Aerobic stability of distillers wet grains as influenced by temperature

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RESULTS: CO$_2$ production and mold colonization indicate that at temperatures near 12 °C, the aerobic stability of DWG was high and that it can be stored for at least a 10-day period. At temperatures close to 22 °C, the onset of increased microbial activity and visible mold colonization occurred between 4 and 7 days and both activity and mold ratings were very high by the ninth day in all three experiments. At 32 °C, 2 days may be a more appropriate limit for storage.

CONCLUSION: Temperature and time interact in a nonlinear fashion that permits the prediction of DWG stability boundaries. The simple visual appearance of mold appears to be a reasonable indicator that correlates well ($r = 0.694$) with CO$_2$ production, a measure of the aerobic stability of DWG.

Keywords
aerobic stability, biofuel, distillers wet grains, ethanol production, storage

Disciplines
Agriculture | Bioresource and Agricultural Engineering

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R Michael Lehman* and Kurt A Rosentrater†

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INTRODUCTION

Distillers grains (non-fermentable solids) are produced in equal mass to the primary product (ethanol) during industrial fuel ethanol production by contemporary dry grind processing and fermentation of raw grain.1 The production of distillers grains has rapidly increased with that of fuel ethanol,2 and income from the sale of distillers grains is pivotal to the overall economic viability of fuel ethanol production.3 If distillers grains are not dried during the production process, they will be saturated with water (with moisture contents between 50% and 65%), and are known as distillers wet grains (DWG). DWG are primarily used as cattle feed at nearby beef and dairy operations where their use is limited due to the short shelf life before the onset of spoilage. Spoilage diminishes the palatability and nutritional value of DWG and increases the potential for mycotoxin production. As a consequence, a large proportion of distillers grains is dried, adding additional expense, so that the dried distillers’ grains can be safely shipped, stored, and used for feed or other uses requiring a longer storage period. It would be economically, energetically and environmentally advantageous to dry as little distillers grains as possible, and market a larger proportion as DWG. However, because there is little published information on the storability of DWG, conservative storage periods are often implemented.

We previously reported on the microbial colonization of DWG following its production, including the types of microorganisms and their numbers over time as DWG aged during open storage at the manufacturing plant.4 In a second study, we measured microbial activity (production of CO2) during open storage to assess the aerobic stability of DWG over time under ambient environmental conditions.5 In this latter study, we found that color parameters may be useful as simple indicators of DWG deterioration following production. In the current study, our primary objective was to measure aerobic stability of DWG as a function of temperature using controlled experimental conditions, since ambient environmental temperatures will change during the annual production cycle. Our secondary objective was to further evaluate the utility of color measurement as an inexpensive and simple tool for estimating DWG spoilage.

MATERIALS AND METHODS

Experimental design and statistical analyses

Freshly produced DWG was collected from an ethanol production plant in eastern South Dakota on three dates: 8 March 2010, 22 September 2010 and 29 November 2010. Sterile polypropylene containers were filled using a sterile scoop and the sealed containers transported to the laboratory to immediately commence experimental procedures. A sub-sample of the DWG collected on each date was subjected to a standard set of physical and chemical
characterization analyses (details below). The remaining DWG was thoroughly mixed, exposing it to air, and 2-g sub-samples were placed into 150 60-mL glass serum vials and capped. Groups of 50 vials containing the DWG were assigned to one of three incubation temperatures: 12 °C, 22 °C and 32 °C. Ten-day incubations were conducted in the dark. At 1-day intervals, five randomly selected vials from each temperature treatment were analyzed for biological gas production (headspace CO$_2$), presence of visible mold, and color. Analysis of variance procedures (ANOVA) were used to test for significant effects of temperature (fixed factor), aging period (fixed factor), and experiment (random factor) on color, mold and CO$_2$ production. Changes in the response variables were plotted with time to visual changes in color, mold and CO$_2$ production during the experiments. To determine significant differences among color parameters measured at different times or temperatures, multiple comparisons of color means were performed for each experiment using Tukey’s post hoc test. Correlation analysis (Pearson linear coefficient) was used to assess the linear relationships among CO$_2$ production, mold, and color parameters within all the vials ($n = 450$) from the three experiments. CO$_2$ production as a nonlinear function of temperature and storage time was modeled using multiple nonlinear regression and visualized as a response surface using TableCurve 3D v.4.0 (San Jose, CA, USA) software.

**Initial characterization of distillers wet grains**

Bulk samples of the DWG were physically and chemically characterized at the outset ($t = 0$ days) of each of the three experiments. Moisture content was determined following Standard Method S352.2, using a forced-convection laboratory oven (Thelco Precision; Jovan Inc., Winchester, VA, USA) at 103 °C for 72 h. Thermal conductivity and diffusivity were determined with a thermal properties meter (KD2; Decagon Devices, Pullman, WA, USA), that utilized the line heat-source probe technique.$^7$ Bulk density was measured by filling a standard 0.5-L bushel tester (Seedburo Equipment Co., Chicago, IL, USA). pH was measured following Standard Method 02 – 52.$^8$ Chemical properties included crude protein, crude fat and crude fiber, which were determined using Official Methods 990.03, 920.39, and 978.10, respectively;$^9$ ash content, which was measured following Standard Method 08-03; and total carbohydrate content, which was determined by difference. Each chemical constituent was determined using four replicate sub-samples.

**Biological gas production over time**

CO$_2$ production (carbon mineralization or respiration) was used to estimate aerobic stability in a manner analogous to silage.$^{10}$ Headspace gas samples (2 mL) were collected via the septum of the 15 serum vials that were pulled daily from the different temperature incubations. Headspace CO$_2$ was quantified by gas chromatography [Shimadzu 14B (Shimadzu, Kyoto, Japan) with a CombiPal AOC-5000 autosampler (CTC Analytics, Zwingen, Switzerland), 2 mL injection loop, an 1/8” stainless steel Porapack Q (80/100 mesh) column operated at 60 °C with ultrapure nitrogen as the carrier gas, and an electron capture detector operated at 260 °C].

**Mold development over time**

For the 15 vials selected on each day, the presence of visible mold in the vials was assessed by inspection using the following progressive rating system: 0, no visible mold; 1, any visible mold (<50% of surface colonized); and 2, extensively colonized (>50% of surface colonized). Mold ratings were square root transformed for statistical analyses.

**Color changes over time**

Each day, the 15 selected serum vials were decapped following gas and mold analysis, and the contents placed on plastic Petri dishes. DWG color was measured using a calibrated spectrophotometer (LabScan XE; Hunter Associates Laboratory, Reston, VA, USA) using the $L, a, b$ opposable color scales according to manufacturer’s guidelines.$^{11}$ To measure color, each Petri dish containing DWG was placed under the machine’s sample observation port, and five reflectance spectra measurements were collected.

**RESULTS AND DISCUSSION**

Maximizing the storability of DWG has great influence on the economic, energetic and carbon balances of fuel ethanol production, yet there is little published data from controlled studies on the deterioration of DWG following production. We used biogenic CO$_2$ production to assess the aerobic stability of DWG collected on three separate occasions (March, September and November) to account for some of the annual variation in production practices that occur at a fuel ethanol plant. The freshly produced DWG had very high moisture content (519–673 g kg$^{-1}$, wet weight basis), high water activity (>0.91), low pH (3–4), and contained about 300 g kg$^{-1}$ crude protein (dry weight basis) (Table 1). These values are within the range typically reported for this product$^{12}$ and should allow for general extension of our results.

Main effects due to temperature and time treatments on CO$_2$ production were significant ($P < 0.001$ (Table 2). As expected, CO$_2$ production was greatest in DWG held at 32 °C, moderate at 22 °C and lowest at 12 °C (Fig. 1). CO$_2$ production generally increased over time at 32 °C and 22 °C; however, at 12 °C, we saw little evidence for CO$_2$ production over the 10-day incubation period.

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<table>
<thead>
<tr>
<th>Property</th>
<th>March</th>
<th>September</th>
<th>November</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content (g kg$^{-1}$, wb)</td>
<td>519 (2)</td>
<td>657 (5)</td>
<td>673 (3)</td>
</tr>
<tr>
<td>Water activity</td>
<td>0.92 (0.01)</td>
<td>0.91 (0.01)</td>
<td>0.91 (0.01)</td>
</tr>
<tr>
<td>Density (g cm$^{-3}$)</td>
<td>0.45 (0.01)</td>
<td>0.30 (0.03)</td>
<td>0.51 (0.02)</td>
</tr>
<tr>
<td>Thermal conductivity (W m$^{-1}$ °C$^{-1}$)</td>
<td>0.19 (0.01)</td>
<td>0.30 (0.03)</td>
<td>0.22 (0.05)</td>
</tr>
<tr>
<td>Thermal diffusivity (mm$^2$ s$^{-1}$)</td>
<td>0.09 (0.01)</td>
<td>0.10 (0.01)</td>
<td>0.10 (0.01)</td>
</tr>
<tr>
<td>Hunter $L$</td>
<td>45.89 (1.57)</td>
<td>54.95 (1.52)</td>
<td>52.70 (1.02)</td>
</tr>
<tr>
<td>Hunter $a$</td>
<td>9.35 (0.18)</td>
<td>7.05 (0.29)</td>
<td>7.74 (0.23)</td>
</tr>
<tr>
<td>Hunter $b$</td>
<td>22.52 (0.75)</td>
<td>23.33 (0.33)</td>
<td>24.45 (0.29)</td>
</tr>
<tr>
<td>pH</td>
<td>3.17 (0.05)</td>
<td>–</td>
<td>4.46 (0.01)</td>
</tr>
<tr>
<td>Crude protein (g kg$^{-1}$, db)</td>
<td>295 (4)</td>
<td>302 (1)</td>
<td>314 (3)</td>
</tr>
<tr>
<td>Crude fat (g kg$^{-1}$, db)</td>
<td>86 (5)</td>
<td>81 (1)</td>
<td>101 (5)</td>
</tr>
<tr>
<td>Ash (g kg$^{-1}$, db)</td>
<td>29 (1)</td>
<td>22 (1)</td>
<td>25 (1)</td>
</tr>
<tr>
<td>Carbohydrate (g kg$^{-1}$, db)</td>
<td>591 (9)</td>
<td>596 (1)</td>
<td>561 (2)</td>
</tr>
<tr>
<td>Crude fiber (g kg$^{-1}$, db)</td>
<td>93 (4)</td>
<td>95 (5)</td>
<td>94 (6)</td>
</tr>
</tbody>
</table>

Results are given as mean values with 1 standard deviation in parentheses ($n = 450$). wb, wet basis. db, dry basis.
Visible mold is an indicator of progressing spoilage that is easily assessed and therefore we measured the development of molds on aging DWG by visual inspection and assignment of a numerical rating. Both temperature and time had significant treatment effects ($P < 0.001$) on mold ratings; the significance of treatment factors and their interactions were identical for mold and CO$_2$ production. When the DWG was held at 32°C, molds first appeared at day 3 in the September and November experiments (Fig. 2). In DWG held at 22°C, molds appeared by day 5 in the September and November experiments. In the March experiment, the appearance of molds at both 32°C and 22°C was between 6 and 7 days, later than the other two experiments. It may be that DWG that was produced in March was immediately subjected to freezing temperatures (0°C, ambient temperature) near the end of winter that reduced colonization and activity in the DWG. In comparison, the highest mold ratings were observed in the samples collected in September when it was warmer (15°C, ambient temperature) near the end of summer, which provided conditions that enabled molds to develop more rapidly. While we did not identify the molds in the current study, past work has shown that fungi colonizing DWG at ethanol plants can include genera that contain species capable of mycotoxin production, i.e. Alternaria, Fusarium and Penicillium.4

In a previous study, Hunter $L$, $a$ and $b$ color parameters were significantly correlated with microbial growth and activity in DWG stored under ambient outdoor conditions.5 In the current study,
Hunter \( L \) changed little over the 10-day storage period at any one of the three fixed temperatures (Fig. 3) and its correlation with \( \text{CO}_2 \) production was weak (Table 3), similar to our earlier study. There was a significant (\( P < 0.001 \)) temperature effect on both Hunter \( a \) and \( b \) with the 32 °C treatment having lower values than either 12 °C or 22 °C (\( P < 0.05 \), Tukey’s multiple comparison test). Storage time had a significant main effect (\( P < 0.001 \)) on Hunter \( a \) and \( b \) values and had significant interactive effects with temperature and experiment. Multiple comparisons found that Hunter \( a \) and \( b \) values significantly decreased (\( P < 0.01 \), Tukey’s) at days 4–6, 3–5 (and 10), and 5–6 in the March, September and November experiments, respectively. Hunter \( a \) and \( b \) values were highly correlated (\( r = 0.851 \), Table 3) and the time periods of changing Hunter \( a \) and \( b \) values corresponded to the first appearance of mold on the DWG in each of these experiments. Since final Hunter \( a \) and \( b \) values were lower than the initial values (September experiment) and decreased during the onset of molding (all three experiments), there is some support for our previous conclusion that decreased Hunter \( a \) and \( b \) values reflect DWG aging. However, linear correlations between Hunter \( a \) (\( r = -0.205 \)) and \( b \) (\( r = -0.355 \)) and \( \text{CO}_2 \) production were weak, partly because of the modest effect of temperature on color parameters compared to mold and \( \text{CO}_2 \) production.

Surface modeling (Fig. 4) using nonlinear, multiple regression clearly illustrated the nonlinear nature of the relationships between time, temperature, and \( \text{CO}_2 \) production. The best-fit equation which described this behavior was:

\[
\text{CO}_2 = 2318.14 + -255.42c + -439.90d + 5.88c^2 + -2.26d^2 + 45.39cd
\]

\( (1) \)

where \( c \) represents temperature (°C) and \( d \) represents time (days). This equation had an \( R^2 \) value of 0.62, which was moderately high. As shown in the graph, as both storage temperature and time increased, \( \text{CO}_2 \) production increased, but at an increasing rate. Furthermore, the interaction between time and temperature had a significant effect (\( P < 0.0001 \)) on \( \text{CO}_2 \) production. Based on our empirical data, at moderate storage temperatures (i.e. 22 °C), appropriate storage times appeared to range from 4 to 7 days, which corresponds to typical storage periods used at commercial ethanol facilities. Using Equation 1, at this temperature and these times, predicted \( \text{CO}_2 \) generation ranged from 1743.38 to 3344.84 mg L\(^{-1}\). Thus, maximum storage times for a given storage temperature can be predicted using Equation 1 by constraining the \( \text{CO}_2 \) level between these maximum and minimum levels. As depicted in Fig. 5, as storage temperature increases, maximum

![Figure 3. Hunter color values over time for each sampling period. Avg denotes average; stdev denotes standard deviation.](image-url)
shelf life decreases following a power law relationship. Additionally, at a given storage temperature, as storage time increases, CO₂ generation (and thus microbial activity and spoilage) increases. Therefore, the predicted maximum storage time for a given storage temperature is provided in the shaded region between the maximum and minimum CO₂ generation levels.

Freshly produced DWG should be nearly sterile having undergone elevated temperatures (65–80 °C), high ethanol concentrations, low pH (<4.5), and exposure to selective antibiotics. However, our previous studies documented viable microorganisms in DWG produced by modern fuel ethanol plants. DWG derived from the fermentation of wheat and corn for the beverage industry were reported to be initially sterile (wheat) or non-sterile (corn) in the only other microbiological studies of freshly produced DWG that we located. In any event, produced DWG is probably rapidly colonized from windblown inocula, the pad itself, and handling equipment, as microbial growth and activity are observed to proceed with even aseptically-handled samples collected from the plant. Our data on CO₂ production and visible mold colonization indicate that at temperatures near 12 °C, the aerobic stability of DWG is high and that it can be stored for at least a ten day period. When temperatures were close to 22 °C, the onset of increased microbial activity and visible mold colonization occurs between 4 and 7 days and both activity and mold ratings were very high by the ninth day in all three experiments. At 32 °C, 2 days may be a more appropriate limit for storage. Our data does not validate or discount the use of color analysis as indicator of spoilage, but the simple visual appearance of mold appears to be a reasonable indicator that correlates well (r = 0.694) with CO₂ production.

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