2007

Use of Swine Manure to Improve Solid-State Fermentation in an Integrated Storage and Conversion System for Corn Stover

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Abstract

Swine manure contains a host of chemical and biological constituents which make it desirable for amending lignocellulosic biomass in storage for year round processing in a biorefinery. Application of swine manure in an integrated biomass storage and conversion system was investigated to determine the potential for improved conversion of corn stover to organic acids and soluble carbohydrates during ensiling. Corn stover- swine manure mixtures were prepared containing swine manure at rates of 0%, 15%, 30%, 45%, and 60% while simultaneously being adjusted to 65% moisture on a wet basis and ensiled for 0, 1, 7, and 21 days. Samples were analyzed for pH, dry matter, water-soluble carbohydrates, and organic acids. All treatments, with the exception of the 60% manure substrate, produced a pH less than 4.5, which is sufficient for stable storage. Water-soluble carbohydrates were highest in the control treatment, producing a level of 3.0% DM at day 21. Lactic acid production was unaffected by the rate of manure, with a concentration of 2.8% DM reached at day 21. Acetic acid production was improved with the manure substrates. Manure amendment rates of 30%, 45%, and 60% produced the highest acetic acid concentration of 1.8% DM. Treatments of 0%, 15%, 30%, and 45% swine manure would be acceptable substrates for use in this system; however, if preservation of fermentable carbohydrates is a higher priority than organic acid production, then the pure corn stover substrate would be the most appropriate material to use.

Keywords

Biomass, Conversion, Corn stover, Ensilage, Fermentation, Iodoform, Lactic acid, Manure, Storage, Swine

Disciplines

Agriculture | Bioresource and Agricultural Engineering

Comments

This article is from *Transactions of the ASABE*, 50, no. 5 (2007): 1901–1906.

USE OF SWINE MANURE TO IMPROVE SOLID-STATE FERMENTATION IN AN INTEGRATED STORAGE AND CONVERSION SYSTEM FOR CORN STOVER

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ABSTRACT. *Swine manure contains a host of chemical and biological constituents which make it desirable for amending lignocellulosic biomass in storage for year round processing in a biorefinery. Application of swine manure in an integrated biomass storage and conversion system was investigated to determine the potential for improved conversion of corn stover to organic acids and soluble carbohydrates during ensiling. Corn stover- swine manure mixtures were prepared containing swine manure at rates of 0%, 15%, 30%, 45%, and 60% while simultaneously being adjusted to 65% moisture on a wet basis and ensiled for 0, 1, 7, and 21 days. Samples were analyzed for pH, dry matter, water-soluble carbohydrates, and organic acids. All treatments, with the exception of the 60% manure substrate, produced a pH less than 4.5, which is sufficient for stable storage. Water-soluble carbohydrates were highest in the control treatment, producing a level of 3.0% DM at day 21. Lactic acid production was unaffected by the rate of manure, with a concentration of 2.8% DM reached at day 21. Acetic acid production was improved with the manure substrates. Manure amendment rates of 30%, 45%, and 60% produced the highest acetic acid concentration of 1.8% DM. Treatments of 0%, 15%, 30%, and 45% swine manure would be acceptable substrates for use in this system; however, if preservation of fermentable carbohydrates is a higher priority than organic acid production, then the pure corn stover substrate would be the most appropriate material to use.*

Keywords. *Biomass, Conversion, Corn stover, Ensilage, Fermentation, Iodoform, Lactic acid, Manure, Storage, Swine.*

Corn stover, the residue remaining after harvest of corn grain, is currently the most abundant agricultural biomass resource in the U.S. (USDA-DOE, 2005) and has been examined extensively as a lignocellulosic feedstock for producing ethanol (Wyman, 2003). The amount of corn stover available annually for collection has been estimated between 73 and 100 million dry metric tons based on different collection scenarios (Chief Executive Assistance, 2000; Gallagher and Johnson, 1995; USDA-DOE, 2005).

Storage of corn stover harvested in a one-pass system or directly following grain harvest is necessary for year-round processing in a biorefinery. Wet storage of corn stover by ensilage has several advantages over dry storage, including reduced weather-related delays during stover harvest, greater harvesting efficiency, lower storage losses, and a more uniform product following storage (Shinners and Binversie, 2004). Ensiling of wet biomass also increases the dry matter density of the material, reduces the required storage area by 10-fold, limits dirt contained in the biomass, and alleviates the fire risk associated with storing dry materials (Atchison and Hettenhaus, 2004).

Ensilage is a solid-state fermentation process in which lactic acid producing bacteria are the predominant fermentation organism. Solid-state fermentation refers to handling of materials as solids as opposed to liquids in conventional fermentation systems. During the initial stage of ensiling, excess oxygen is consumed by a mixed flora of indigenous aerobic organisms (Woolford, 1984), producing an anaerobic environment. During the second stage, typically occurring during a period lasting from one day to three weeks, soluble carbohydrates are converted to lactic acid, acetic acid, ethanol, mannitol, acetaldehyde, and carbon dioxide by homofermentative and heterofermentative lactic acid bacteria (Roberts, 1995). After this period, significant acetic and lactic acid accumulation results in pH declining to a level that inhibits further microbial growth, and the silage is considered to be stable. A stable range from pH 4.2 to 4.5 has been suggested for producing good-quality silage (Woolford, 1984). If a satisfactory pH level is not reached in sufficient time, then clostridial spoilage can occur in which clostridia bacteria convert sugars and lactic acid to butyric acid, propionic acid, carbon dioxide, and hydrogen gas (Moser, 1980; Pitt, 1990). Butyric fermentation is detrimental to the ensilage process because butyric acid is a weak acid for preserving silage and considerable (>20%) losses in energy from the silage occurs (Jaster, 1995).

In addition to its use for storing wet biomass, ensilage could be used as a low-cost technology to produce multiple

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Submitted for review in September 2006 as manuscript number BE 6686; approved for publication by Biological Engineering Division of ASABE in August 2007.

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ISSN 0001-2351

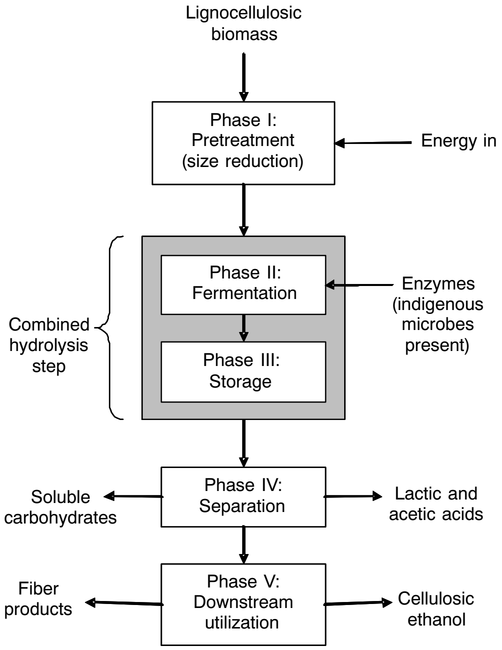

Figure 1. Schematic of integrated storage and conversion system for biomass.

organic compounds from biomass (Murphy, 2006). After removal from storage, organic acids, soluble sugars, and other fermentation products of interest could be extracted from the silage and the remaining high-fiber residue used for further processing into fiber products or for the production of cellulosic ethanol. The two likely most abundant extracted compounds, lactic and acetic acid, can be used as chemical building blocks to produce a wide range of products, including plastics, paint, adhesives, and resins (Brown, 2003). Wet storage of biomass is preferred by the pulping industry because pulping characteristics are greatly improved during ensilage (Atchison and Hettenhaus, 2004). An integrated process for storage and conversion of corn stover is described in figure 1.

Availability of soluble carbohydrates and macronutrients in corn stover harvested during or following grain harvest may limit the robustness of the fermentation when the material is ensiled. Swine manure has potential as an amendment for corn stover to alleviate these limitations and provide additional benefits to the fermentation process. Commercial lactic acid fermentation requires a soluble carbohydrate source, which is typically glucose, and sources of nitrogen, magnesium, potassium, and phosphorus (Maisch, 2003). Swine manure contains limited amounts of soluble carbohydrates but significant concentrations of macronutrients and many micronutrients (*ASAE Standards*, 2005a). The buffering capacity of swine manure is much higher than that of corn stover (Murphy et al., 2006). This may help to resist pH change and prolong the fermentation period, thus increasing lactic and acetic acid production. Swine manure also contains a plethora of bacterial species that are capable of fermenting a range of monosaccharides, oligosaccharides, and polysaccharides (Cotta et al., 2003). Investigation of swine manure as a source of nutrients, buffering capacity, and fermentation organisms to enhance fermentation during the ensilage process is needed.

The objectives of this experiment were to determine the effects of various levels of swine manure on fermentation product yields during ensilage of corn stover and to determine possible application rates for its use.

Materials and Methods

An experiment was conducted to determine the effects of swine manure content on water-soluble carbohydrate, lactic acid, and volatile fatty acid production in corn stover-manure substrates ensiled for a period of 21 days. Substrates containing 0%, 15%, 30%, 45%, and 60% manure on a wet basis by weight (w.b.) and ensilage periods of 0, 1, 7, and 21 days were evaluated using a split-plot design with three replications. Manure treatments were applied to whole samples, and ensilage time was applied to subsequent subsamples.

Treatment Procedure

Large round bales of corn stover (hybrid Pioneer 34H31 produced during the 2004 cropping season) were obtained from a grain producer near State Center, Iowa. The whole-plant stover was fractionated using a portable shredder (MTD Products, Inc., Valley City, Ohio) equipped with a screen having 12 × 80 mm slots to produce a smaller, more uniform particle size for improved fermentation (Ren et al., 2006). The initial moisture content of the corn stover was 15% (w.b.), as determined by drying 100 g of material at 60°C in a forced-air oven for 72 h (*ASAE Standards*, 2005b).

Swine manure was acquired from the Iowa State University Bilsland Swine Research Farm near Madrid, Iowa. The manure was obtained from a storage pit located beneath a confinement building for finishing swine, which is pumped into a lagoon every two weeks. The manure contained approximately 10% solids (w.b.), which is similar to the average solids content for finishing swine manure (*ASAE Standards*, 2005a). Solids content of the manure was determined by drying 100 g of sample at 103°C in a forced-air oven for 4 h, which is based on a standard method for solids determination in wastewater (Clesceri et al., 1998).

Swine manure was added to corn stover at rates 0%, 15%, 30%, 45%, and 60% (w.b.) of the total stover-manure mixture, and the mixtures were simultaneously adjusted to a desirable 65% moisture content (w.b.) for ensiling (Pitt, 1990) by addition of water. Each mixture was treated with iodoform at a rate of 0.23 g/kg dry matter (DM) to inhibit butyric fermentation (Murphy et al., 2006). Iodoform was applied by preparing a solution containing 1 g iodoform in 60 mL of cold ethanol. A hemicellulase-cellulase enzyme mixture (Multifect A40, Genecor, Cedar Rapids, Iowa) was applied to all samples at a rate of 5 and 12.5 IU/g DM of hemicellulase and endocellulase activity, respectively, to ensure adequate sugars for fermentation (Ren et al., 2006; Richard et al., 2002). Treatments were applied to triplicate 2 kg samples and separated into equal 500 g subsamples, corresponding to each of the four ensilage periods. Subsamples were then vacuum-sealed in polyethylene bags and incubated for 0, 1, 7, or 21 days at 37ºC, which is the standard temperature for laboratory-scale ensilage experiments (Ren, 2006).

Laboratory Analyses

After ensiling, samples were removed from the polyethylene bags, mixed thoroughly, and analyzed for pH, dry matter, water-soluble carbohydrates (WSC), and organic acids. Dry matter of the subsamples was determined by drying 100 g of material at 60°C in a forced-air oven for 72 h (*ASAE Standards*, 2005b). Dried samples were ground using a Wiley mill (Thomas Scientific, Inc., Swedesboro, N.J.) fitted with a 1 mm sieve and used for WSC analysis. Dry matter was also determined for ground samples by drying 1 g of sample at 103°C in a forced-air oven for 4 h to make moisture corrections for WSC. Samples for pH determination were prepared with a 10:1 mass dilution (H_2O:sample) and allowed to stand for 30 min prior to measurement with a pH electrode (Moore et al., 1985).

Water-soluble carbohydrates were determined using a modification of the method described by Guiragossian et al. (1977). Water extracts were prepared by shaking 0.25 g of the ground material with 100 mL of distilled water for 30 min followed by filtering through No. 42 filter paper (2.5 μm pore size) (Guiragossian et al., 1977). To a 1 mL aliquot of the filtrate, 1 mL of 5% phenol solution and 5 mL of 18 M sulfuric acid were added, and the solution's absorbance was measured at 490 nm (Guiragossian et al., 1977) using a UV-1601 spectrophotometer (Shimadzu Corp., Columbia, Md.) equipped with a rectangular 10 mm light path cell. Sample values were calculated from a standard calibration curve prepared using equimolar concentrations of glucose and xylose to determine carbohydrate levels (Murphy et al., 2007).

Organic acid concentrations were analyzed using a method similar to that described by Moore et al. (1985). Water extracts were prepared by shaking 50 g of sample with 200 mL of distilled water for 4 h, refrigerating at 5°C for 20 h, and filtering through four layers of cheese cloth. A 45 mL aliquot of the filtrate was acidified with 25% metaphosphoric acid and centrifuged for 15 min at 15,000 rpm. The resulting supernatant was collected and analyzed using gas-liquid chromatography. Separations of acetate, propionate, butyrate, and iso-butyrate were made using an SP-1200/H_3PO_4 column (Sigma-Aldrich Co., St. Louis, Mo.) operated at 120°C using N_2 as the carrier gas at a flow rate of 30 mL/min with an injection block temperature of 170°C. Quantification of the acids was done using a flame ionization detector operating at 180°C. Lactate concentrations were determined using a similar procedure. Separation was done using an SP-1000/H_3PO_4 column (Sigma-Aldrich Co., St. Louis, Mo.) with the initial oven temperature set at 100°C for 1 min and increased to 120°C at a rate of 10°C/min. Prior to injection, lactate was methylated by combining 2 mL of filtrate, 4 mL of methanol, and 0.8 mL of 50% sulfuric acid in a test tube and heating for 30 min at 60°C (Supelco, 1998). After cooling, 2 mL of water and 2 mL methylene chloride were added to the test tube, and the resulting bottom layer of methylene chloride was sampled. All other operating conditions were the same as described above.

STATISTICAL ANALYSIS

Statistical analysis was done using SAS software (SAS, 2003). The significance of the effects of ensiling time, manure rate, and their interaction on pH, WSC, lactate, acetate, and butyrate was determined using the general linear model (GLM) procedure. Differences between treatments for significant main effects or interactions were further investigated using an appropriate least significant difference (LSD). All comparisons were determined to be significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

PH LEVEL

Acidification occurred with all treatments during the first 7 days of ensiling, and the pH levels remained virtually constant the reminder of the 21-day fermentation period (fig. 2). Similar pH results were observed by other researchers ensiling corn stover substrates with hemicellulase and cellulase enzyme mixtures (Ren et al., 2006; Richard et al., 2002). Throughout the ensiling period, the 60% manure treatment maintained a higher pH than all other treatments and did not reach a pH of less than 4.5, which has been found to be adequate for long-term storage of corn stover (Ren et al., 2006). All other treatments reached a stable storage pH by day 7. Overall, the increase in the manure concentration of the substrate increased the stable pH of the silage produced. This is most likely due to the approximately 5-fold higher buffering capacity of swine manure relative to corn stover (Murphy et al., 2006). Increasing the buffering capacity of an ensilage substrate requires an increasing accumulation of organic acids in order to achieve a satisfactory storage pH level. In the subsequent organic acid data (figs. 4 through 6), the 60% manure treatment produced approximately one percentage point DM more organic acids than the control treatment, mostly due to an increase in acetate. However, this additional acetate content was not sufficient to compensate for the buffering effect of the manure.

WATER-SOLUBLE CARBOHYDRATES

Water-soluble carbohydrates ranged from 1.1% to 3.1% DM during ensiling (fig. 3). The control consistently produced higher WSC levels than the 45% and 60% manure treatments throughout the 21-day period. From day 7 to 21, WSC in the manure treatments converged to a level of 1.4% DM, with WSC in the control treatment increasing from 2.4% to 3.1% DM over the same period.

The increase in WSC produced by the control treatment (0% manure) compared to the manure treatments at day 21 roughly correlates to the increase in acetate and butyrate observed in the manure treatments relative to the control (figs. 5 and 6). Overall, addition of swine manure likely did not significantly improve or inhibit hydrolysis of structural carbohydrates (hemicellulose and cellulose) to fermentable

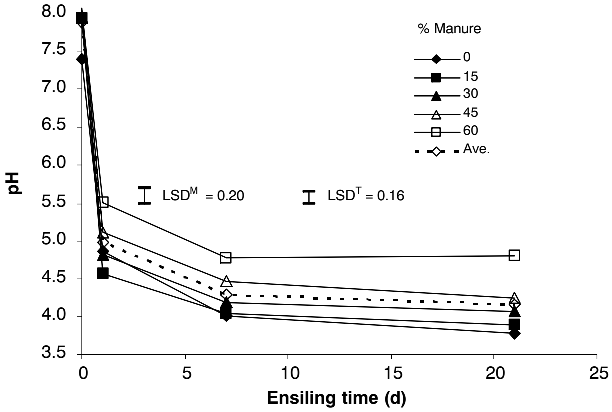

Figure 2. pH of corn stover-manure substrates containing 0%, 15%, 30%, 45%, and 60% manure at 0, 1, 7, and 21 days of ensiling (*n* = 3). $LSD^M_{0.05}$ is appropriate for comparison of manure treatments within a time and $LSD^T_{0.05}$ is appropriate for comparison of ensiling times averaged across all manure treatments.

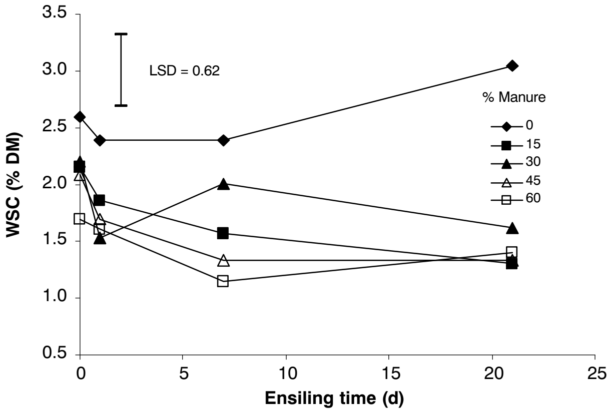

Figure 3. Concentrations of water-soluble carbohydrates (WSC) in corn stover-manure substrates containing 0%, 15%, 30%, 45%, and 60% manure at 0, 1, 7, and 21 days of ensiling (*n* = 3). LSD$_{0.05}$ is appropriate for comparison of manure treatments within a time.

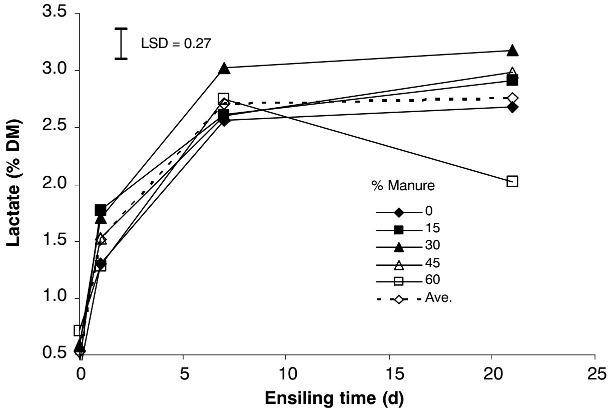

Figure 4. Average concentrations of lactate in corn stover-manure substrates containing 0% to 60% manure at 0, 1, 7, and 21 days of ensiling (*n* = 3). LSD$_{0.05}$ is appropriate for comparison of ensiling times averaged across all manure treatments.

sugars by either the cell-wall degrading enzymes added prior to ensiling or by enzymes produced by microbes present in the manure or corn stover.

The accumulation of WSC observed from day 7 to 21 in the control is important because accumulations of fermentable sugars during storage could be recovered for various downstream processes, including fermentation to ethanol. Ren et al. (2006) also reported an increase in WSC using a similar substrate and ensiling conditions, excluding iodoform application, but accumulation was not observed until 63 days of ensiling.

ORGANIC ACIDS

Organic acid production was dominated by lactate and acetate, with much lower concentrations of butyrate being produced (figs. 4 through 6). Lactate concentrations were unaffected by treatment with manure, with concentrations averaged over all treatments displayed in figure 4. Increases in lactate were observed between day 0 and day 7 and remained at a level of 2.8% DM for the remainder of the 21-day period.

Trends in acetate concentrations were very similar to trends in lactate concentrations (fig. 5). Treatments containing manure increased in acetate during the first 7 days and maintained similar levels for the rest of the fermentation period. The control treatment behaved differently, reaching a sustained level of acetate at day 1. At day 21, acetate concentrations in the 30%, 45%, and 60% manure treatments, at 1.8% DM, were all higher than in the 15% manure treatment. The control produced a significantly lower acetate level of 0.8% DM.

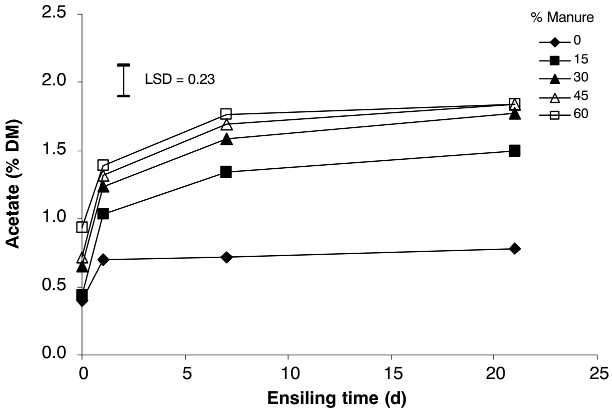

Figure 5. Concentrations of acetate in corn stover-manure substrates containing 0%, 15%, 30%, 45%, and 60% manure at 0, 1, 7, and 21 days of ensiling (*n* = 3). LSD$_{0.05}$ is appropriate for comparison of manure treatments within a time.

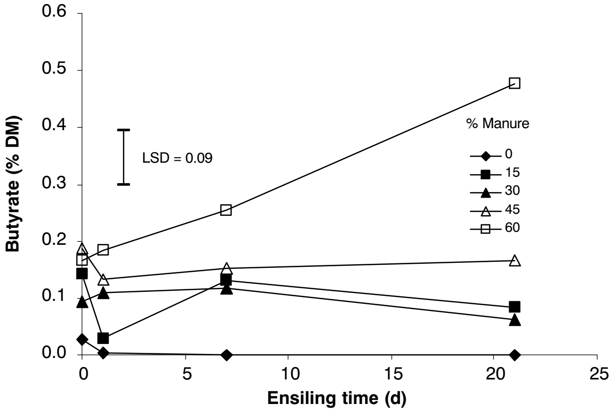

Figure 6. Concentrations of butyrate in corn stover-manure substrates containing 0%, 15%, 30%, 45%, and 60% manure at 0, 1, 7, and 21 days of ensiling (*n* = 3). LSD$_{0.05}$ is appropriate for comparison of manure treatments within a time.

Acetic acid is primarily produced from the fermentation of pentose sugars during ensiling; however, 97% of the bacterial species isolated in swine manure produce acetic acid (Cotta et al., 2003). Increases in acetate observed in manure treatments relative to the control suggest that either addition of acetic acid fermenting bacteria contained in the swine manure increased acetic acid formation or fermentation of pentose sugars by lactic acid bacteria is improved by application of manure. It is unclear if one or both scenarios were occurring, but it is more plausible that addition of acetic acid bacteria was responsible for the increase in acetic acid production in the manure treatments.

Butyrate concentrations remained below 0.2% DM in all treatments except for the 60% manure treatment, which produced a concentration of 0.5% DM at day 21 (fig. 6). Higher butyrate levels observed in the 60% manure treatment are a result of not attaining a desirable storage pH of less than

4.5, with a more neutral pH increasing butyric fermentation and presumably clostridia activity (Pitt, 1990). A significant decrease in butyrate was observed between day 0 and day 1 in the 15% manure treatment. Secondary fermentation of butyrate is not a process normally identified with ensiling; therefore, it is unclear the reason for this decrease.

Based on the organic acid results, the 30% and 45% manure treatment would be the most appropriate substrates for use in a hybrid ensilage system where bioconversion is a high priority due to increased acetic acid production and acceptable storage conditions. Acetic acid can be recovered and converted to mixed alcohols (Brown, 2003). Acetic acid also improves the aerobic stability of ensiled materials after they are removed from storage by inhibiting growth of yeasts, which hasten spoilage and heating in silages (Moon, 1983; Woolford, 1990). In a traditional ensilage system for preserving feedstuffs, aerobic deterioration of fermentation products and fiber constituents after unloading may be as high as 15% (McDonald et al., 1991).

CONCLUSIONS

Rapid acidification occurred in all treatments within the first day of ensiling, and by day 21 all treatments, except for 60% manure substrate, produced a stable storage pH of less than 4.5. Because of a 21-day pH of 4.8, as well as increased butyric acid levels relative to the other treatments, the 60% manure treatment would not be an acceptable substrate for ensiling. Water-soluble carbohydrates were highest in the substrate containing no manure, reaching a concentration of 3.1% DM at day 21 compared to 1.4% DM for manure substrates. Lactic acid production was unaffected by application of manure, and acetic acid production was improved an average of 1.0 percentage points DM at day 21 with application of manure, suggesting that manure may improve fermentation of pentose sugars. Treatments of 0%, 15%, 30%, and 45% manure would be acceptable substrates for use in a hybrid ensilage conversion system; however, if fermentable carbohydrates are a more desirable ensilage product than organic acids, then a corn stover substrate without manure would be the most appropriate material to use.

ACKNOWLEDGEMENTS

Support for this research provided by the Iowa Energy Center is appreciated.

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