Affinity of Hyperammonia-Producing Bacteria To Produce Bioammonium/Ammonia Utilizing Five Organic Nitrogen Substrates for Potential Use as an Organic Liquid Fertilizer

Brian K. Ward, Robert J. Dufault, Richard Hassell, and Matthew A. Cutulle*

Department of Plant and Environmental Sciences, Horticulture, Clemson University Clemson Coastal Research and Education Center, 2700 Savannah Hwy, Charleston, South Carolina 29414, United States

ABSTRACT: This research was conducted to create a plant-available nitrogen nutrient solution utilizing ruminant bacteria for ultimate use as a liquid nitrogen fertilizer for precision fertigation of vegetable crops. Three hyperammonia-producing ruminant bacteria, Clostridium aminophilum, Peptostreptococcus anaerobius, and Clostridium sticklandii, were cultured anaerobically using five different organic nitrogen substrates to determine their efficiency in producing bioammonium/ammonia (BAA), a term defined here as a biologically produced solution containing both ammonium and ammonia. These bacteria were chosen because of their ability to produce ammonium at rates not experienced by any other bacteria. The five substrates were soy protein isolate (SPI), blood meal, feather meal, dried fish, and yeast extract (Y) used alone and in combination with Y. C. aminophilum and SPI were selected for further experimentation in an attempt to maximize BAA production. These substrates were chosen because they are commonly fed to cattle and they are also used as organic fertilizer amendments. C. aminophilum was cultured with SPI rates from 0.8 g·10 mL−1 salt solution reaching SPI’s maximum solubility level at 1.6 g·10 mL−1 salt solution at 0.2 g intervals, and the BAA content was measured every 24 h for 168 h. It was concluded that there was no significant benefit in cultivating C. aminophilum with more than 1.0 g·10 mL−1 for more than 96 h to achieve maximum BAA concentrations.

INTRODUCTION

Public awareness and demand for healthier foods have led to an increased consumption of organic vegetables, resulting in one of the fastest growing agricultural markets in the United States.1 The increased demand for organic produce necessitates exploration and optimization of organic sources of fertilizer. Plants can only absorb nutrients in mineralized forms such as ammonium or nitrate.2 Organic sources of nitrogen (N) are found in proteins, polypeptides, and amino acids; prior to uptake by a plant, microorganisms must convert these sources into mineral forms.3 Many different species of microorganisms accomplish this conversion in a variety of terrestrial, aquatic, and symbiotic environments. In typical agricultural settings, these microbial-mediated transformations occur in the soil rhizosphere of the plant. These transformations are highly variable and dependent on a healthy soil food web and favorable climatic and environmental conditions.4 These conditions are especially lacking in sandy soils common on South Carolina’s coastal plain region (personal communication with Powell Smith, Extension Agent, Clemson University). Infertile soils in this region also impede the success of conventional growers transitioning to organic production. Organic crops growing on these infertile soils may demand more available N than the soils can provide.5 It is challenging to conduct comparative studies with organic and conventional vegetables because of the mineralization state of the fertilizers. Essentially, bound N found in organic fertilizer amendments cannot benefit plant growth until mineralized and made plant available. Lack of available N during critical growth stages can result in nutrient deficiencies, delayed maturity, lack of uniform maturity, lower yields, and quality changes.

The first step in the mineralization of organic matter and N-rich proteins in the rhizosphere is the production of ammonia. In an effort to quantify mineralization rates of organic fertilizers in soils, Hartz and Johnstone5 compared the mineralization rate of feather meal (FM), dried fish (DF), and blood meal (BM) mixed with field soil incubated for 8 weeks at 10, 15, 20, and 25 °C, respectively. Nitrogen mineralization rates were not significantly different at differing temperatures and occurred rapidly for the first 2 weeks. Across all temperatures, 8 week N mineralization averaged 60, 62, and 66% for FM, DF, and BM, respectively. Although slow mineralization rates can be an advantage in certain scenarios such as for use in slow-release fertilizers, the mineralization rates of these soil amendments in the above-mentioned study are still far too slow to effectively fertilize vegetable crops.
through drip fertigation. Therefore, microorganisms existing naturally in the soil and Johnstone’s experiment failed to mineralize these organic fertilizers fast enough for optimal crop growth. Microorganisms capable of accomplishing more efficient rates of mineralization in bioreactor situations for controlled mineralization of these organic N substrates need further investigation.

The largest source of ammonia production in natural environments is not terrestrial or aquatic but is in ruminant digestion as the loss of protein-based urea N through the symbiotic metabolism of both rumen microorganisms and metabolism of feed by rumen microorganisms. The efficiency of dietary N is approximately 15% of the total N consumed in the diet of ruminants, and the remaining 85% can be lost through excretion in which scientists have estimated that as much as 50% of N in ruminant feed is lost as ammonia. In ruminant nutrition, amino acid deamination is nutritionally wasteful. Early attempts by Russell et al. to isolate bacteria responsible for excess ammonia production in the rumen concluded that *Peptostreptococcus* and *Clostridium* species were the organisms responsible when soybean meal was an ingredient in ruminant feed. This early work eventually gave rise to the isolation and characterization of a novel new group of obligate amino acid-fermenting bacteria designated as hyperammonia-producing (HAP), and of particular interest was the discovery of three new HAP species: *Peptostreptococcus anaerobius*, *Clostridium sticklandii*, and *Clostridium aminophilum*. In a continuous culture, these bacteria had developed an affinity for yeast extract (Y) at 0.5 g L⁻¹ (J. B. Russell, personal communication). Recent interest in the HAP bacterial species has developed strategies to inhibit their growth because the cost of feed supplements to cattle producers is expensive and the loss of nitrogen from these organisms is very wasteful.

BM, FM, DF, Y, and soy protein isolate (SPI) are used not only as feed supplements but also as organic fertilizer amendments because they are protein-based and rich in nitrogen. All of them are rich in N and contain specific amino acids that are conducive to rapid growth of the HAP species; they also produce large amounts of ammonia byproducts that may be used in organic crop production (Table 1). The goal in this experiment was to orchestrate the conversion of a protein source by an HAP species in some novel bioreactor, which is similar to a ruminant digestion, with various organic N substrates to capture and produce BAA at a known concentration and use this product to precision-fertilize vegetables organically.

The objective of this study was to evaluate the ammonia-producing ability of *P. anaerobius*, *C. sticklandii*, and *C. aminophilum* grown in vitro in pure culture on BM, FM, DF, Y, and SPI to subsequently identify one bacteria species and protein substrate that produces maximum ammonia concentrations for its ultimate use as an ammonia/nitrate fertilizer in organic crop production. A subsequent study will then focus on the bacteria’s ability to produce maximum BAA using minimal protein substrate amounts over various time frequencies.

### RESULTS AND DISCUSSION

**Bacteria Selection Assay.** The relative importance of each source of variation and interaction in the analysis of variance (ANOVA) were partitioned (Table 2). Of all sources, bacteria species accounted for the greatest amount of variation (47.1%; \( P < 0.0001 \)). Replication accounted for only 2.9% of the variation, indicating that repetitions of this experiment were very similar and predictable to each other. The substrate was also highly significant at the \( P \leq 0.0001 \) level, accounting for 4.2% of the variation. The interaction of HAP species with the substrate was highly significant and accounted for 17.0% of the variation, which indicated that not all bacteria responded similarly to the substrate. The main effect of time on BAA evolution was also highly significant and accounted for 9.7% of the variation. The interaction of HAP species with time was highly significant, accounting for 4.0% of the variation. Because

### Table 1. Percentages of Amino Acid, Total Protein, and Total Nitrogen of the Five Nitrogen Substrates (As Verified by Independent Lab Analysis: ISO13903:2005) Used To Produce BAA

| amino acid | BM (%) | FM (%) | DF meal (%) | SPI (%) | Y (%) |
|------------|--------|--------|-------------|---------|-------|
| alanine    | 7.7    | 3.5    | 4.6         | 4.3     | 5.4   |
| arginine   | 4.2    | 5.9    | 4.4         | 8.9     | 2.6   |
| aspartic acid | 7.2  | 4.9    | 6.4         | 11.4    | 5.4   |
| cystine    | 1.2    | 3.7    | 0.7         | 1.1     | 0.1   |
| glutamic acid | 8.8  | 9.9    | 9.2         | 18.9    | 10.4  |
| glycine    | 4.5    | 6.8    | 5.4         | 4.1     | 2.9   |
| histidine  | 6.5    | 0.4    | 2.0         | 4.1     | 1.2   |
| isoleucine | 3.2    | 4.2    | 3.0         | 4.4     | 3.8   |
| leucine    | 11.4   | 6.5    | 5.1         | 7.8     | 4.7   |
| lysine     | 7.9    | 1.8    | 5.6         | 6.2     | 4.6   |
| methionine | 1.1    | 0.8    | 2.3         | 1.5     | 0.8   |
| phenylalanine | 6.2  | 3.5    | 2.8         | 5.1     | 3.6   |
| proline    | 4.6    | 8.0    | 3.5         | 5.2     | 1.9   |
| serine     | 4.1    | 9.5    | 2.7         | 5.2     | 1.7   |
| threonine  | 3.5    | 3.8    | 3.0         | 3.7     | 1.6   |
| tryptophan | 1.2    | 0.3    | 0.7         | 1.1     | 0.5   |
| tyrosine   | 2.3    | 1.9    | 2.2         | 4.0     | 0.8   |
| valine     | 7.1    | 6.0    | 3.5         | 4.4     | 4.1   |
| total protein | 86   | 84    | 82         | 90      | 77    |
| total nitrogen | 13.8| 13.4  | 13.1       | 14.4    | 12.3  |

---

**Table 2. Sources of Variation in the ANOVA of HAP Bacteria Species, Nitrogen Substrate, Sample, and Time on BAA Production**

| source | BAA \( (%) \) total | significance |
|--------|----------------------|-------------|
| Rep    | 27.4                 | 2.9         | **** 🟢 |
| HAP species (A) | 441.9 | 47.1 | **** 🟢 |
| substrate (B) | 39.1 | 4.2 | **** 🟢 |
| AB     | 159.8                | 17.0        | **** 🟢 |
| sample (C) | 0.2 | 0.0 | NS 🟢 |
| AC     | 0.1                  | 0.0         | NS 🟢 |
| BC     | 0.6                  | 0.0         | NS 🟢 |
| ABC    | 1.2                  | 0.1         | NS 🟢 |
| time (D) | 90.5 | 9.7 | **** 🟢 |
| AD     | 37.5                 | 4.0         | **** 🟢 |
| BD     | 7.1                  | 0.7         | NS 🟢 |
| ABD    | 23.7                 | 2.5         | NS 🟢 |
| CD     | 0.1                  | 0.0         | NS 🟢 |
| ACD    | 0.4                  | 0.0         | NS 🟢 |
| BCD    | 0.9                  | 0.1         | NS 🟢 |
| ABCD   | 1.5                  | 0.1         | NS 🟢 |
| error  | 105.8                | 11.2        | NS 🟢 |
| CV     | 31.1                 |             |             |

*Units are sum of squares from ANOVA. ** indicates significance at the \( P \leq 0.0001 \) level. NS indicates no significance at \( P = 0.05 \).*
of lack of replication and small sample size, an uncontrolled error accounted for 11.3% of the variation. The coefficient of variation (CV) for this experiment was considered low at 31.1%.

Of greatest importance in this experiment was the interaction between HAP bacteria and substrates on BAA production (Figure 1). All three HAP species grew with nine organic N substrate combinations and produced BAA; however, there was a clear superiority among the HAP species. *P. anaerobius* was the least efficient bacteria, producing significantly equal amounts of BAA with all the nine organic N substrate combinations. *C. sticklandii* tended to produce intermediate BAA concentrations compared to *P. anaerobius* and *C. aminophilum* in all experimental combinations, producing significantly the same BAA concentrations on SPI + Y, BM + Y, FM + Y, DF, DF + Y, and Y alone and the lowest BAA concentrations on the SPI and FM. Consistent with the literature, *C. aminophilum* produced the highest BAA concentrations compared to *P. anaerobius* and *C. sticklandii.*

*C. aminophilum* produced significantly the highest BAA concentration when cultured on SPI alone (7.23 mM) and significantly higher than any other HAP species or organic N substrate combination. *C. aminophilum* produced significantly less BAA when Y was added to SPI and BM, indicating the suppressive effect of Y with these two organic N substrates; however, when Y was added to FM and DF, the decrease was not statistically significant. *C. aminophilum,* when cultured with Y alone, produced the lowest BAA concentrations. When pooled over organic N substrates, *C. aminophilum* produced higher BAA concentrations at 96 h than the other two HAP species; *C. sticklandii* produced equal BAA concentrations at 96 h when compared to *C. aminophilum* at 24 h (Figure 2). *P. anaerobius* significantly produced the lowest BAA concentrations and was unaffected by time and very low growth rates as indicated by the low production of BAA. The initial goal was to determine one HAP species and one organic N substrate combination capable of producing the highest BAA concentrations in a minimum duration of time, which was achieved with *C. aminophilum* when cultured on SPI. Further, temporal analysis was performed at 96 h. The interaction between *C. aminophilum,* substrate, and time was highly significant at \( P \leq 0.0001 \) (Table 3). The total error accounted for only 7.8% of the total variation with a small CV (14.4%). Finally, there was a clear indication that *C. aminophilum* produced more BAA when cultured with SPI at 96 h (Figure 3) than any other organic N substrate combinations.

*C. aminophilum* SPI Rate Study. *C. aminophilum* was cultured with five SPI concentrations with a zero control for 168 h to determine the maximum BAA concentration produced in the shortest time period using the lowest SPI concentration. A rate of 0.8 g was chosen as the baseline rate because preliminary experiments indicated that this is where BAA concentrations first experienced curvilinear responses (data not shown); SPI rates were increased at 0.2 g intervals to the maximum SPI solubility at 1.6 g·10 mL\(^{-1}\), and the sampling interval time was extended to 168 h.

When analyzing all sources of variation, the time factor accounted for the greatest amount of variation with the SPI rate accounting for a far less variation (Table 4). The uncontrolled error accounted for a significant portion of the total variation and may be attributed to the low viscosity of the higher SPI rates with salt solution and the lack of agitation between samplings.

Incremental polynomial regression models for each rate were then selected based on the model that best explained the

---

**Figure 1.** Interaction of HAP bacteria species and nitrogen substrates (pooled over time) on BAA production. Letters above columns indicate significant differences of means by least significant difference (LSD) at \( P = 0.05 \).

**Figure 2.** Interaction of HAP bacteria species and time (pooled over organic nitrogen substrates) on BAA production. Letters above columns indicate significant differences of means by LSD at \( P = 0.05 \).

**Table 3. Sources of Variation in the ANOVA of the Nitrogen Substrate and Time on BAA Production of *C. aminophilum***

| source | BAA\(^a\) | (%) total | significance |
|--------|-----------|-----------|--------------|
| Rep    | 3.2       | 1.0       | NS\(^b\)     |
| substrate (A) | 161.2  | 52.1      | ****c        |
| time (B) | 99.7   | 32.2      | ****        |
| AB     | 20.8      | 6.7       | ****        |
| error  | 24.2      | 7.8       |             |
| CV     | 14.4      |           |             |

\(^a\)Units are sum of squares from ANOVA. \(^b\)NS indicates no significance at \( P = 0.05 \). \(^c****\) indicates significance at the \( P \leq 0.0001 \) level.
data (Figure 4). SPI rates of 0.8 and 1.0 g exhibited a curvilinear third-order model response with $R^2$ values of 0.65 and 0.69, respectively. A rate of 1.0 g plateaued, displaying nearly flat-line BAA levels after 96 h and slightly more BAA than 0.8 g for each sampling interval. When the SPI rate was increased to 1.2 g·10 mL$^{-1}$ salt solution, a third-order model was chosen to describe the data, exhibiting unstable BAA levels in the later sampling intervals with nearly identical BAA levels to the 1.0 g SPI rate at 96 and 144 h, respectively. SPI rates of 1.4 and 1.6 g displayed second- and fourth-order models, respectively, with BAA production decreasing after 96 h, indicating variability and instability.

Stability and consistent plateauing of BAA production were achieved with an SPI rate of 1.0 g·10 mL$^{-1}$ salt solution at the sampling interval of 96 h. The other SPI rates and sampling intervals were unstable and were not predictable enough with higher SPI rates.

### CONCLUSIONS

The initial goal for conducting this research was to create a biologically derived source of ammonium nitrate for precision fertilization of specialty vegetable crops. This was achieved by scrutinizing various HAP bacteria and substrates for their ability to produce BAA. Essentially, a novel group of HAP bacteria was discovered from the cattle rendering industry, which negatively affect ruminant metabolism but can positively be used in generating organic N fertilizers. Specifically, *C. aminophilum* and SPI yielded the greatest BAA. Furthermore, taking into account solubility, bioreaction stability, and reliability, it was determined that the 1.2 g SPI rate was practically identical to the 1.0 g rate at the sampling interval of 96 h, and the additional cost would not warrant the use of this rate in upscaled production scenarios. Future research will focus on field studies evaluating the efficacy of this bacteria substrate-derived N source.

### EXPERIMENTAL SECTION

#### Generating Anaerobic Conditions

Specific anaerobic conditions were developed according to the work pioneered by Hungate [11] and published in the Anaerobe Laboratory Manual 4th ed. 1977, Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, VA. Anaerobic gas utilized throughout the process consisted of 10% CO$_2$, 10% H$_2$, and 80% N$_2$ (Airgas National Welders Supply Company, Charleston, SC). To scrub out all oxygen contamination of the gases, the above-mentioned gas was passed through a copper oxidation/reduction potential (ORP), and a pH color indication solution were prepared. All chemicals and salts obtained from Sigma-Aldrich, Inc., St. Louis, MO. Salt solution A contained (per liter) distilled H$_2$O: 7.3 g (K$_2$HPO$_4$·3H$_2$O), 12.0 g (NH$_4$)$_2$SO$_4$, 12.0 g sodium chloride (NaCl), 2.5 g (MgSO$_4$·7H$_2$O), and 1.6 g (CaCl$_2$·H$_2$O). The ORP solution contained (per 100 mL) distilled H$_2$O: 100 μg resazurin as an indicator of anaerobiosis.

To prepare the salt solution anaerobically, 50 mL of salt solution A and 50 mL of salt solution B were added to a 1.0 L
Erlenmeyer vacuum flask with a side arm. The flask was topped with a butyl rubber stopper with one hole drilled through it containing a 6.4 mm diameter stainless steel tube extending 12.7 mm from the bottom of the flask to 50.8 mm above the stopper. This tube was attached to butyl rubber hosing in which the anaerobic gas supply was allowed to enter. The side arm of the flask was fitted with butyl rubber hosing and wrapped with an aluminum foil allowing gas to escape. Eight hundred and fifty milliliters of distilled H$_2$O and 1 mL of the stock ORP solution were added to the flask to visually aid in determining that proper ORP and pH were achieved. The solution immediately turned from clear to purple. The combined salt solution was then heated to the boiling point and allowed to boil for 1 min with continuous gassing. The solution was then immediately placed in an ice bath while continuing to be gassed.

A buffering solution was prepared by adding 4 g of sodium carbonate (Na$_2$CO$_3$) to a 250 mL Erlenmeyer flask, 50 mL of distilled H$_2$O was then added, and the flask was covered with an aluminum foil. Attached to anaerobic gas via butyl rubber hosing, an 18-gauge 22.9 cm needle (model 9875; Cadence Science, Inc., Lake Success, NY) was then inserted through the foil until the syringe touched the bottom of the flask. Anaerobic gas was bubbled through the solution, heated until the boiling point, and then rapidly cooled in an ice bath as the gas continued to bubble through the solution. This buffering solution was then transferred to the cooling salt solution using a pipette that had been repeatedly flushed with anaerobic gas to limit oxygen contamination during the transfer process. The solution then turned from blue to bright pink, followed by a bleached pink coloring. Next, 0.6 g of L-C$_3$H$_7$NO$_2$S, remained in an ice bath and continued to be gassed with anaerobic salt solution and 15 mL borosilicate septum anaerobic test tubes (model 2048-18150; Bellco Glass, Vineland, NJ) (Table 1). The vitamin solution contained 15 g of Y per 50 mL of distilled H$_2$O was added directly to the flask by cracking the stopper, causing the solution to turn bright fluorescent pink. This solution, containing the ORP-indicating solution, the buffering solution, and L-cysteine hydrochloride monohydrate, remained in an ice bath and continued to be gassed for 1 h until the solution turned totally clear with an ORP of ~250 mV and a pH of 6.26.

After 1 h of gassing, the flask containing the now reduced anaerobic salt solution and 15 mL borosilicate septum anaerobic test tubes (model 2048-18150; Bellco Glass, Vineland, NJ) were gassed with anaerobic gas using an 18-gauge stainless steel needle as described before to purge the atmospheric oxygen. Next, 10 mL of the now reduced anaerobic salt solution was added via a pipette as described before. The pipette was removed, whereas the needle was still purging the atmospheric air from the test tube. A butyl rubber stopper was inserted simultaneously as the needle was removed, and an aluminum septum cap was mounted and crimped over the test tube. Once all test tubes were prepared, they were autoclaved for 20 min at 121 °C. The test tubes were removed from the autoclave, placed in a Precision shaking water bath (model 51221080; Thermo Fisher Scientific, Inc., Waltham, MA) incubation chamber at 39 °C for a total of 168 h.

BAA Analysis. For the measurement of BAA production, 0.5 mL aliquots of cultures were removed anaerobically with an anaerobically purged syringe as described before at 24 and 96 h and immediately stored at −20 °C until analysis. Prior to determining BAA production, the samples were removed from the freezer, allowed to thaw to room temperature, particulates were allowed to settle to the bottom of the syringe, and the supernatant was removed for the analysis. Time 0 samples were analyzed for BAA and were nondetectable. Ammonia concentrations were determined by the colorimetric method of Chaney and Marbach, as modified by Cotta and Russell. This method was modified again by maximizing the standard curve and diluting the samples by a factor of 3. Each sample for each rep was run three separate times as independent syringe samples and then analyzed to obtain an accurate reading. Ammonia concentrations were measured with a Molecular Devices SpectraMax Plus 384 spectrophotometer microplate...
reader at 630 nm$^{-1}$ (Molecular Devices, Devices, MDS Analytical Technologies, Inc., Sunnyvale, CA).

**C. aminophilum** SPI Rate Study. For determining the maximum BAA efficiency by *C. aminophilum*, the increasing rates of SPI in 10 mL salt solutions were arranged in a completely randomized block design within the test tube racks with two replications and two tubes per treatment. SPI was weighed and placed in anaerobic test tubes in 0.1 g increments from 0.1 g to 1.0 g·10 mL$^{-1}$ salt solution. Then, 10 mL of the reduced anaerobic salt solution was added with a pipette as described before in the first experiment. The pipette was removed, while the needle was still purging the atmospheric air from the test tube. A butyl rubber stopper was inserted simultaneously as the needle was removed, and an aluminum septum cap was mounted and crimped over the test tube. Once all test tubes were prepared, they were autoclaved for 20 min at 121 °C (model STM-EL; Market Forge Co., Everett, MA). The test tubes were removed from the autoclave, placed in a precision water bath (model S1221080; Thermo Fisher Scientific, Inc., Waltham, MA) incubation chamber at 39 °C, and allowed to equilibrate. The test tubes were individually inoculated with 0.5 mL of 18 h mid-log phase stock *C. aminophilum* culture using an anaerobically purged syringe needle as before and placed randomly within the test tube racks and placed back into the water bath.

For the measurement of BAA production, 0.5 mL aliquots of treatment cultures were removed with an anaerobically purged syringe as described before at 24, 48, 72, 96, 120, and 168 h, respectively, and immediately stored at −20 °C until analysis. Prior to determining BAA production, the samples were removed from the freezer, allowed to thaw to ambient room temperature, and particulates settled to the bottom of the syringe before the supernatant was removed for the analysis. BAA concentrations were determined by the colorimetric method of Chaney and Marbach,18 as modified by Cotta and Russell.13 This method was modified in the first experiment by maximizing the standard curve and diluting the samples by a factor of 3. This method was modified again in the SPI rate experiment by diluting the samples by a factor of 6 because of the outlying data points on the standard curve observed during the spectrophotometric analysis in the first experiment. Each sample for each rep was independently sampled three separate times to obtain an accurate reading. Concentrations of BAA were measured spectrophotometrically with a microplate reader at 630 nm$^{-1}$ (model SpectraMax Plus 384; Molecular Devices, MDS Analytical Technologies, Inc., Sunnyvale, CA).

**Statistical Analysis.** All data were analyzed using the MSTAT-C version 1 (Mich. State Univ., East Lansing, MI) using ANOVA. The three-way ANOVA, with independent variables being time, HAP species, and N source, was partitioned into individual sources of variation in the model to determine the relative importance of each source of variation. In the SPI rate study, data were then subjected to polynomial regression using Sigmaplot (Systat Software Inc., San Jose, CA) to see how the SPI rate affected BAA production over time. Appropriate regression models for each rate were then selected based on incremental regression significant $P$ values at a defined level of significance of $P < 0.05$ that best explained the data.

**ACKNOWLEDGMENTS**

The authors would like to thank the Agricultural Society of South Carolina and the Clemson University Research Foundation for their support of this project. Dr. Dufault provided guidance to Dr. Ward during the experimental phase of this project; however, Dr. Dufault has since retired and was not able to be reached during the construction of this manuscript.

**ABBREVIATION**

bioammonium/ammonia, (BAA)

**REFERENCES**

1. Zehnder, G.; Hope, C.; Hill, H.; Hoyle, L.; Blake, J. H. An assessment of consumer preferences for IPM and organically grown produce. *J. Text.* 2003, 41, 2.
2. Marschner, H. *Mineral Nutrition of Higher Plants*, 2nd ed.; Academic Press: London, 1995; pp 1–20.
3. Gaskell, M.; Smith, R. Nitrogen sources for organic vegetable crops. *HortTechnology* 2007, 17, 431–441.
4. Hanselman, T. A.; Graetz, D. A.; Obreza, T. A. A comparison of in situ methods for measuring net nitrogen mineralization rates of organic soil amendments. *J. Environ. Qual.* 2004, 33, 1098–1105.
5. Berry, P. M.; Sylvester-Bradley, R.; Phillips, L.; Hatch, D. J.; Cuttle, S. P.; Rayns, F. W.; Gosling, P. Is the productivity of organic farms restricted by the supply of available nitrogen? *Soil Use Manag.* 2002, 18, 248–255.
6. Hartz, T. K.; Johnstone, P. R. Nitrogen availability from high-nitrogen-containing organic fertilizers. *HortTechnology* 2006, 16, 39–42.
7. Asman, W. A. H.; Sutton, M. A.; Schijorring, J. K. Ammonia: emission, atmospheric transport and deposition. *New Philol.* 1998, 139, 27–48.
8. Bierman, S. J. *Nutritional Effects on Waste Management*. MS Thesis; University of Nebraska: Lincoln; 1995; pp 5–30.
9. Russell, J. B.; Strobel, H. J.; Chen, G. Enrichment and isolation of a ruminal bacterium with a very high specific activity of ammonia production. *Appl. Environ. Microbiol.* 1988, 54, 872–877.
10. Paster, B. J.; Russell, J. B.; Yang, C. M. J.; Chow, J. M.; Woese, C. R.; Tanner, R. Phylogeny of the Ammonia-Producing Ruminal Bacteria Peptostreptococcus anaerobius, Clostridium sticklandii, and Clostridium aminophilum sp. nov. *Int. J. Syst. Bacteriol.* 1993, 43, 107–110.
11. Hungeate, R. E. The anaerobic mesophilic cellulolytic bacteria. *Bacteriol. Rev.* 1950, 14, 1–49.
12. Salanitro, J. P.; Fairchild, I. G.; Zgornicki, Y. D. Isolation, culture characteristics, and identification of anaerobic bacteria from the chicken cecum. *Appl. Microbiol.* 1974, 27, 678–687.
13. Wilkins, T. D.; Chalgren, S. Medium for use in antibiotic susceptibility testing of anaerobic bacteria. *Antimicrob. Agents Chemotherapy.* 1976, 10, 926–928.
14. Chaney, A. L.; Marbach, E. P. Modified reagents for determination of urea and ammonia. *Clin. Chem.* 1962, 8, 130–132.
15. Cotta, M. A.; Russell, J. B. Effect of peptides and amino acids on efficiency of rumen bacterial protein synthesis in continuous culture. *J. Dairy Sci.* 1982, 65, 226–234.