This was likely due to oxidation of NH_4 to NO_3 by nitrifying bacteria (Paul and Clark, 1989). The delay can be accounted for by the time required for the nitrifying bacteria populations to build up in response to the initial occurrence of NH_4 in the substrate.

Microbial hydrolysis of whole feathers with *B. licheniformis* brought about desirable reductions during the first 5 week period and increases during the 8- to 11-week period of N release. The shifts, however, were not sufficiently large to warrant use of microbial hydrolysis, as conducted in this experiment, for developing slow-release fertilizer from feathers. Further studies with keratinase active microorganisms are warranted and treatment of ground rather than whole feathers should be tested.

Experiment 3. Formaldehyde treated feathers. Release of N for slow-release fertilizer purposes was best, although not completely satisfactory, from raw feathers (Fig. 3A). There was a period of N release through the first four weeks, followed by little release during weeks 4 through 6, and a second period of release during weeks 7 through 12. The slope of the cumulative curve for N release from raw feathers (Fig. 3B) came closer to matching the continually increasing slope sought for a slow-release fertilizer than any other treatment. Feathers steam hydrolyzed for 150 min released a larger quantity of N during the first 6 weeks than raw feathers and nearly no N thereafter (Fig. 3A). This was more suitable for an immediately available fertilizer but less desirable for a slow-release fertilizer than raw feathers since the cumulative release of N was increased from 16% in raw feathers to 45% in hydrolyzed feathers (Fig. 3B).

Steam hydrolyzed feathers reacted with 0.1%, 0.5%, and 1.0% formaldehyde had similar N release patterns to feathers that were only steam hydrolyzed (Fig. 3A). Steam hydrolyzed feathers reacted with 5% and 10% formaldehyde released less N during the first 4 weeks than feathers only hydrolyzed and a similar low amount in subsequent weeks, thus, these treatments were inferior to hydrolyzed feathers for both types of fertilizers. Hydrolyzed feathers reacted with 0.005%, 0.01%, and 0.05% formaldehyde had a virtually identical N release pattern to feathers that were only steam hydrolyzed (data not shown). Overall, cross-linking of steam hydrolyzed feather keratin with formaldehyde offered no advantages for an immediately available fertilizer over feathers hydrolyzed only because total N release was similar and rendered feathers less desirable for slow-release fertilizer than raw feathers due to the lower N release during weeks 8 through 12.

The release of PO_4-P and Ca into the eluents followed almost identical patterns and quantitative levels to those in Fig 3 of the previous paper (Choi and Nelson, 1996) (data not shown). Again, the sources appeared to be the H_3PO_4 and CaO used in the formaldehyde reaction. Most PO_4 was present at time 0. Little PO_4 was released during weeks 1 through 5, but during weeks 6 through 12 there was an increase in PO_4 release in the treatments where H_3PO_4 was used. Calcium release declined over time. The level of Ca release related well with the amount of formaldehyde used in the reaction with feathers. Again, this suggests that less supplemental PO_4 and Ca would be required if a crop were fertilized with a formaldehyde processed feather product.

In summary, steam hydrolysis of feathers resulted in a four fold increase in N release which was good for an immediate release fertilizer but did not add to the 12-week profile of slow N release that was sought since the increased release occurred during the first 5 weeks. Cross linking of amino groups in the feather protein with formaldehyde after steam hydrolysis did not improve the slow-release properties of feathers either. The one procedure that was successful in increasing the slow-release property of feathers was microbial hydrolysis with *B. licheniformis* which increased N release during the 8- through 11-week period. While the increase was not sufficient to yield a commercial product, it indicated that further investigation of microbial hydrolysis was warranted.

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